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ABSTRACTS

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BIOLOGY AND MANAGEMENT OF *MELOIGOGYNE HAPLA* ON CARROTS, ONIONS AND LETTUCE IN NEW YORK. **Abawi, G. S., T. L. Widmer, J. W. Ludwig, and N. A. Mitkowski.** NYSAES, Cornell University, Geneva, NY.

In recent years, the northern root-knot nematode (NRNK) has become widely distributed and an important factor in the production of vegetables, especially on organic soils. In commercial fields heavily infested with NRKN, marketable yields of carrots and onion were reduced by as much as 45% and 70%, respectively. Lettuce weight was reduced by 26% in field microplots at an infestation level of > 2 eggs/cc soil (damage threshold density) and heads of lettuce rarely reached marketable size in heavily infested sections of commercial fields. Vydate applied as a broadcast or drench application was found highly cost-effective on carrots. Similar results were obtained on onions with Vydate and a request for its registration for use in New York has recently been made. Green manures of sudangrass were found effective in suppressing the population of NRKN and its damage to these vegetables. Commercially grown cultivars of these vegetables are susceptible to NRKN. Available preparations of biocontrol organisms have not been effective against NRKN. Current crop rotations on organic soils are of limited value due to the susceptibility of crops grown. Thus, the development of an integrated management program against NRKN is being emphasized.

OCCURRENCE OF *PASTURIA PENETRANS* IN AL-QASSIM, SAUDIA ARABIA. **Al-Rehiayani, S., A. A. Farahat, and M. M. Belal.** Plant Protection Department, King Saud University, Al-Qassim, Saudi Arabia.

A survey study has been initiated to determine the presence and distribution of *Pasturia penetrans* on nematodes in Al-Qassim fields. *Pasturia penetrans* was detected in a grape field in the Malieda area in Al-Qassim, and was observed attached to juveniles of *Meloidogyne* spp. This is the first report of *P. penetrans* in Saudi Arabia.

DEVELOPMENTAL RATES OF TWO PATHOTYPES OF *MELOIDOGYNE ARENARIA* ON GRAPE. **Anwar, S. A., and M. V. McKenry.** Department of Nematology, University of California, Riverside, CA 92521.

Penetration, development and reproduction of second-stage juveniles from two resistance-breaking populations of *Meloidogyne arenaria* were compared with non-aggressive populations of *M. incognita* and mixed *Meloidogyne* spp. Hosts included susceptible Cabernet Sauvignon as well as the two newly susceptible rootstocks. Differences among nematodes in rate of penetration, development and reproduction were observed for 46 days. Mixed *Meloidogyne* spp. were unable to invade Freedom and Harmony rootstocks, whereas *M. incognita* penetrated but could not develop beyond underdeveloped third-stage juveniles. The *M. arenaria* pathotypes reproduced equally well on Cabernet Sauvignon. A significantly greater number of *M. arenaria* pt. Freedom juveniles penetrated on both Freedom and Harmony rootstocks compared to that of *M. arenaria* pt. Harmony; however, the number of adult females was statistically equal. Reproduction factor (Pf / Pi) and fecundity (eggs/g root) of *M. arenaria* pt. Freedom was 4.11 and 9.2 times greater on Freedom than on Harmony, respectively. Currently, these pathotypes are morphologically and biochemically indistinguishable; however, they commonly occur within 15 years of planting these rootstocks into sandy soils.

RESISTANCE OF TEN GRAPE ROOTSTOCKS AGAINST SIX *MELOIDOGYNE* SPP. **Anwar, S. A., M. V. McKenry, and S. Kaku.** Department of Nematology, University of California, Riverside, CA 92521.

Ten grape rootstocks were evaluated in microplots for reactions to non-aggressive populations of *Meloidogyne incognita* R3, *M. chitwoodi*, mixed *Meloidogyne* spp., and aggressive populations of *M. arenaria* pt. Freedom, *M. arenaria* pt. Harmony and *Meloidogyne* sp. pt. Ramsey. The following grape rootstocks were screened for resistance to root-knot nematodes: 6-19B, 10-17A, 10-23B, RxS-3, RxS-2, Teleki 5C and Ramsey. The rootstocks Freedom and Harmony were used as a commercially resistant check, and Cabernet Sauvignon was used as a susceptible check. Each replicate was inoculated with 716 (211–1,500) second-stage juveniles per 250-cm^3 soil of field population of each species/populations. Fourteen months after inoculation, number of eggs and root weight were determined. The rootstocks were rated for egg production on a 0–5 scale; where 0 = no eggs, 1 = 1 or 2, 2 = 3–10, 3 = 11–30, 4 = 31–100, and 5–100 eggs per gram of root. The resistance of 10-23B was observed common against all *Meloidogyne* spp. and pathotypes tested. The rootstocks 6-19B, 10-17A, 10-23B, and RxS-3 also demonstrated broader root-knot resistance than the commercially available rootstocks.

GNOTOBIOTIC CULTURE OF *PASTEURIA* SP. ON *BELONOLAIMUS LONGICAUDATUS*. **Bekal, S.,[1] R. M. Giblin–Davis,[2] and J. O. Becker.[1]** [1]Department of Nematology, University of California, Riverside, CA 92521, and [2]University of Florida, Ft. Lauderdale, FL 33314.

Sting nematodes isolated from an infested golf course in the Coachella Valley, CA, were added to soil containing *Pasteuria* sp. (S-1) endospores. This specific bacterial parasite of *Belonolaimus longicaudatus* was obtained from an infested golf course in Florida. After 20 days, nematodes were encumbered with endospores and extracted from soil. Adult nematodes with an average of 28 *Pasteuria* endospores attached were surface sterilized by migration through 1.2% water agar and transferred to excised corn roots grown in Gamborg's B5 medium. After 45 days at 28 °C, one-third of the nematodes were filled with mature endospores. Filled nematodes were collected from the culture and broken open. Newly formed endospores released from nematodes attached to all sting nematode juvenile stages and adults. Development of *Pasteuria* sp. was observed in third- and fourth-stage juveniles and in adults of the sting nematode.

EFFECTS OF PRE-FREEZING FLOODING AND FREEZING TEMPERATURES ON LARVAE AND EGG MASSES OF *MELOIDOGYNE HAPLA*. **Bélair, G.** Agriculture and Agri-Food Canada, Saint-Jean-sur-Richelieu, Quebec, Canada.

Meloidogyne hapla second-stage juveniles and egg masses were exposed to pre-freezing flooding and freezing temperatures in an organic soil. On a 32-day period, infested soil samples were deposited in Styrofoam cups and exposed to a lowering temperature regime brought down from 18° to -8EC with a 4–6 °C drop every four days. Flooding was performed when soil temperatures had reached 18°, 14°, 10°, 4° or 0 °C. A second set of cups was lowered from 18° to 4 °C and flooded at those pre-freezing temperatures. Non- flooded checks were included for both the freezing and the 4 °C regimes. The assay was performed with two different inocula, one with second-stage juveniles and the other with egg masses. Each treatment was repeated eight times in a randomized, complete block design. After 32 days, all cups were drained for seven days at 25 °C. A 2-week tomato cv. Rutgers seedling was transplanted in each cup and grown for 21 days at 23 °C in the greenhouse. The survival of *M. hapla* was estimated by counting the number of nematodes inside the acid-fuschin-stained root system and the number of larvae per 100 cc of soil recovered with the pan method. Working with the second-stage juvenile inoculum, pre-freezing flooding and freezing temperatures significantly reduced by 86% and 68%, respectively, the number of juveniles in the soil when compared to the non-flooded and non-frozen soil samples. Pre-freezing flooding made at 18 °C reduced by 98% the number of juveniles in the soil, which was significantly different from the 14°, 10°, 4° and 0 °C treatments with 94%, 93%, 93% and 92% reduction, respectively.

Working with the egg-mass inoculum, pre-freezing flooding and freezing temperatures had no significant effect on the number of nematodes in roots and on the number of juveniles in the soil. These results indicate that, under Québec's winter soil temperatures, fall flooding of *M. hapla*-infested organic soils could significantly reduced second-stage juvenile populations, but is likely to have a low impact on egg-mass populations.

A HOMOLOGUE OF *CAENORHABDITIS ELEGANS* CALNEXIN PRECURSOR GENE FOUND IN *GLOBODERA PALLIDA*. **Bendezu, I. F.,[1] K. Evans,[2] and S. J. Turner.[3]** [1]Department of Plant Pathology and Microbiology, Texas A&M University, College Station, TX 77843, [2]IACR–Rothamsted, Harpenden, Hertfordshire AL5 2JQ, UK, and [3]DANI–Newforge Lane, Belfast, UK.

RAPD-PCR patterns of DNA from a British population of the potato cyst nematode *Globodera pallida* reared for several generations on either resistant (*Solanum vernei* (Vtn)2 62.33.3) or susceptible (Arran Banner) potato cultivars were compared. From 66 Operon random primers, the primer I-13 gave banding patterns that showed differences between the populations, including one band of approximately 860 bp that differentiated between selected and unselected populations from Little Ouse (pathotype Pa2). This DNA fragment was cloned and sequenced and may be a part of the gene encoding a *Globodera pallida* homologue of a *Caenorhabditis elegans* calnexin precursor gene. The *G. pallida* sequence, comprising 148 amino acids, showed 57% identity with the *C. elegans* sequence. The protein coded by this gene has been reported to have a major role in the quality control apparatus of the endoplasmic reticulum by retaining incorrectly folded glycoproteins, a type of protein which has been found to be related to the existence of nematode feeding structures in cells of plant roots. As this gene or allele was found only in virulent individuals, it is proposed that the failure of non-virulent individuals to become established in roots is due to the lack of this allele, which results in a defective glycoprotein that does not perform its intended physiological role.

IMPACT OF AN INTRODUCED C4 PLANT ON THE NEMATODE COMMUNITY STRUCTURE OF THREE, "OLD FIELD" COMMUNITIES. **Berney, M. F.,[1] G. W. Bird,[1] and W. L. Goodfriend.[2]** [1]Department of Entomology, Michigan State University, East Lansing, MI 48824, and [2]Kellogg Biological Station, Michigan State University, Hickory Corners, MI.

Andropogon gerardi, a deep-rooted C4 bunch grass was transplanted to three "old field" succession sites at the W.K. Kellogg Biological Station in Hickory Corners, MI. Five years after establishment, soil and root samples were taken for nematode analysis from the rhizosphere of *A. gerardi* and from the adjacent, naturally occurring plant community. The samples were taken at plant senescence, and replicated six times at each of the three sites. Nematodes were extracted from the soil and associated root tissue using the Berman funnel technique. The nematodes recovered were identified to genus and assigned to one of the following seven life history groups: plant parasite, plant associate, bacterial feeder, fungal feeder, algal feeder, omnivore or carnivore. Several measures of community structure were used for analysis of the resulting data. Significant differences were found in the nematode community associated with *A. gerardi*, compared to the nematode community supported by the naturally occurring vegetation. These differences included the ratio of bacterial feeding to fungal feeding nematodes. This ratio was significantly higher in the *A. gerardi* community than in the native community. There were also differences in community structure among the three research sites.

MICRO-PLOT EVALUATION OF THE PEST STATUS OF *PRATYLENCHUS COFFEAE*, *HELICOTYLENCHUS MULTICINCTUS* AND *MELOIDOGYNE* SPP. ON PLANTAIN (*MUSA* AAB, CV. APANTU-PA) IN GHANA. **Brentu, C. F.,[1] P. R. Speijer,[1] K. R. Green,[1] and B. M. S. Hemeng.[2]** [1]International Institute of Tropical Agriculture (IITA), Croydon, England, and [2]Crop Science Department, University of Science and Technology, Kumasi, Ghana.

The pest status of three nematode species was determined on Apantu-pa, the preferred cultivar of plantain (*Musa* AAB) in Ghana. Hot-water treated suckers, planted in 3 L bags containing sterilized soil, were inoculated one month after planting with a single species, a species mixture or not inoculated. Single species populations of *Pratylenchus coffeae*, *Helicotylenchus multicinctus* and *Meloidogyne* spp. were used at 1,000 or 10,000 nematodes per plant or in a mixture of 3,000 nematodes for each species per plant. Three months after planting, the inoculated suckers were transplanted into micro-plots (0.7 m^3 concrete containers filled with sterilized soil). All species significantly ($P < 0.05$) reduced the bunch weight, when compared to the non-inoculated control. High inoculation densities of *H. multicinctus* and *Meloidogyne* spp. reduced production by 26% and 30%, respectively, while the species mixture reduced production by 47%. Production losses exceeding 70%, compared to the control ($P < 0.05$), occurred under high inoculation densities of *P. coffeae*. This reduction was, in particular a result of the high toppling incidence (60%) of plants carrying bunches in the *P. coffeae*-infested plots. Given that *P. coffeae* is the most widespread and abundant nematode species on plantain in Ghana, it is evident that this species represents a major production constraint.

IMMUNOLOCALIZATION OF PROTEINS INVOLVED IN THE ATTACHMENT OF *PASTEURIA PENETRANS* TO *MELOIDOGYNE ARENARIA.* **Brito, J. A.,[1] J. F. Preston,[2] D. W. Dickson,[1] D. Williams,[2] H. C. Aldrich,[2] R. M. Giblin–Davis,[1] and J. D. Rice.[2]** [1]Entomology and Nematology Department, and [2]Microbiology and Cell Science Department, University of Florida, Gainesville, FL 32611.

An IgM monoclonal antibody has been selected on the basis of its ability to block the attachment of *Pasteuria penetrans* (isolate P-20) spores to the cuticle of *Meloidogyne arenaria*, and to recognize an epitope shared on several polypeptides separated by SDS-PAGE. This IgM was used to follow the appearance of adhesins during sporogenesis and to localize the adhesins involved in attachment. Transmission electron microscopy was used to examine thin sections of twenty-day-old healthy and P-20-infected females, and P-20 spores attached to second-stage juveniles. Nematodes were fixed, dehydrated, and embedded in LR White resin. The labeling was performed with anti-P-20 IgM and anti-mouse IgM conjugated with colloidal gold. Sections were post-stained with aqueous uranyl acetate and lead citrate. Antigens bearing the epitope were uniformly distributed in the sporangium, exosporium and parasporal fibers. The labeling of adhesins was more abundant in mature spores than in early developmental stages. No labeling was observed in the vegetative cells. The appearance of antigens during the development supports our previous studies by ELISA, which indicates a temporal synthesis of the epitope during sporogenesis. The uniform distribution of the epitope suggests a potential role in the apolar attachment of the spores to receptors on the cuticle of the nematodes.

DO GROWTH REGULATORS OR DEFENSE COMPOUNDS PRESENT IN POTATO HAVE A ROLE TO PLAY IN *GLOBODERA ROSTOCHIENSIS* HATCH? **Byrne, J. T., and B. B. Brodie.** U.S. Plant, Soil and Nutrition Laboratory, Cornell University, Ithaca, NY 14853.

Large scale *Globodera rostochiensis* hatch occurs in response to specific compounds present in potato root diffusate (PRD). Two classes of potato-specific defense compounds were found to significantly influence *in vitro* hatching behaviour. An observed reduction in the hatch activity of PRD collected from the H1- resistant variety ‘Kanona’ after J2 invasion may be due to a H1-dependent increase in phytoalexin production. The phytoalexins caused significant *in vitro* hatch inhibition effective down to a 0.005 ppm concentration. The potato glycoalkaloids (alpha-solanine, alpha-chaconine and beta-2-chaconine) initiated significant hatch responses at 10-3 and 10-4 M concentrations. PRD from one-week-old ‘Superior’ plants was found to have a 3 × 10-9 M glycoalkaloid concentration. Several growth regulators and a large number of organic compounds were screened both alone and in various combinations for hatch activity. Zeatin riboside was found

to synergise both PRD and glycoalkaloid-induced hatch. Diffusates were collected from several tomato mutants. Those deficient in gibberellicacid produced a diffusate with reduced hatch activity towards *G. rostochiensis*.

NEMATODE SPECIFIC GRAVITY PROFILES AND APPLICATIONS TO FLOTATION EXTRACTION AND TAXONOMY. **Carta, L. K., and D. G. Carta.** Nematology Laboratory, ARS, USDA, Beltsville, MD 20705.

A technique was developed that refines the standard sugar flotation procedure used to isolate nematodes from their surroundings. This allows the extraction of information about nematode density that can be used as a physical character. By centrifuging nematodes in a number of increasing specific gravity solutions and plotting the fraction floating, the cumulative probability distribution of the population's specific gravity is generated. By assuming normality, the population mean, F, and standard deviation, s, can be found by a non-linear, least squares procedure. Mean and standard deviation pairs (F, s) were found for the specific gravities of the adult stage of the plant parasites *Pratylenchus agilis* (1.068, 0.017), *P. scribneri* (1.074, 0.027), *P. penetrans* (1.060, 0.015) and the bacterial-feeder *Caenorhabditis elegans* (1.091, 0.016).

INFLUENCE OF GIBBERELLIN ON REPRODUCTION AND EGG HATCHING OF SOYBEAN CYST NEMATODE. **Casta, L., W. Anderson, C. Shirk, and P. M. Tefft.** Department of Biology, St. Joseph's University, Philadelphia, PA 19131.

The development of the soybean cyst nematode is dependent on many factors, including the physiological status of the host plant. Because plant growth regulators have profound effects on plant physiology, we studied the effect of the hormone gibberellin on nematode development. In one experiment, three groups of plants (24 plants per group) were foliage sprayed with either 10^{-6}M/week gibberellin and 10^{-4}M/week gibberellin or distilled water as controls. These plants were inoculated with an equal aliquot of nematode eggs in suspension (30 cysts/plant). Nematode development was assessed by sampling infected plants weekly over a four-week period. Plant roots were cleared and stained with acid fuchsin. Juveniles were counted and developmentally staged. Morphogenic effects of the hormone on soybean plants were monitored by the determination of plant height, number of nodes and root weight. All of the gibberellin treated plants yielded about 50% fewer nematodes compared to the untreated controls. In another experiment one group of soybean plants were treated with gibberellin (10^{-4}M/week) and a control group was sprayed with distilled water. These plants were used to collect root diffusates. Surface disinfested nematode eggs were exposed to diffusates calibrated to 1 RGH from the roots of treated and untreated plants. Hatching response was monitored after two weeks and compared to hatching in water and zinc chloride (3 mM). Hatching in the diffusate derived from plants treated with gibberellin were significantly less than hatching in diffusates from untreated controls.

SURFACE DISINFESTATION OF HETERODERA GLYCINES EGGS AFFECTS HATCH BEHAVIOR. **Charlson, D. V., and G. L. Tylka.** Department of Plant Pathology, Iowa State University, Ames, IA 50011.

In 1972, T. Okada reported that *Heterodera glycines* egg homogenates stimulate hatching of *H. glycines* eggs. However, during our initial investigations, stimulation from *H. glycines* egg homogenates was not detected in eggs surface disinfested with 0.5% chlorhexidine diacetate. Consequently, hatch of *H. glycines* eggs was further studied in laboratory experiments. Soil infested with *H. glycines* was collected from a field near Ames, IA, in May 1998 and stored at 20–24 °C until eggs were isolated from cysts. Free eggs were either surface disinfested or left untreated. Eggs were surface disinfested by incubating in 0.5% chlorhexidine diacetate for 15 min at 20–24 °C, followed by rinsing several times with sterile distilled water. Surface disinfested and untreated eggs were incubated in darkness at 20–24 °C in either sterile distilled water, 3 mM zinc sulfate, one of

three concentrations of soybean root diffusate, or one of three concentrations of *H. glycines* egg homogenates. Eggs were transferred to new, sterile incubation solution every two days for 18 days. Hatched juveniles were counted after each transfer, and percent cumulative hatch was determined for each treatment. Surface disinfestation with chlorhexidine diacetate reduced hatch of *H. glycines* eggs relative to hatch of untreated eggs in soybean root diffusate and egg homogenates, but surface disinfestation did not affect hatch in water or 3 mM zinc sulfate. Hatching of both surface disinfested and untreated eggs was stimulated by 3 mM zinc sulfate, but not by soybean root diffusate. Surface disinfested eggs were stimulated to hatch only in the highest concentration of egg homogenate, where as untreated eggs were stimulated by the two highest concentrations. Results indicate that treatment of eggs with chlorhexidine diacetate may influence egg hatch response to egg homogenates and may indicate that hatch stimulation in zinc sulfate is not a biological response.

FUNGI ASSOCIATED WITH THE SOYBEAN CYST NEMATODE IN MINNESOTA. **Chen, F. J., and S. Y. Chen.** University of Minnesota Southern Experiment Station, Waseca, MN 56093.

A survey was conducted in 1996–97 to determine species and frequency of fungi colonizing females, cysts and eggs of the soybean cyst nematode (SCN), *Heterodera glycines*, in Minnesota. A total of 45 soil samples from 26 counties across southern Minnesota were examined. Soybeans were grown in pots containing the soil with the addition of 20,000 SCN second-stage juveniles per pot in a growth room. Cysts and females were extracted from the soil and soybean roots after two months. One hundred cysts, 100 females and about 1,000 eggs from each soil sample were examined for fungal colonization. Fungal colonization in cysts, females and eggs varied among the samples. Fungi colonized 29% to 89% (average 55%) of the cysts. A total of 53 species were identified. *Cylindrocarpon destructans* (18%), *Fusarium solani* (8%), *Pyrenochaeta terrestris* (7%) and *Fusarium oxysporum* (6%) were frequently encountered in cysts. Frequency of fungi colonizing females was usually low, from 0 to 41% (average 3.5%). More than 26 species were isolated from the females. *Fusarium solani, F. oxysporum* and *C. destructans* were the most common fungi in females. An average of 0.9% of eggs was colonized and more than 21 fungal species were recovered. *Cylindrocarpon destructans, P. terrestris, F. solani, F. oxysporum* and *Exophiala pisciphila* were common in eggs.

COLONIZATION OF SOYBEAN ROOTS BY FUNGI PATHOGENIC TO *HETERODERA GLYCINES* EGGS. **Chen, F. J., and S. Y. Chen.** University of Minnesota Southern Experiment Station, Waseca, MN 56093.

The ability of a nematode egg-parasitic fungus to colonize plant roots is an important trait for an effective biocontrol agent. Nine fungal strains of five species with various degrees of pathogenicity to *Heterodera glycines* (SCN) eggs were evaluated in the greenhouse for their ability to colonize soybean roots. Soybeans were planted in 10-cm-diameter pots containing non-sterilized field soil with the addition of a test fungus cultured on corn grits. Three thousand SCN second-stage juveniles (J2) were added to each pot one week after planting. Soybean roots were removed from the soil two weeks after the addition of J2. Two pieces of root, 5 mm long, were cut from each primary root and a total of 60 pieces of root from each treatment were examined for fungal colonization. Fungi colonized 32% to 60% of the root segments. Percentage of root segments colonized by the test fungi were 0 to 23%, depending on fungal strains and species. *Verticillium chlamydosporium* was isolated from 23% of the root segments. Two strains of *Cylindrocarpon destructans* were recovered from 3% and 7% of the root segments, respectively. One strain of *Fusarium solani* was isolated from 3% and another from 13% of the root segments. Percentages of root segments colonized by three stains of *Fusarium oxysporum* were 0%, 13%, and 15%, respectively. *Phoma* sp. colonized 7% of the root segments.

THE EFFECT OF FUNGAL-FEEDING NEMATODES ON FUNGAL BIOMASS DURING DECOMPOSITION OF ORGANIC MATTER. **Chen, J.,[1] H. Ferris,[1] K. M. Scow,[2] and K. J. Graham.[2]** [1]Department of Nematology, and [2]Department of Land, Air and Water Resources, University of California, Davis, CA 95616.

Fungi (*Rhizoctonia solani* and *Trichoderma* sp.) and nematodes (*Aphelenchus avenae* and *Aphelenchoides composticola*), isolated from field soils, were introduced into alfalfa/cellulose/sand microcosms with C:N ratios of 11:1, 20:1, 30:1 and 40:1. The six treatments included each fungus alone or with each nematode. Fungal biomass was measured by identification and quantification of phospholipid fatty acids (PLFAs) extracted from living cell membranes through gas chromatography. Principal component analysis was used to identify key PLFA patterns associated with fungal colonizers and their nematode grazers. Fatty acid 18:2w6c, a fungal indicator, consisted of 52.2% + 3.8 of total PLFAs in pure broth cultures, and 10.2% + 2.3 in alfalfa/cellulose/sand for *R. solani* in the absence of nematodes. Biomass of *R. solani*, as indicated by fungal PLFAs, was reduced in the early stage of decomposition of the organic matter in the presence of *A. composticola*, but was not reduced by day 21. There was more N mineralized in the presence of nematodes grazing on *R. solani* than in their absence. Fungal biomass of *R. solani* was lower at C:N ratios of > 30:1 than at < 20:1 on day 21, but C:N effects were inconsistent for *Trichoderma* sp.

INVESTIGATION OF FUNGAL ANTAGONISTS OF *HETERODERA GLYCINES* IN MINNESOTA. **Chen, S. Y., F. J. Chen, X. Z. Liu, and C. D. Reese.** University of Minnesota Southern Experiment Station, Waseca, MN 56093.

During 1996–98, research was done to determine the impact of fungal antagonists on the *Heterodera glycines* (SCN) in Minnesota. A total of about 50,000 eggs, 6,000 females and 9,000 cysts from 47 soils collected from 26 counties in Minnesota were examined for fungal colonization. About 7,000 isolates, belonging to more than 80 species of fungi, were isolated and identified. Fungi that were frequently encountered in cysts, females and/or eggs were tested for their pathogenicity to the nematode eggs. Fungal parasites of the second-stage juveniles (J2) of the nematode were also investigated in Minnesota soybean fields. A total of 435 soil samples from 270 fields were examined for parasites of J2. Two fungal species, *Hirsutella rhossiliensis* and *Hirsutella* sp., were frequently encountered in SCN J2. About 39% of the soil samples were infested with *H. rhossiliensis* and/or *Hirsutella* sp. A total of 22 isolates, belonging to 16 species of egg parasites and 18 isolates of J2-parasitic fungi, *Hirsutella* species, were evaluated in the greenhouse. A few fungal species significantly reduced the nematode population density. An egg-parasitic fungus, ARF18, reduced egg density by 98.4% and one isolate of *H. rhossiliensis* reduced egg density by 85.5% to 99.9% in greenhouse soils. In 1998 five fungal species were evaluated in a field as biocontrol agents. The J2-parasitic fungi, *H. rhossiliensis* and *Hirsutella* sp. significantly reduced the nematode density. The efficacy of *Hirsutella* sp. in reducing egg density (58% reduction) at the end of season was similar to the efficacy of the nematicide aldicarb (64% reduction). Our studies suggest that fungal antagonists may have promising potential against SCN as biocontrol agents.

EVALUATION OF TRAPPING CROP FOR MANAGING THE 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 Experiment Station, Waseca, MN 56093, [2]University of Minnesota Southwest Experiment Station, Lamberton, MN, and [3]Department of Plant Pathology, University of Minnesota, Minneapolis, MN.

An experiment was conducted at two field sites at Waseca and Lamberton, Minnesota, in 1998 to evaluate potential of trapping the soybean cyst nematode (SCN) with soybean plants in the corn year to manage the SCN. The experiment was a factorial design including four seeding rates (0, 123,500, 247,000 and 494,000 seeds/hectare) and four dates (3, 4, 5 and 6 weeks after planting) of killing soybean plants with a herbicide. Six replicates were used. Each plot consisted of four 76-cm corn rows, 7.6 m long. SCN-resistant soybean cultivar Pioneer-9234 was drilled before planting

corn at the same day. The plots were separated by four rows of corn without soybean. Nematode egg densities were determined at planting, one month and two months after planting, and at harvest. Corn yield was measured. No significant difference in the nematode population density was observed among the treatments at both sites. Average initial nematode density was 1,900 and 10,852 eggs per 100 cm^3 of soil and average final nematode density was 1,776 and 8,903 eggs per 100 cm^3 of soil at Waseca and Lamberton, respectively. All the treatments did not affect corn yield. These results suggest the use of soybean as trapping crop during the corn year was not an effective means for management of the SCN.

SOME MARINE AND TERRESTRIAL NEMATODES FOUND IN COASTAL REGIONS OF SONOMA COUNTY, CALIFORNIA. **Chitambar, J. J., and E. O. Duarte.** Plant Pest Diagnostics Center, California Department of Food and Agriculture, Sacramento, CA 95832.

Samples collected from Horseshoe Cove in Bodega Bay and the coastal regions of Port Sonoma in Sonoma County, California, yielded a number of free-living and plant parasitic nematodes associated with various hosts. A profile of nematode species associated with mussels in tide pools, green and white algae, iceplant, cordgrass, wild clover and sedge plants from the above regions was obtained through surveys conducted in 1996 and 1999. Detailed morphological descriptions were made of *Monoposthia* sp. from mussels, *Metancholaimus* sp. from green and white algae, and *Hirschmaniella* sp. from sedge plants.

FAILURE TO DETECT MONOGLYCOSYLCERAMIDES IN CYSTS OF *HETERODERAGLYCINES*. **Chitwood, D. J.** Nematology Laboratory, ARS, USDA, BARC-West, Beltsville, MD 20705.

Nematode glycolipids have putative structural and bioregulatory roles; monoglycosylceramides (MGCs) are simple glycosphingo lipids that consist of a single sugar residue attached to a long chain sphingoid base connected to a fatty acid molecule via an amide linkage. Our previous research demonstrated that MGCs comprised 0.17% (dry wt basis) of eggs of *Meloidogyne incognita* and mixed stages of *Caenorhabditis elegans*. In our current research, two attempts were made to isolate similar compounds from cysts of the soybean cyst nematode, *Heterodera glycines*. Cysts were extracted three times with hexane:isopropanol 3:2 (v:v). The crude lipid extract was fractionated on a silica column with increasing percentages of acetone in chloroform in order to obtain glycolipids. Glycolipids were analyzed by two methods. First, fractions were analyzed on a reversed phase (octadecyl) high-performance liquid chromatography column with a solvent of 1.0% water in methanol. Second, the fraction containing putative MGCs was applied to a thin-layer chromatoplate developed with chloroform: 95% ethanol 70:30. Surprisingly, MGCs in cysts of *Heterodera glycines* were not detected with either method (threshold of detection = 0.005% of dry weight.) The results are interesting in view of the reported lack of lipase activity in hatching of eggs of other *Heterodera* species, in contrast to the lipase activity that reportedly accompanies root-knot nematode egg hatching.

MARKER-ASSISTED SELECTION OF NEMATODE RESISTANCE IN PEANUT. **Church, G. T.,[1] C. E. Simpson,[2] and J. L. Starr.[1]** [1]Department of Plant Pathology and Microbiology, Texas A&M University, College Station, TX 77843, and [2]Texas Agricultural Experiment Station, Stephenville, TX 76401.

To increase the efficiency of breeding peanuts resistant to *Meloidogyne arenaria*, the utility of two RFLP loci linked to resistance in identifying individuals homozygous for resistance was determined. Two tetrafoliate leaf samples were collected from each of 548 space planted individuals from three segregating breeding lines and DNA was successfully extracted from 82.5% of the individuals with the first attempt. Extraction of the second sample resulted in DNA from a total of 94.5% of the plants. The DNA concentration was determined for each sample, was digested with EcoR I, and Southern blotted to Hybond-N+ membranes. The membranes were probed with the

RFLP specific probe R2430E, then stripped and reprobed with the probe R2545E. Samples for which no data was obtained due to problems in extraction, digestion or hybridization ranged from a low of 14.4% for breeding line TP301-1-8 probed with R2430E to 38.9% for TP294-4-4 probed with R2545E. For the three lines TP294-4-4, TP293-3-3 and TP301-1-8, 65.1%, 27.6% and 29.5%, respectively, were identified as being homozygous for resistance with R2430E. The second marker, R2545E, identified 50%, 24.5% and 23.5%, respectively, individuals homozygous for resistance. Differences between the two RFLP probes were due to unreadable data and differences in putative genotype. The use of these RFLP loci can aid in identification of genotype of individuals in a segregating population, but the data are not unambiguous.

PREDICTIVE MODELS IN MANAGEMENT OF *BELONOLAIMUS LONGICAUDATUS* IN A POTATO PRODUCTION SYSTEM. **Crow, W. T.,[1] D. W. Dickson,[1] and D. P. Weingartner.[2]** [1]Entomology and Nematology Department, University of Florida, Gainesville, FL 32611, and [2]Agriculture Research and Education Center, University of Florida, Hastings, FL 32145.

Potato in northeast Florida is grown in the winter–spring months followed by a summer cover crop of sorghum–sudangrass. *Belonolaimus longicaudatus* (sting nematode) is the nematode most strongly correlated with yield loss of potato in this production system. This research used data from field studies to generate damage functions and predictive models of population increase and decline for sting nematode in this system. These models were applied to management decisions faced by potato growers. Economic threshold densities of sting nematode were established to predict the cost-effectiveness of nematicide applications. The sorghum–sudangrass cover crop was identified as the major contributing factor to maintaining sting nematode problems. Alternatives to sorghum–sudangrass (fallow, non-host cover crops) were evaluated as management options. Based on our models, use of fallow or a non-host cover crop between potato crops should be sufficient to reduce levels of sting nematode below economic threshold densities.

INTERACTIONS BETWEEN NEMATODE CUTICLES AND POTENTIAL MICROBIOLOGICAL CONTROL AGENTS. **Davies, K. G., and P. Mendoza de Gives.** Department of Entomology and Nematology, IACR-Rothamsted, Harpenden, Hertfordshire AL5 2JQ, UK.

The Gram-positive bacterium *Pasteuria penetrans* and the nematode-trapping fungus *Duddingtonia flagrans* are micro-organisms that have potential to be developed as biological control agents of nematodes. In standard assays, spores of the bacterium exhibited varying degrees of attachment to a range of plant parasitic nematodes, but were not found to attach to animal parasitic nematodes, the free living nematode *Caenorhabditis elegans* or srf mutants of *C. elegans*. Extensive tests with *Pasteuria penetrans* against several different species of root-knot nematode (*Meloidogyne* spp.) populations showed a high degree of variation that did not correlate to nematode phylogeny. Standardised trapping assays using *Duddingtonia flagrans* and other nematode-trapping fungi against a range of animal- and plant-parasitic nematodes, *C. elegans* and its srf mutants showed that all groups of nematodes were eventually trapped by the fungi. However, differences in the rate of trapping were observed in the first 24 hrs and all fungi tested trapped the srf mutants of *C. elegans* more effectively than the wild type. Similar differences in trapping behaviour were observed not only between different species of plant-parasitic nematodes, but also between sheathed and exsheathed larvae of animal-parasitic nematodes. The results show the importance of cuticle heterogeneity in nematode microbial interactions.

RESISTANCE TO *MELOIDOGYNE INCOGNITA* AND *M. ARENARIA* IN CORN HYBRIDS. **Davis, R. F.,[1] and P. Timper.[2]** [1]Department of Plant Pathology, University of Georgia, Athens, GA 30602, and [2]NWCRU, ARS, USDA, Tifton, GA 31793.

Corn (*Zea mays*) is commonly grown in rotation with cotton and peanut for suppression of soil-borne pathogens, including *Meloidogyne* species. Resistance to *M. incognita* race 3 and *M. arenaria* race 1 in 24 corn hybrids was evaluated in separate greenhouse experiments with six

replications per test. The corn hybrids were chosen because they are grown on more than 90% of the corn acreage in Georgia. Two isolates each of *M. incognita* and *M. arenaria* were tested in two experimental trials per isolate. Pots (15-cm-diameter) were inoculated with 8,000 eggs approximately 7–10 days after corn emergence. Nematode eggs were extracted and counted approximately 56 days after inoculation. Reproduction of *M. incognita* was similar in all hybrids regardless of nematode isolate with a mean of 252,154 eggs per pot. Reproduction of *M. arenaria* differed among hybrids with a mean of 2,142 eggs per pot for the eight most resistant hybrids and 15,537 for the eight most susceptible hybrids. Reproduction of *M. arenaria* was not affected by nematode isolate. It is likely that the most resistant hybrids could be useful in managing *M. arenaria* populations. Though tested in separate experiments, it appears that these corn hybrids allow significantly less *M. arenaria* than *M. incognita* reproduction.

THREE NEW SPECIES OF *NOTHACROBELES* (NEMATODA: CEPHALOBIDAE) FROM THE MOJAVE DESERT, CALIFORNIA. **De Ley, I. T.,[1] P. De Ley,[2] J. G. Baldwin,[1] M. Mundo–Ocampo,[1] and S. A. Nadler.[3]** [1]Department of Nematology, University of California, Riverside, CA 92521, [2]Vakgroep Biologie, Universiteit Gent, B-9000 Gent, Belgium, and [3]Department of Nematology, University of California, Davis, CA 95616.

First results are presented of a long-term project to explore cephalobid diversity, including inventories of *Cephalobina* in Southern California. Three new species of *Nothacrobeles* are described from localities in the Mojave desert and comparative detailed scanning electron micrographs of the lip region are included. *Nothacrobeles* n. sp.1 is characterized by the presence of long post-vulval sac and three apparently unique tubular adoral projections. Both *Nothacrobeles* n. sp. 2 and *Nothacrobeles* n. sp. 3 are smaller than any other known species within the genus. *Nothacrobeles* n. sp. 2 has labial probolae that are short and spatulate without a basal ridge, whereas those of *Nothacrobeles* n. sp. 3 are flattened and plate-like. Furthermore, *Nothacrobeles* n. sp. 3 is unique by its extremely short corpus (less than 25 μm long in adult females), especially relative to the isthmus, and the small size of its guarding processes. Tables of measurements are included for morphometric comparison. The genus diagnosis of *Nothacrobeles* will need to be emended to accommodate some of the distinctive characteristics of these new species.

PHYLOGENETIC ANALYSES OF INTERNAL TRANSCRIBED SPACER REGION SEQUENCES WITHIN *MELOIDOGYNE*. **De Ley, I. T.,[1] G. Karssen,[2] P. De Ley,[3] A. Vierstraete,[3] L. Waeyenberge,[1] M. Moens,[1] and J. Vanfleteren.[3]** [1]Agricultural Research Centre, Merelbeke, Belgium, [2]Plant Protection Service, Wageningen, The Netherlands, and [3]University of Ghent, Belgium.

Complete ITS1, 5.8S and ITS2 sequences were determined and aligned for 27 populations of *Meloidogyne*, representing 14 nominal species: *M. ardenensis, M. arenaria* (races 1 and 2), *M. artiellia, M. chitwoodi, M. duytsi, M. fallax, M. graminicola, M. hapla* (races a and b), *M. hispanica, M. ichinohei, M. incognita, M. javanica, M. maritima, M. morocciensis* and *M. naasi*. Two groups of species have nearly identical ITS region sequences: the Fallax group (*M. fallax* and *M. chitwoodi*) and the Arenaria group (*M. hispanica, M. incognita, M. javanica, M. morocciensis* and both races of *M. arenaria*). Each of the remaining species has clearly different sequences. *M. duytsi, M. hispanica* and *M. morocciensis* display significant levels of sequence polymorphism. Length of the entire ITS region ranges from 474 base pairs in one sequence variant of *M. hispanica*, to 574 bp in *M. ichinohei*. Analyses of five pruned alignments, including *Nacobbus aberrans* as outgroup but excluding repetitive sequences and all but the most conserved positions, alternately placed *M. ichinohei* or *M. artiellia* as sister taxon to the other *Meloidogyne* species. Excluding *N. aberrans* and using either *M. ichinohei* or *M. artiellia* as outgroup, further analyses were then performed on five alignments containing the complete ITS region sequence of at least one population from each distinct species or species group. In all these analyses, the Fallax group was sister

taxon to a clade consisting of *M. naasi* and *M. graminicola*. Relationships of this robust crown group with the other species were less clearly resolved, depending greatly on choice of alignment and tree construction method. *M. ardenensis* and *M. maritima* were each other's sister taxon in most analyses.

DIFFERENCE IN GROWTH REDUCTION OF THREE INDIAN POPULATIONS OF *MELOIDOGYNE JAVANICA* ON PEANUT. **DiVito, M.,[1] F. Lamberti,[1] G. Zaccheo,[1] F. Catalano,[1] and S. B. Sharma.[2]** [1]Istituto di Nematologia Agraria, CNR, Bari, Italy, and [2]ICRISAT, Patancheru, Andhra Pradesh, India.

An experiment was undertaken to investigate the effect of a range of inoculum densities of three Indian populations of *Meloidogyne javanica* (from Makila, Pallipalem and Tiripurati) on the growth of peanut in pots. The inoculum consisted of a mixture of finely chopped tomato roots, infested with one of the root-knot nematode population, and steam sterilized sandy soil. Proper amounts of this inoculum were thoroughly mixed with the soil of each one liter pot to give initial population densities of 0, 0.125, 0.25, 0.5, 1, 2, 4, 8, 16, 32, 64, 128, 256 and 512 eggs and juveniles/cm^3 soil. One seed of the peanut cv. ICG 7827 was then sown per pot. Peanut growth parameters and nematode reproduction rates differed among nematode populations. Data of height and fresh weight of the plants fitted the Seinhorst's model $y = m + (1\text{-}m)z^{P\text{-}T}$. According to this model, tolerance limits (T) of peanut to the three nematode populations were similar: 3.27 and 2 eggs/ml soil for plant height and top weight, respectively. Minimum relative yields (m) of peanut, instead, greatly differed among nematode populations; they were 0.3, 0.4 and 0.75 for plant height and 0.15, 0.15 and 0.3 for fresh top plant weight, for the populations of Makila, Tiripurati and Pallipalem, respectively, and occurred at Pi > 128 eggs/cm^3 soil. Maximum reproduction rates (Pf/Pi) were <1 for the Makila population at all initial population densities and 127.2 and 47.2-fold, at lowest Pi, for the Pallipalem and Tiripurati population, respectively.

TEN-STATE EVALUATION OF SELECTED AGRONOMIC PRACTICES ON SOYBEAN CYST NEMATODE. **Donald, P. A.,[1] G. R. Noel,[2] H. Melakeberhan,[3] N. Atibalentja,[2] T. R. Anderson,[4] S. Y. Chen,[5] J. Faghihi,[6] J. M. Ferris,[6] C. R. Grau,[7] D. E. Hershman,[8] A. E. MacGuidwin,[7] T. L. Niblack,[1] R. D. Riggs,[9] W. C. Stienstra,[5] G. L. Tylka[10], and T. Welacky.[4]** [1]University of Missouri, [2]University of Illinois, ARS, USDA, [3]Michigan State University, [4]Agriculture Canada, [5]University of Minnesota, [6]Purdue University, [7]University of Wisconsin, [8]University of Kentucky, [9]University of Arkansas, and [10]Iowa State University.

Agronomic practices have changed in the 40 years since soybean cyst nematode (*Heterodera glycines*) (SCN) was discovered causing plant damage in the southern United States. We investigated the effects of no-tillage, row spacing and host resistance on SCN. The goal was to find production practices that would reduce SCN egg population densities while maintaining soybean yield. SCN-soybean interactions differ among sites and, to explain these differences, we measured soil and plant nutrients plus other diseases present at each location in addition to SCN egg-population density, SCN race, and soybean yield. Over two years in 88% of the sites, resistant varieties had statistically greater grain yield than susceptible varieties in cyst-infested fields. No-tillage plots had greater yields than conventional tillage plots in 55% of the sites in 1998. In all sites, row spacing had an effect on yield, but the rate of SCN reproduction did not follow the row spacing trends. Although brown stem rot, *Phialophora gregata*, was present in plant tissues in many plots, visual symptoms were not widespread in 1997. Data from these 18 sites indicate that certain information, such as the benefit of growing resistant soybean varieties, can be extrapolated across the North Central states, but the effects of other factors are site specific.

APPLICATION OF THE POLYMERASE CHAIN REACTION FOR ROOT-KNOT NEMATODE SPECIES IDENTIFICATION. **Dong, K., R. A. Dean, B. A. Fortnum, and S. A. Lewis.** Department of Plant Pathology and Physiology, Clemson University, Clemson, SC 29634.

RAPD-PCR was tested for screening the species-specific DNA fragments. Twenty-five different single-egg-mass nematode isolates, including 7 *M. arenaria*, 2 *M. hapla*, 11 *M. incognita* and 5 *M. javanica*, were used for the test. Species-specific DNA fragments have been observed in many random primer reactions and different isolates of the same species can be grouped according to the polymorphism. Three optimum RAPD-PCR primers were selected, and each of them can amplify strong polymorphic DNA fragments to differentiate the four common species. When standard nematode DNAs were used as control for PCR and gel running, the selected PCR primers can detect the species in small nematode samples, e.g., the J2s from 500 cm^3 soil samples. A mixture of more than one root-knot nematode species in the samples can also be detected by this PCR method. In addition to RAPD-PCR, species-specific DNA fragments were collected from the agarose gels and cloned into TA vector. We are trying to develop a set of species-specific PCR primers and further adapt them for the colorimetric assay system.

GENETIC AND MORPHOLOGICAL RELATIONSHIPS AMONG ISOLATES OF *PRATYLENCHUS COFFEAE* AND *P. GUTIERREZI.* **Duncan, L.,[1] R. Inserra,[2] K. Thomas,[3] D. Dunn,[1] L. M. Frisse,[3] K. Morris,[3] I. Mustika,[1] and D. T. Kaplan.[4]** [1]University of Florida, IFAS, Lake Alfred, FL 33850, [2]FDAC, DPI, Gainesville, FL 32611, [3]University of Missouri, Kansas City, MO 64110, and [4]ARS, USDA, Orlando, FL 32803.

Morphological and genome variation were characterized among four nematode isolates with morphological characteristics of *P. gutierrezi* collected from Central America and 25 isolates with morphological characteristics of *Pratylenchus coffeae* collected throughout the New World and in the Middle East, Africa and Asia. Sequence homology within the D2/D3 expansion segment of the 28s rDNA gene suggest that both groups of isolates are species complexes. The DNA sequences of a topotype isolate of *P. gutierrezi* and isolates from Costa Rica and Guatemala all differed. Although numerous *P. coffeae* isolates from diverse hosts and localities have an identical D2/D3 sequence, three different sequences were detected among the remaining isolates. Six lesion nematode isolates with numerous males and two lip annules, collected from coffee in eastern Java (the type locality of *P. coffeae*), also represent a species complex. Viewed *en face* with scanning electron microscopy, the first lip annule is divided into lateral and median sectors in one isolate from Java, while in five other isolates the first lip sector is smooth. DNA analysis of these isolates from Java is pending.

FACTORS AFFECTING *GLOBODERA ROSTOCHIENSIS* HATCHING IN SUPPRESSIVE AND CONDUCIVE SOILS. **El-Sherif, M. A., and B. B. Brodie.** Department of Plant Pathology, Cornell University, Ithaca, NY 14853.

Globodera rostocheinsis J2s emerged from cysts more in suppressive soil extract (SSE) than in conducive soil extract (CSE). However, storing CSE at 20 °C for two months increased its hatching potential compared with that stored at 5 °C for a similar period. Afterwards, CSE hatch activity declined with time and reached a minimum after four months. A 0.1% filtrate solution from a crude culture of organisms isolated from the CSE inhibited hatch in both SSE and potato root diffusate (PRD). However, filtrate of an unidentified *Actinomycete* isolated from the suppressive soil plot significantly increased the hatching activity of a diluted PRD preparation. Water extract of potato fruit balls also increased the PRD hatch activity when added at a rate of 0.1%. G-10 Sephadex fractionation of concentrates from both soils showed some hatch activity. However, the extracts differed with respect to their most hatch-active fractions. SSE continued to slowly stimulate hatching until planting time. In the presence of the potato crop, hatch stimulation gradually increased in both soil plots and reached a maximum in August. A month after harvest, SSEs possessed a hatch activity four times that of CSEs.

ULTRASTRUCTURAL STUDY OF THE FEMALE REPRODUCTIVE SYSTEM OF THE LESION NEMATODE, *PRATYLENCHUS PENETRANS.* **Endo, B. Y.,[1] U. Zunke,[2] and W. P.**

Wergin.[1] [1]Nematology Laboratory, Beltsville Agricultural Research Center (BARC), ARS, USDA, Beltsville, MD 20705, and [2]Universtät Hamburg, Institut für Angewandte Botanik, Marseiller, Hamburg, Germany.

An electron microscopic study of the reproductive system of adult females of *Pratylenchus penetrans* revealed details of the oocyte development and the transformation of oocytes into eggs. Oogonial development into oocytes was distinctive in that most of the nuclei of ovarian cells were in prophase I of meiosis. In the mid-section of the ovary, the oocytes increased in number, enlarged, and accumulated in a single row. Next, the oocytes entered a muscular oviduct and accumulated lipid bodies and protein granules. The plasma membrane of the oviduct became plicated and formed cisternae; centralized membrane junctions established openings for oocytes to enter the spermatheca. Spermatozoa traversed the lumen of the uterus and accumulated in the spermatheca. Each oocyte passed through the spermatheca and then traversed between columnar cells. The posteriad region of the columnar cells attached to other uterine cells to form the lumen of the uterus that extended beyond the vaginal opening and into the post-vulvar uterine branch of the reproductive system. The fertilized egg was deposited to the exterior after passing between cuticle-lined vaginal and vulval walls supported by anteriad and posteriad muscle bands. These observations may provide clues for modifying or disrupting nematode reproduction and could lead to new methods of control for economically destructive species.

INVESTIGATION OF THE NATURE OF THERMOSTABLE, SDS-INSOLUBLE EPICUTICULAR DETERMINANTS OF *PASTEURIA PENETRANS* ENDOSPORE ATTACHMENT TO *MELOIDOGYNE INCOGNITA.* **Esnard, J., J. T. Chin, and K. E. Schwarz.** Department of Plant Pathology, Cornell University, Ithaca, NY 14853.

The highly specific attachment mechanism of *Pasteuria penetrans* endospores to the cuticle of *Meloidogyne incognita* second-stage juveniles (J2) is not based on readily degradable or soluble components of the bacterial or nematode surface. Nematode–endospore–nematode (NEN) clumping (attachment) bioassays showed that pretreatment of endospore-studded and/or -free (*Meloidogyne incognita*) race 1 (Mi1) and 3 (Mi3) J2 by heating for 16 h at 65 °C in 20% SDS, did not prevent attachment (Esnard, 1998, *Phytopathology* 88:S26). Studies were conducted with a monoclonal antibody to collagen I ($MAb_{Col\text{-}I}$), periodic acid, and glycosaminoglycans (GAGS) to further elucidate the nature of these thermostable, detergent insoluble determinants of attachment by pretreating "stripped J2." "Stripped J2" were J2 whose surface coats were removed by heating at 97 °C for 2 h in 20% SDS. Pretreatment of stripped Mi1 J2 with $MAb_{Col\text{-}I}$ blocked NEN clumping to untreated (non-stripped) Mi1 J2 encumbered with strain P100 endospores. NEN clumping was not prevented by $MAb_{Col\text{-}I}$ when Mi3 J2 were tested. Pretreatment of stripped Mi1 or stripped Mi3 with 10 mM periodic acid (in 2 mM sodium acetate) for 18 h prevented NEN clumping with non-stripped $Mi1_{P100}$ (Mi1 encumbered with P100 endospores). Periodic acid pretreatment of non-stripped $Mi1_{P100}$ or non-stripped $Mi3_{P100}$ did not block NEN clumping with $Mi1_{P100}$ or with periodate-treated $Mi1_{P100}$. Non-stripped but periodate-treated $Mi1_{P100}$ clumped with non-stripped Mi1. GAGs hyaluronate, chondroitin-4-sulfate, chondroitin-6-sulfate, and heparin (500 μg/ml) did not block NEN clumping of Mi1. NEN clumping (attachment) is blocked when Mi1 or Mi3 J2 are pretreated for $\geq$ 2.25 h in 20% SDS at 97 °C. Endospores P100 and P20 caused NEN clumping of pre-parasitic J2, parasitic J2 and late J2, but not adult Mi1 or Mi3. These results suggest that stage-specific collagenous glycoprotein(s) in the J2 cuticle (below the surface coat) may be the determinant(s) of specific attachment.

PRELIMINARY ACCOUNT OF NEMATODE BIODIVERSITY IN COSTA RICAN PROTECTED AREAS. **Esquivel, A.** Instituto Nacional de Biodiversidad, Escuela de Ciencias Agrarias, Universidad Nacional, Costa Rica.

The Instituto Nacional de Biodiversidad (INBio), in collaboration with the Nematology Laboratory at Universidad Nacional (U.N.A.) of Costa Rica, is carrying out the Nematode Inventory of

Costa Rican wildlands. One of the most important mission of INBio is knowing, ordering and systematizing taxonomic information about Costa Rica's biodiversity and promoting this knowledge for the benefit of society use. The study was carried out in five Conservation Areas of Costa Rica: Arenal, Tempisque, Osa, Amistad Pacífico and Guanacaste. A total of 180 samples were taken at random in different ecosystems and microhabitats in national parks and biological reserves that belong to these areas. The samples were processed using Cobb's modified decanting and sieving method, and the water suspension with nematodes was gathered after 24 and 48 hours for terrestrial and fresh water samples, respectively. The nematodes were fixed and passed to pure glycerin using the Seinhorst's rapid method. Permanent mountings in Cobb's slides were prepared using the paraffin-wax ring method. The results showed the most frequent nematode order in natural ecosystems in Costa Rica is *Dorylaimida* with 56% of the total nematodes registered; on the other hand, the lowest percentages obtained belong to *Chromadorida* and *Monhysterida* with 2% and 1%, respectively. Analyzing the feeding type distribution, omnivorous, bacterial and animal feeders were predominant, while plant, hyphal and unicellular eucaryote feeders were maintained in low percentages.

PERFORMANCE OF GERMPLASM PUSCN14 WITH HARTWIG RESISTANCE IN A FIELD HIGHLY INFESTED WITH SOYBEAN CYST NEMATODE. **Faghihi, J.,[1] R. A. Vierling,[2] V. R. Ferris,[3] and J. M. Ferris.[3]** [1]Department of Entomology, [2]Indiana Crop Improvement Association and Department of Agronomy, and [3]Department of Entomology, Purdue University, West Lafayette, IN 47907.

Back cross progenies from a Williams 82 ×Hartwig cross were selected based on their agronomic performance in a non-infested field in 1997. They were screened individually in the greenhouse with a race 4 phenotype true SCN inbred. In 1998, seeds showing complete resistance (no cyst development) were planted in a field highly infested with 28,000 eggs/250cc of soil of race 1 phenotype SCN. Plants segregated to determinate and indeterminate growth type with maturity ranging from group 1 to late group 3. Plants were harvested individually based on their desirable agronomic characteristics. When they were screened with a race 4 phenotype of a true SCN inbred, nearly all showed complete resistance. The highest yield (3,932 kg/h) was from one of the indeterminate PUSCN14 back crosses with a mid-group 3 maturity growth type. Resistant variety Jack produced 3,058 kg/h, while susceptible variety Williams 82 yielded 1,484 kg/h in the same field. PUSCN14 has shown complete resistance in greenhouse screening to 58 field isolates of SCN, selected randomly regardless of their race phenotype.

NEMATODE FAUNAL INDICATORS OF SOIL FOOD WEB CONDITION. **Ferris, H.,[1] T. Bongers,[2] and R. G. M. de Goede.[3]** [1]Department of Nematology, University of California, Davis, CA 95616, [2]Laboratory of Nematology, Wageningen Agricultural University, Wageningen, The Netherlands, and [3]Sub-department of Soil Science and Plant Nutrition, Wageningen Agricultural University, Wageningen, The Netherlands.

Various indicators, each with advantages, disadvantages and constraints, have been used for assessment of soil nutritional status and the condition of the soil food web. We propose, and demonstrate, a system based on the abundance of nematodes in functional guilds, weighted along r- and K-trajectories. We assert that the weighted guilds are indicators of the characteristics of the community food web in which they function. Survivalist bacterial-feeders (predominantly Cephalobidae) and fungal-feeders (Aphelenchina and Anguinidae) indicate the basal characteristic of the food web and the level to which it is stressed or resource-depleted. Other guilds, including longer-lived, larger-bodied carnivores and omnivores, indicate structure in the food web and measure its maturity. Opportunistic bacterial-feeders (predominantly Rhabditidae and Panagrolaimidae) indicate resource availability and enrichment of the food web. In concert, the indicators allow graphical analysis of the state of the food web with regard to abundance and nature of detrital

substrate, physical or environmental perturbation, or pollution. Higher resolution analyses are provided by ratios among the weighted abundance of guilds to indicate the enrichment/depletion status of the web and whether fungal or bacterial decomposition channels predominate. The guild-based analyses are not taxonomically intensive, requiring resolution at the family level only. The higher resolution analyses are based only on readily recognized families of bacterial- and fungal-feeding nematodes. The analyses provide a framework for planning, and monitoring the effects of, soil management activities.

EFFECTS OF SELECTED VA-MYCORRHIZAL FUNGI ON GROWTH OF APPLE ROOTSTOCKS IN *PRATYLENCHUS PENETRANS*-INFESTED SOIL UNDER GREENHOUSE AND FIELD CONDITIONS. **Forge, T., A. Muehlchen, C. Hackenberg, G. Neilsen, and T. C. Vrain.** Pacific Agri-Food Research Centre, Agriculture and Agri-Food Canada, Summerland, British Columbia V0H 1Z0, Canada.

The effects of four species of Vesicular–Arbuscular Mycorrhizal fungi (VAM) on growth of Ottawa 3 apple rootstocks and population growth of *Pratylenchus penetrans* were determined in two greenhouse experiments. In addition, a field experiment was initiated in spring of 1998 to evaluate effects of inoculation with *Glomus intraradices* and *G. mosseae* on growth of apple rootstocks transplanted into fumigated and non- fumigated plots in a *P. penetrans*-infested field. *G. mosseae* increased rootstock dry weights in both greenhouse experiments, and *G. intraradices* and *G. etunicatum* each increased dry weights in one of the two greenhouse experiments. Rootstocks inoculated with *G. mosseae* and *G. intraradices* supported significantly fewer *P. penetrans* per g root than *G. etunicatum* and *G. clarum*, but were not different from controls. Plant dry weights were reduced by *P. penetrans* in one experiment. Colonization of roots by VAM was not affected by the presence of *P. penetrans* in either experiment. In the field experiment, *G. intraradices* and *G. mosseae* each increased stem diameters and heights of apple rootstocks in both fumigated and non-fumigated plots. Inoculation with VAM increased uptake of phosphorus, copper and zinc, and reduced uptake of potassium, in fumigated plots only. Rootstocks inoculated with *G. mosseae* also tended to support fewer *P. penetrans* per g root than *G. intraradices*-inoculated and non-inoculated rootstocks, but the differences were not statistically significant. Colonization of roots by VAM at the end of the first growing season was significantly greater for VAM-inoculated rootstocks than for non-inoculated rootstocks in both fumigated and non-fumigated field plots.

MICROSATELLITES IN THE MODEL NEMATODE *CAENORHABDITIS ELEGANS*. **Frisse, L. M.,[1] L. L. Vassilieva, M. Lynch,[2] and W. K. Thomas.[1]** [1]School of Biological Sciences, University of Missouri, Kansas City, MO 64110, and [2]University of Oregon, Eugene, OR.

Microsatellites—small, direct repeats—are a ubiquitous feature of eukaryotic genomes. The high mutation rate of microsatellite loci has made them useful in genetic mapping and evolutionary studies. Despite their widespread use, little is known about the origin of microsatellites, factors that affect microsatellite frequency and distribution, and microsatellite mutation rates and patterns. Understanding the rates and patterns of microsatellite evolution is critical for their use in studies of evolution, genetic mapping and mutational mechanisms. The recent completion of the *Caenorhabditis elegans* genome allows the testing of many of the hypotheses concerning microsatellite evolution. We have identified all microsatellites with 2–5 bp repeats, which are at least 10 perfect repeat units in length. These loci have been physically mapped allowing us to investigate the frequency and distribution of microsatellites across an entire metazoan genome. In order to understand the rates and patterns of microsatellite mutations, we assayed 31 microsatellite loci in a set of 80 mutation accumulation lines of *Caenorhabditis elegans* propagated for 140 generations. Our findings suggest that the mechanisms responsible for microsatellite formation may be unique to each organism; however, the patterns and processes of microsatellite mutation may be a universal feature of all organisms.

THE IDENTIFICATION OF MOLECULAR DIFFERENCES BETWEEN VIRULENT AND AVIRULENT ROOT-KNOT NEMATODES (*MELOIDOGYNE* SPP). **Gleason, C., and V. Williamson.** Nematology Department, University of California, Davis, CA 95616.

The gene-for-gene model of plant–parasite interactions predicts that pathogen avirulence gene products elicit defense responses in plants with the cognate resistance gene products. In other words, plant resistance genes have a corresponding gene in the pathogen. Our lab has recently cloned and characterized the tomato gene Mi that confers resistance to three species of *Meloidogyne: M. arenaria, M. incognita* and *M. javanica*. Based on the gene-for-gene model, we predict that there is a nematode (a)virulence gene that corresponds to Mi. We are using two nearly isogenic strains of *M. javanica* that differ only in their ability to reproduce on Mi-carrying plants. We hypothesize that the only difference between these two strains is the molecular determinants of (a)virulence. To find differences between the virulent and avirulent strains at the level of DNA, we employed a method of DNA fingerprinting called amplified fragment length polymorphisms (AFLPs). The DNA fingerprints showed no reproducible differences and confirmed that the two strains were nearly identical. The difference between the two strains may be at the level of gene expression. We used cDNA-AFLPs to create cDNA fingerprints of the two nematode strains. Presently, we have identified four differentially expressed cDNA fragments. We are working on cloning and sequencing these fragments and are continuing our work with cDNA-AFLPs. The identification of nematode (a)virulence genes may elucidate plant–nematode interactions and may lead to insights in engineering durable plant resistance.

BETA-1,4-ENDOGLUCANASE PRODUCTION BY *GLOBODERA TABACUM*, THE TOBACCO CYST NEMATODE. **Goellner, M.,[1] G. Smant,[2] J. M. de Boer,[3] T. J. Baum,[3] and E. L. Davis.[1]** [1]Plant Pathology Department, North Carolina State University, Raleigh, NC 27695, [2]Nematology, Wageningen Agricultural University, Wageningen, The Netherlands, and [3]Plant Pathology Department, Iowa State University, Ames, IA 50011.

Two beta-1,4-endoglucanase (EGase) genes were isolated from *Globodera tabacum* and sequenced. GT-eng1 (1414bp ORF) encoded a predicted precursor protein of approximately 52 kDa. GT-eng2 (1188bp ORF) encoded a predicted precursor protein of approximately 43 kDa. The 5N ends of these two genes were 92% identical in the first 1,050 nucleotides. Each protein consisted of a signal peptide, catalytic domain, and linker region. GT-ENG1 contained an additional 96aa carboxy terminal sequence with strong homology to type II cellulose binding domains. *In situ* hybridizations with DIG-labeled riboprobes localized EGase transcription to the subventral esophageal gland cells. In addition, the riboprobes and polyclonal antisera specific to recombinant cyst EGases were used to determine expression patterns of EGase transcripts and proteins throughout the nematode life cycle. EGase transcripts and proteins were detected in J2 within eggs just prior to hatching, in pre-parasitic J2 hatched in water, and in parasitic J2 that had invaded host roots. EGase transcripts and proteins were not detected after the molt to the J3 life stage, nor in J4 males and females. Upon the molt to the adult male stage, however, EGase transcription and protein production resumed in the male subventral glands. These data suggest a role for tobacco cyst nematode EGases during intracellular migration upon parasitism of tobacco roots.

RELATIONSHIP BETWEEN TEMPERATURE ADAPTATION, DESICCATION TOLERANCE, AND TREHALOSE ACCUMULATION IN INFECTIVE JUVENILE ENTOMOPATHOGENIC NEMATODES. **Grewal, P. S.** Department of Entomology, Ohio State University, Wooster, OH 44691.

Desiccation survival of infective juveniles of a cold- and warm-adapted entomopathogenic nematode species was compared at a range of temperatures. The cold-adapted *Steinernema carpocapsae* survived desiccation at 5°, 15°, 25° and 35 °C, whereas the warm-adapted *Steinernema riobrave* did not survive desiccation at 5 °C. Exposure of *S. riobrave* infective juveniles to cold-temperatures in water suspension for two or more days enhanced their subsequent desiccation

survival at 5 °C. Both nematode species accumulated trehalose in cold storage: *S. carpocapsae* accumulated more trehalose than *S. riobrave*. The cold-induced trehalose may be responsible for cold-temperature desiccation survival of entomopathogenic nematodes.

A COMPARATIVE ANALYSIS OF EXTRACTION PROCEDURES FOR THE RECOVERY OF SEED-BORNE NEMATODES FROM SEED SAMPLES. **Griesbach, J. A.,[1] J. J. Chitambar,[2] M. J. Hamerlynck,[1] and E. O. Duarte.[2]** [1]Plant Division, Oregon Department of Agriculture, Salem, OR, and [2]Plant Pest Diagnostics Center, California Department of Food and Agriculture, Sacramento, CA 95832.

In order to develop a standard test that could be used in official certification laboratories for the timely and efficient extraction of seed-borne nematodes from seed samples, four procedures were compared in their efficacy to extract *Anguina* sp. larvae from commercial grass seed. These procedures included new ones, as well as those currently used by the State Regulatory Laboratories of Oregon and California. Eleven seed lots of *Agrostis tenuis* (bentgrass) and *Dactylis glomerata* (orchardgrass) naturally infested with varied levels of *Anguina* sp. larvae were individually analyzed. The procedures included extractions from water pre-soaked and aerated seeds which were then: 1) germinated on water agar, macerated and separated by sucrose centrifugation, 2) macerated and sieved, 3) placed under intermittent mist, and 4) macerated and placed over a funnel containing continuously aerated water and host leaf tissue. Only procedure 4 yielded larvae in all 11 samples. In comparison, although the other three procedures resulted in greater numbers of larvae per gram seed, and procedure 2 yielded the greatest numbers, all three failed to yield any nematodes in as many as four seed lots with low infection levels. Each procedure had its operational advantages and disadvantages, and was additionally assessed for suitability as a live recovery test that could be used to demonstrate seed treatment efficacy.

IDENTIFYING NEMATODES: QUARANTINE, PHYTOSANITARY REQUIREMENTS AND REGULATIONS. **Hackney, R. W.** California Department of Food and Agriculture, Sacramento, CA 95832.

In this Symposium on Regulatory Diagnostics, it is pertinent to recognize how, at all levels, of the regulatory process identifications are accomplished, because at the farm gate, millions, if not billions, of dollars of commodity depend upon timely and accurate laboratory results. It is also important to recognize how identifications should be performed and what new biotechnology is available. As scientists we should know what biotechnology is being developed and what is on the horizon. These topics have not been addressed adequately during professional meetings of our professional societies in contributed paper sessions. As a professional society, we have not held adequate symposia, colloquia or discussion sessions that even begin to deal with these topics. We do not license or certify our members to practice in the area of regulatory diagnostics. To put the value of this in some perspective, California alone had a farm gate income in 1997 of $26.8 billion, with about $7.0 billion in exports. It is estimated that in California the state's agricultural industry could lose more than $600 million annually in crop losses if certain plant parasitic nematodes not known or if limited occurrence in the state would become widespread. There is no uniform Standard for Regulatory Diagnostics! The accreditation of private and governmental laboratories for regulatory diagnostics is in its embryonic stages. In the international scientific community cachet in regulatory diagnostics is largely subjective. Hence, higher production costs or limited production could be reflected in higher costs to the consumer. It could also require a greater use of nematicides. Limiting the spread of common pests by maintaining nursery standards of pest cleanliness and certification contributes to the economy.

LABORATORY ACCREDITATION FOR TESTING AND PHYTOSANITARY SERVICES. **Hackney, R. W.** Plant Pest Diagnostics Center, California Department of Food and Agriculture, Sacramento, CA 95832.

A program is currently being established and implemented whereby non-government facilities can become accredited to perform specific laboratory testing or phytosanitary inspection services. The State of California has already enacted a law (i.e., AB 2252) effective January 1, 1999, so that when all of the appropriate Federal Rule Making has been accomplished, the California Department of Food and Agriculture's Plant Pest Diagnostic Center (PPDC) can become an official accrediting agent for the United States Department of Agriculture (USDA).

DETERMINATION OF THRESHOLD LEVEL OF ROOT-KNOT NEMATODE *MELOIDOGYNE CHITWOODII* IN NATURALLY INFESTED SOIL ON POTATO 'RUSSET BURBANK' UNDER IDAHO CONDITIONS. **Hafez, S. L., P. Sundararaj, and M. Larkin.** Parma Research and Extension Center, University of Idaho, Parma, ID 83660.

Experiments were undertaken to determine whether the current economic threshold for treatment of *Meloidogyne chitwoodi* could be validated for Idaho potato production. Six initial nematode population levels (0, 3, 5, 25, 50 and 250) were established under greenhouse and microplot conditions and potatoes were grown to determine yield and percent nematode infection. Significant reduction in yield occurred when initial population levels were as low as 3 nematodes/500 cc of soil in microplot. Yield differences observed under greenhouse conditions were not statistically significant. Only the microplot trial displayed tuber symptoms of nematode infection. It was found that 3 or 5 nematodes per 500 cc resulted in enough damaged potatoes that the field could potentially be considered unmarketable.

SCREENING RAPESEED TISSUE FOR NEMATICIDAL ACTIVITY. **Halbrendt, J. M.,[1] N. O. Halbrendt,[1] and J. Brown.[2]** [1]Department of Plant Pathology, The Pennsylvania State University, Biglerville, PA 17307, and [2]Department of Plant, Soil and Entomological Sciences, University of Idaho, Moscow, IA 83843.

Crops that follow *Brassica* (e.g., rapeseed, mustard) often benefit from a phenomenon known as the mustard effect. The mustard effect has been attributed to the suppression of diseases and nematodes by isothiocyanates resulting from the decomposition of glucosinolates. This hypothesis has been supported by bioassays that evaluated the toxicity of extracted glucosinolates and the end products of their hydrolysis. A modified bioassay was developed to screen for nematicidal activity in *Brassica* under more natural conditions. The tissue (e.g., foliage, root) was fresh frozen, lyophilized and ground into a fine powder. The powder was weighed and mixed into dry, sterile sand in increasing amounts to establish a range of concentrations that killed between 10% and 90% of the nematodes tested. The nematodes were hand picked into vials of sterile water, followed by the addition of the sand/plant powder mixture. The vials were sealed and kept at 24 °C for 24 hours, after which all nematodes (alive and dead) were recovered. Probit analysis was used to establish a mortality curve and calculate LD50 values. The test was superior to aqueous extracts, which may stress nematodes due to oxygen depravation. Bioassays of rapeseed against *Xiphinema americanum* showed variation in toxicity between different cultivars and between foliage and root of the same cultivar. Typically, root tissue was more toxic to nematodes than leaf tissue. For example, leaf tissue from cv. Dwarf Essex gave an LD50 of 12 mg/cc sand, whereas cv. Ericka showed an LD50 of 8 mg/cc sand. Root tissue from Ericka had an LD50 of only 1.5 mg/cc sand. The nematicidal activity correlated with separate tests that determined glucosinolate content of the tissues.

MORPHOLOGICAL, DEVELOPMENTAL AND MOLECULAR CHARACTERIZATION OF *BELONOLAIMUS LONGICAUDATUS* CULTURED ON *IN VITRO* CORN ROOTS. **Han, H.–R.,[1] D. W. Dickson,[1] D. P. Weingartner,[2] and A. Jeyaprakash.[1]** [1]Department of Entomology and Nematology, University of Florida, Gainesville, FL 32611, and [2]Hastings Research and Education Center, Hastings, FL 32145.

Belonolaimus longicaudatus is one of the most damaging soil pathogens of agronomic and horticultural crops in the southeastern United States. The objective of this research is to compare

isolates obtained from different host plants and geographical locations. Three isolates were collected from Florida—Gainesville (turf), Hastings (potato) and Lake Alfred (citrus)—and one isolate from Georgia—Tifton (cotton). The isolates are being compared based on morphology, developmental biology and molecular biology. Mophometrically, the Hasting isolate is different from the other isolates in body length, body width, and stylet-tail ratio ($P \leq 0.05$). The Georgia isolate has a shorter developmental time from egg to the adult than the other isolates. The DNA was extracted from a single female by rupture in sterile water. The ribosomal DNA of ITS-1 region between 18S and 5.8S was amplified by polymerase chain reaction (PCR). The genes are being cloned though *E. coli* and they will be sequenced.

PASTEURIA SP. PARASITIZING *CRICONEMELLA* SP. IN FLORIDA. **Han, S.–C.,[1] T. E. Hewlett,[2] and D. W. Dickson.[2]** [1]Agricultural Biology, Andong National University, Andong, Korea, and [2]Entomology and Nematology, University of Florida, Gainesville, FL 32611.

A population of *Criconemella pelerentsi* in a peanut field was found to be infected with an unknown *Pasteuria* sp. Endospores are pyramid shape. Sporangia diameter, central body diameter and height were 3.03 ± 0.04, 1.08 ± 0.08, and 1.93 ± 0.08 mm, respectively, which were different from *P. penetrans* (3.93 ± 0.21, 1.57 ± 0.18, and 1.90 ± 0.14 mm). The average number of endospores found in *C. pelerentsi* bodies was 25,000. Attachment studies have shown that this *Pasteuria* sp. attached to *C. pelerentsi*, but not to *M. incognita* (race 1) and *M. arenaria* (races 1 and 2). Endospores on the nematode body can be stained with fluorescein.

DETECTION OF GENETIC VARIABILITIES THAT EXIST WITHIN M. INCOGNITA DIFFERENT POPULATIONS FROM YAYOUM GOVERNORATE, EGYPT, BY USING RIBOSOMAL DNA. **Haroon, S. A.** Plant Protection Department, College of Agriculture, Cairo University, Fayoum, Egypt.

The internal transcript spacer (ITS) and restriction fragment length polymorphism (RFLP) of ribosomal DNA sequences were used to distinguish between different species of root-knot nematode. DNA fragments containing the internal transcribed spacer (ITS) rDNA were amplified from DNA genome of 97 *Meloidegyne* spp. isolates that infest vegetable crops in Fayoum Governorate, Egypt. Twenty-five females were sufficient to amplify PCR product. When primers 5368 and 5367 were used for amplification of the ITS region, every isolate from the *Meloidogyne* spp. gave one major product of approximately 760 bp. When nested primers P195 and P194 were used, a strong signal of 570 bp amplified region was obtained; this signal was 190 bp shorter than the one obtained from ITS amplification. ITS regions of all isolates were digested with restriction enzymes by using RFLP test the size of the DNA fragment using Hind III was strong band at 560 bp and another weak band at 200 bp that is typical for *M. incognita* and *M. javanica* with Hind 1, bands at 440 and 320 bp was obtained, while 520, 240 bp band were cleared when EcoR1 was used. Four bands of 220, 200, 180 and 160 bp were obtained when Dra 1 restriction enzyme was used. When scan primer was used to differentiate between *M. javanica* and *M. incognita*, only one strong band positive control for *M. javanica* was obtained. While no signals from all Fayoum isolate that indicate the presence of *M. incognita* species in the majority of Fayoum isolates. In multiplex test, a fragment of 415 bp was obtained when *M. incognita* was used as a template (positive control). Ten primers were evaluated for their powerfulness in identifying the genetic variability within populations of *Meloidogyne incognita* by using RAPD markers. Colonies were established from a single egg mass for each population, it was maintained in tissue culture on the susceptible tomato cv. Castle Rock; after three months, 25 female nematode were collected and stored at −20 °C until used for DNA extraction. Primer G_2 produced four distract DNA fragments (bp 1550, 1100, 800 and 750), which were consistently present in almost all *M. incognita* populations. Seven more bands were detected. The coefficient of similarity between different populations was arranged from 0.63 to 8.00. These results are in agreement with phylogenetic trees derived from different char-

acters. RAPD marker is very powerful tool for genetic mapping applications as well as for genetic diagnoses.

MRNA ABUNDANCE DIFFERENCES WITHIN *HETERODERA SCHACHTII*-INFECTED *ARABIDOPSIS THALIANA* ROOTS. **Hart, J., D. Hermsmeier, S. R. Rodermel, and T. J. Baum.** Plant Pathology, Iowa State University, Ames, IA 50011.

Infection by cyst nematode (*Heterodera* spp.) decreases yields of commercially important crops such as soybean and sugar beet. The syncytium, or feeding site, of the nematode is formed from susceptible root cells. It is thought that the nematode commandeers the genetic systems of the host in order to redirect the purpose of these cells to providing food for the parasite. Combating this plant–pathogen interaction would be greatly facilitated by understanding the roles of genes that are up- or down-regulated at the site of infection, either as a part of the plant defense response or as a component of the unknown regulatory system imposed by the nematode. To further knowledge in this area, differential display PCR was performed on RNA from *Arabidopsis thaliana* root sections harvested 3–4 days after inoculation with *Heterodera schachtii*. Clones were identified based on RNA abundance changes when RNA from syncytia, with attached nematodes, was compared with RNA from immediately adjacent segments of the same roots, which did not contain syncytia. Fifty-eight cDNA clones were isolated, corresponding to mRNA species with abundance differences in the tested adjacent root segments. Twenty-seven of these clones were of *A. thaliana* origin. mRNA abundance levels of these 27 clones were assayed via northern blot analysis with *A. thaliana* total RNA from infected and uninfected shoots and roots. Eight of these clones proved abundant enough to exhibit banding patterns on northern blots. One cDNA showed close sequence match to transcription factors involved in the plant defense response. Differential display showed this clone to be locally down-regulated at the site of infection, while northern blot analysis showed a systemic increase in mRNA abundance in infected roots when compared to uninfected roots. *In situ* hybridization analysis was undertaken to gain a further understanding of the possible function of this clone in the plant defense response.

INTEGRATED CROP MANAGEMENT PROTOCOLS AND THE MANAGEMENT OF POTATO CYST NEMATODES. **Haydock, P. P. J.,[1] and K. Evans.[2]** [1]Crop and Environment Research Centre, Harper Adams University College, Newport, Shropshire, UK, and [2]IACR-Rothamsted, Harpenden, Hertfordshire AL5 2JQ, UK.

The multiple retailers (supermarkets) market in excess of 65% of the fresh potatoes sold in the UK. The supermarkets require growers to comply with specific production protocols. The NFU-retailer protocol and Tesco's Nature's Choice protocol for fresh potatoes refer specifically to the management of the potato cyst nematodes (PCN) *Globodera pallida* and *G. rostochiensis*. Both protocols require an integrated approach to PCN management, which includes soil sampling, species identification, and the use of crop rotation, resistant cultivars and nematicides when necessary. The minimum crop rotation length of one year in five will result in many growers having to change cropping practices, which will be of long-term benefit in allowing a greater natural decline of PCN between potato crops. Crop rotation is more effective for *G. rostochiensis* than for *G. pallida*. While protocols favour the use of resistant potato cultivars, there is a dearth of cultivars with partial resistance to *G. pallida* that are also acceptable to the supermarkets. The use of nematicides is not favoured by supermarkets and is usually only allowed as a part of an integrated approach in moderately or highly infested soils. However, their use should not be confined to situations where a yield benefit is seen in crops to which they are applied: if PCN populations are to be maintained at low levels in the long term, then the use of nematicides at low-population densities, where they are most effective in controlling nematode population increase, can be justified.

DYNAMICS OF *PASTEURIA PENETRANS* ON *MELOIDOGYNE ARENARIA* IN FIELD SOIL. **Hewlett, T. E., and D. W. Dickson.** Entomology and Nematology Department, University of Florida, Gainesville, FL 32611.

Pasteuria penetrans suppresses root-knot on agricultural crops in the southeastern United States. Our objective was to determine the effect of different crops and chemicals on the dynamics of *P. penetrans* and *Meloidogyne arenaria* race 1. The experimental design was a split-plot with plantings of peanut, eggplant, okra, soybean, field corn and sweet corn as the main plots, and 1,3-D, chloropicrin and an untreated control as sub-plots. Soil was collected at the end of each crop cycle for nematode extraction and soil and plant assays. Recently hatched J2 were added to the soil for these assays. In nematode extraction samples, eggplant and peanut plots had the highest percentage of J2 with spores attached than other crops in year 1 (71% and 65%, respectively, $P < 0.01$) and peanut was the highest (65%, $P < 0.01$) in year 2. For year 1, in the soil assay, peanut soil had the highest percentage of J2 with spores attached (71%,) and the highest number of spores attached per J2 (13) compared to all other crops ($P < 0.01$). Control plots had a greater percentage of J2 with spores attached (51%, $P < 0.01$) than chloropicrin treated plots. For year 1, in the plant assay, peanut soil produced the lowest number of galls, and peanut and eggplant soil had greater numbers of spore-filled females (28% and 21%, respectively, $P < 0.01$) than other crops. Chloropicrin treated plots had lower numbers of spore-filled females than the control or 1,3-D treated plots ($P < 0.01$). The results suggest that peanut is a good crop for amplifying *P. penetrans*. Chloropicrin affects the host–parasite relationship of *P. penetrans* with *M. arenaria*.

ORIGIN OF *MELOIDOGYNE INCOGNITA* SURFACE COAT. **Hu, G., M. A. McClure, and M. E. Schmitt.** Department of Plant Pathology, University of Arizona, Tucson, AZ 85721.

The surface coat (SC) of plant nematodes is thought to originate either from the living hypodermis or from secretory glands associated with the excretory system or nervous system. In this study, we investigated the origin of the SC of *M. incognita*, by immunolocalization with a monoclonal antibody (MISC1) raised against the surface coat of the pre-parasitic juveniles (J2). Under the electron microscope, strong labeling was found on the cuticular surface and in the rectal dilation of the J2, while labeling was absent in other parts of the nematode, including the hypodermis, excretory system, nervous system and digestive system. Because the rectal glands are known to be the origin of the gelatinous egg matrix produced by adult females of *Meloidogyne*, we also examined thick sections of mature females from monoxenic cultures of *Arabidopsis thaliana*. Labeling of the female occurred in the rectal glands and in the gelatinous matrix exuded from the anus. At the ultrastructural level, gold particles were mainly deposited in multivesicular structures that appeared to be associated with the Golgi bodies of the rectal glands. Our results suggest that the SC of the J2 originates from the rectal gland cells and that the SC of the J2 shares common epitopes with the gelatinous egg matrix of mature females.

NEMATODE-REPELLING METABOLITES PRODUCED BY *XENORHABDUS* SPP. AND *PHOTORHABDUS* SPP. **Hu, K., and J. M. Webster.** Department of Biological Sciences, Simon Fraser University, Burnaby, Vancouver, British Columbia, Canada.

3,5-dihydroxy-4-isopropylstilbene (ST) and indole, two nematicidal metabolites produced by the bacterial symbionts, *Xenorhabdus* spp. and *Photorhabdus* spp., influence the behaviour of infective juveniles (IJs) of entomopathogenic nematodes at a dose as low as 0.1 μg/disc (filter paper, diameter 0.6 cm) in Petri dish bioassays. ST repelled the IJs of some *Steinernema* spp. but not of *Heterorhabditis* spp. tested, and indole repelled the IJs of some species of both *Steinernema* and *Heterorhabditis*. ST was produced by all five strains and species of *Photorhabdus* cultured in tryptic soy broth (~25–40 μg/ml) and in nematode-infected larval *Galleria mellonella* (~670–4,000 μg/g wet insect), but indole was produced in only broth culture by some species of *Xenorhabdus and Photorhabdus*. ST was present in infected larval *G. mellonella* 24 h post-infection (25 °C in

the dark) and maintained a relatively high and stable level throughout the infection cycle. It is hypothesized that some secondary metabolites that are produced *in vivo*, such as ST, may play a role in minimizing competition among nematode species.

FEEDING AND ROOT-TIP GALL INDUCTION OF *LONGIDORUS AFRICANUS* ON HOST SEEDLINGS IN AGAR CULTURE. **Huang, X., and A. T. Ploeg.** Department of Nematology, University of California, Riverside, CA 92521.

The feeding behavior of *Longidorus africanus* on sugar beet seedlings in agar culture was studied. The agar culture was kept in 8-hour lighting period at 26°. *L. africanus* began feeding on the seedling root as early as six hours after the seedling was introduced into the agar plate, and preferred the root tip as the feeding site. The complete feeding process consisted of exploration, stylet penetration, inactivity (salivation), ingestion, and stylet retraction. The duration of each phase was also recorded. Feeding lasted for as long as 450 minutes. The incomplete feeding resembled the complete feeding except that the nematodes withdrew before ingestion occurred. The attacked root then began to swell and galled after feeding, resulting in the termination of the root growth within a few days after feeding. Also, necrotic lesions appeared at the feeding site. No feeding was observed on corn, lettuce, soybean or tomato.

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

The needle nematode *Longidorus africanus* is a pathogen on head lettuce in the Imperial Valley of southern California. Plants are generally affected in the seedling stage, resulting in reduced size of lettuce head or even plant death. 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 0–1,000 nematodes per plant (200 ml soil), and under the influence of soil temperature (17°, 20°, 25°, 30 °C) at 0–400 nematodes per plant (60 ml soil). Fresh and dry weights of lettuce top and root were determined 30 days post-nematode inoculation. Plant weights were expressed relative to the non-inoculated controls and data were fitted according to the "Seinhorst model," and the minimum yield and nematode tolerance level were estimated. When seedlings were inoculated within 20 days after seeding, within 1 nematode/200 ml soil of *L. africanus* already significantly reduced top and root dry weight. The tolerance level of seedlings inoculated with nematodes at 30 days after seeding increased to 25.6 nematodes/200 ml soil as measured by dry top weight loss, and slightly increased to 1.6 nematodes/200 ml soil as measured by dry root weight loss. There was a positive correlation between the age of lettuce seedlings at time of inoculation and estimated minimum yields. At 25 °C, the estimated minimum yields of both top and root dry weight was less than at any other temperatures, while the tolerance level was within 1 nematode/60 ml soil at all temperatures. These results indicate that *L. africanus* is very destructive to lettuce and provide a preliminary basis for predicting damage to lettuce caused by *L. africanus*. It can be concluded also that early sowing at lower temperatures or a delay in exposure of lettuce seedlings to *L. africanus*, e.g., by using transplants or by temporarily immobilizing the nematodes with nematicides, is likely to reduce yield loss.

NEMATODE PARASITISM OF PLANTS. **Hussey, R. S.** Department of Plant Pathology, University of Georgia, Athens, GA 30602.

Evolutionary adaptation of nematodes for plant parasitism has led to the development of a hollow, protrusible feeding structure, the stylet, in the nematode stoma, as well as marked morphological and physiological modifications of the esophagus. These biotrophic parasites use their stylet to pierce the plant cell wall to inject secretions through the stylet orifice into plant tissue and

to ingest nutrients from plant cells. Migratory plant nematodes may feed as ecto- or endoparasites on plant tissue and ingest plant cell contents directly with their stylets. Nematodes that enter root tissue also use their stylets to cut openings and/or inject secretions to dissolve or weaken the cell wall or middle lamella to facilitate intracellular or intercellular migration, respectively. Plant nematodes that have evolved sedentary feeding habits modify plant cells for feeding, including elaborate changes in plant cell morphology and gene expression induced by some sedentary endoparasites. The gland cells in the esophagus of plant nematodes have evolved into enlarged secretory cells and are the principal source of the molecular mechanisms and signals involved in plant parasitism. These gland cells changed considerably in morphology for parasitism and the functions of the secretions have evolved to enable nematodes to invade plant tissue and to modify cells for feeding. The key to understanding nematode parasitism of plants is the characterization of the secretory molecules that are synthesized in the esophageal gland cells and released through the stylet into host tissue. Parasitism genes encoding products in stylet secretions are now being cloned by constructing gland-specific cDNA (EST) libraries that have been reverse-transcribed from the mRNA in the cytoplasm microaspirated directly from the esophageal gland cells. An understanding of the nature of the secretions and their function in parasitism is now beginning to emerge, aided by the availability of new molecular tools.

EFFICACY OF CITRONELLAL AND LIMONENE AGAINST PLANT-PARASITIC NEMATODES. **Hutchinson, C. M., and J. O. Becker.** Department of Nematology, University of California, Riverside, CA 92521.

Limonene and citronellal are constituents of citrus peel oil. These compounds have relatively low mammalian toxicity and are commonly used as flavorings and fragrances. Initial results from our laboratory indicate that these plant oils may provide excellent plant-parasitic nematode control. Three-week-old tomatos (*Lycopersicon esculentum* cv. Orange Pixie) were transplanted into 20-cm diameter pots. Two-weeks after transplanting, 7,000 root-knot nematode eggs (*Meloidogyne incognita*) were mixed with either citronellal or limonene (0.01, 0.1, 0.5 or 1.0 M) in 400 ml water. Tomato plants were drenched with the nematode/nematicide mixture. Nine-weeks after treatment, plant roots were washed. Nematode egg masses were stained and counted. Citronellal (10 mM) and limonene (100 mM) reduced root-knot nematode populations on tomato by 98% compared to controls. Both plant oils were phytotoxic at 0.5 and 1.0 M. In a second set of *in vitro* laboratory experiments, citrus nematode (*Tylenchulus semipenetrans*, J2) was treated with 100, 10, 1.0 and 0.1 mM citronellal. After treatment, nematodes were rinsed and extracted on a Baermann funnel. No nematodes were recovered from the citronellal treatments.

MOLECULAR CHARACTERIZATION OF THE ROOT-KNOT NEMATODE RESISTANCE GENE MI-1 IN TOMATO "HAIRY ROOTS." **Hwang, C.–F., A. Bhakta, G. Truesdell, and V. Williamson.** Center for Engineering Plants for Resistance Against Pathogens, University of California, Davis, CA 95616.

Mi-1 is a single dominant gene that confers resistance to several root-knot nematode species (*Meloidogyne* spp.) in tomato. Recombinant analysis localized the Mi-1 gene to a ~65 kb region. This region was isolated as bacterial artificial chromosome (BAC) clones and 52 kb of contiguous DNA was sequenced. Three open reading frames were identified with similarity to other cloned R-genes. Two, Mi-1.1 and Mi-1.2, appear to be intact genes; the third is a pseudogene. The predicted proteins, Mi-1.1 and Mi-1.2, are 91% identical in amino acid sequence and belong to the LZ-NBS-LRR family of plant resistance genes. Complementation studies using cloned copies of Mi-1.1 and Mi-1.2 indicated that Mi-1.2, but not Mi-1.1, is sufficient to confer resistance to a susceptible tomato line. Current research includes producing modifications in Mi-1 by site-directed mutagenesis and domain swapping of sequences from Mi-1.1 and Mi-1.2. For this work, a relatively rapid, reliable assay system was needed that takes into account the requirement of differentiated root tissue for nematode infection. We have established such an assay by using *Agrobac-*

terium rhizogenes-based transformation to produce transgenic "hairy roots." The hairy roots of resistant and susceptible cultures respond as expected to root-knot nematode infection. This assay reduced the time required to test *in vitro* modifications of the Mi-1 gene from more than six months to less than two months. Alterations of Mi-1.2 and domain swaps between Mi-1.1 and Mi-1.2 have been constructed and their phenotypes tested in this system.

PHYTOPARASITIC NEMATODES ASSOCIATED WITH PALM TREES AND SOME ORNAMENTAL PLANTS IN EGYPT. **Ibrahim, I. K. A.** Department of Plant Pathology, College of Agriculture, Alexandria University, Alexandria, El-Shatby, Egypt.

A nematode survey was conducted in northern Egypt to identify phytoparasitic nematodes associated with palm trees and some ornamental plants. About 22 nematode genera were found in the collected root and soil samples. The nematode species *Criconemella sphaerocephala, Helicotylenchus microcephalus, H. pseudorobustus, Hemicriconemoides cocophilus, Hemicycliophora thienemanni, Hoplolaimus aegypti, Meloidogyne arenaria, M. incognita, M. javanica, Paratrichodorus minor, Pratylenchus thornei, Tylenchorhynchus* sp., and *Xiphinema ensiculiferum* were identified on date palm trees. Also, *Hoplolaimus columbus, Nothocriconemella mutabilis, Paratylenchus projectus, Rotylenchulus reniformis, Scutellonema brachyurum, Tylenchorhynchus ebriensis, T. clarus, T. goffarti, Tylenchus exiguus* and *Xiphinema basilgoodeyi* were found on certain ornamental plants. The results show new host plant records for these nematode species in Egypt.

CADUSAFOS INHIBITS THE HATCHING, INVASION AND MOVEMENT OF THE POTATO CYST NEMATODE *GLOBODERA PALLIDA.* **Ibrahim, S. K., and P. P. J. Haydock.** Crop and Environment Research Centre, Harper Adams University College, Newport, Shropshire, UK.

The potato cyst nematodes *Globodera pallida* and *G. rostochiensis* are serious pests of potatoes in the United Kingdom. *Globodera pallida* is more difficult to control than *G. rostochiensis* because there are no fully resistant potato cultivars and non-fumigant nematicides may be less effective against this species when compared with *G. rostochiensis*. This is due to the relatively short persistence of nematicides in soil and the prolonged period of emergence of *G. pallida* juveniles. Cadusafos is an organophosphorus insecticide and nematicide used worldwide to control a range of nematodes species. The effect of the nematicide cadusafos on the hatching of the potato cyst nematode *Globodera pallida* in potato root diffusate, soil leachate and distilled water was investigated. Cadusafos had a significant effect on the hatching, migration, movement and root invasion by the second-stage juveniles. Hatching was completely inhibited at low concentrations of cadusafos (0.002–0.004 μg/ml), but hatching resumed a week after removing the nematicide. At concentrations of 0.05 μg/ml and higher of analytical grade cadusafos, the inhibition of hatching was permanent.

PERSISTENCE OF THE NEMATICIDE CADUSAFOS IN DIFFERENT SOIL TYPES AND CONTROL OF THE POTATO CYST NEMATODE *GLOBODERA PALLIDA.* **Ibrahim, S. K., and P. P. J. Haydock.** Crop and Environment Research Centre, Harper Adams University College, Newport, Shropshire, UK.

The potato cyst nematodes *Globodera pallida* and *G. rostochiensis* are serious pests of potatoes in the United Kingdom. *Globodera pallida* is more difficult to control than *G. rostochiensis* because there are no fully resistant potato cultivars and non-fumigant nematicides may be less effective against this species when compared with *G. rostochiensis*. This is due to the relatively short persistence of nematicides in soil and the prolonged period of emergence of *G. pallida* juveniles. Cadusafos is an organophosphorus insecticide and nematicide used worldwide to control a range of nematodes species. Experiments were carried out in field and glasshouse conditions where cadusafos was added to soils and samples taken at regular intervals. Cadusafos residues were then extracted and quantified, using gas chromatography, to determine the persistence of cadusafos in the soil. Cadusafos was detectable in field and glasshouse soils for up to four months after

application. The rates of degradation of cadusafos in different soil types, at different depths in the soil profile, and under different conditions of temperature and moisture content are discussed.

POSSIBLE INTEGRATION OF BENEFICIAL NEMATODES WITH MICROBIAL AGENTS FOR BIOLOGICAL CONTROL OF SOIL PESTS. **Ishibashi, N., and H. Murakami.** Department of Applied Biological Sciences, Faculty of Agriculture, Saga University, Saga 840, Japan.

Aiming at a development of integrated biocontrol of soil-borne fungal diseases and soil insect pests, compatibility of fungivorous nematode *Aphelenchus avenae* (Aa) or entomopathogenic nematodes (EPNs), *Pasteuria penetrans* (Pp) and *Bacillus thuringiensis* (Bt), was evaluated in water and soil. 1) Aa and Pp did not interfere with each other in control of disease complex induced by plant-parasitic fungi and root-knot nematode *Meloidogyne incognita* (Mi). Moreover, mixed application of the two biological agents suppressed the root invasion by Mi more efficiently than did each agent alone. 2) The biocontrol efficacy of Aa and Bt was not affected by their mixed application on the simultaneous control of soil diseases and insect pests. 3) Attachment of Pp endospores onto Mi cuticle was reduced under the high concentration of Bt, but practically no problem. 4) Aa, EPNs, Pp and Bt were practically compatible in the control of plant diseases and soil pests. The integration of these agents will be realized as a substitute for chemicals such as methylbromide or other soil fumigants, if these agents are easily mass-produced at a reasonable cost.

ENCHYTRAEIDS, NEMATOPHAGOUS FUNGI, AND BIOLOGICAL CONTROL OF PLANT-PARASITIC NEMATODES. **Jaffee, B. A.** Department of Nematology, University of California, Davis, CA 95616.

When added to non-sterile soil as biological control agents against plant-parasitic nematodes, nematophagous fungi often fail to establish. Poor establishment of nematophagous fungi in research plots on the campus of UC–Davis has been experimentally associated with enchytraeid worms, but data from commercial agricultural fields are lacking. In the present study, I determined whether enchytraeids suppressed nematophagous fungi in three commercial tomato fields and in two commercial vineyards. Pelletized hyphae (alginate pellets with no additional nutrient base) of the nematophagous fungi *Hirsutella rhossiliensis* or *Monacrosporium gephyropagum* were added to heat-treated (enchytraeids removed) or non-heat-treated soil, and the soil was packed into cages. Each cage consisted of PVC pipe (6 cm long and 3 cm wide, 80 cm^3 volume) sealed at the ends with fine (20 Fm) or coarse (480 Fm) mesh; the fine mesh excluded enchytraeids, whereas the coarse mesh allowed enchytraeids to enter the cages. The soil in the cage had been collected from the same fields where the cages were to be buried. To prevent disturbance from cultivation, the cages were buried about 22 cm deep in the tomato planting row or adjacent to vines. After 10–50 days, depending on soil temperature and rainfall or irrigation, the cages were recovered and fungi and enchytraeids were quantified. Twenty-one independent experiments were performed (12 with *M. gephyropagum* and 9 with *H. rhossiliensis*). Fine mesh excluded enchytraeids in all experiments, but enhanced fungus population density in only three experiments. In contrast, heat treatment of soil enhanced fungus population density in 16 experiments. The data suggest that organisms smaller than 20 Fm, rather than enchytraeids, suppressed growth of the fungi from the pellets. Enchytraeid numbers were 10x less in commercial fields than in-campus plots, which may explain their relative insignificance in the tomato fields and vineyards.

EFFECT OF MILLET AND SORGHUM HYBRIDS AS ROTATIONAL CROPS ON POPULATIONS OF ROOT-LESION NEMATODE (*PRATYLENCHUS PENETRANS*). **Jagdale, G. B.,[1] B. Ball–Coelho,[2] J. Potter,[3] J. Brandle,[4] and R. C. Roy.[4]** [1]Department of Entomology, Ohio State University, OARDC, Wooster, OH 44691, [2]Southern Crop Protection and Food Research Center (SCPFRC), Agriculture and Agri-Food Canada, Delhi, [3]SCPFRC, Agriculture and Agri-

Food Canada, Vineland Station, L0R 2E0 Ontario, Canada, and [4]SCPFRC, Agriculture and Agri-Food Canada, Delhi, Ontario, Canada.

In 1997, a two-year crop rotational experiment was initiated at SCPFRC, Delhi, on a fox loamy sand to study the effect of millet and sorghum hybrids on populations of *P. penetrans* infesting a subsequent high-value crop. Treatments included forage millet (FM 2), grain millet (GM 83) and grain sorghum (GS 7) hybrids as rotational crops, as well as rye, which was included as a susceptible check crop. There were two rye plots so that one could be fumigated as a nematicidal check in the subsequent tobacco crop year (1998). Population densities of *P. penetrans* were significantly influenced by rotation crops in the rotation year and the subsequent tobacco crop year. Rye and GS 7 crops supported a significantly greater number of nematodes than did forage and grain millet hybrids. Greatest reduction in population of *P. penetrans* was in FM 2 plots, where there were 20 and 50 times fewer nematodes than in rye and GS 7 plots, respectively, by fall 1997. The suppressive effects of FM 2 and GM 83 on root-lesion nematodes persisted the following year (1998). Nematode populations were significantly lower in the plots with millet–tobacco rotations than in plots with sorghum–tobacco or rye–tobacco (non-fumigated) rotations. Fumigation treatment with Vorlex CP prior to transplanting of tobacco significantly reduced *P. penetrans* populations infesting tobacco crop during 1998. Suppressive effects of the FM 2 and GM 83 rotations were comparable to that of fumigation; the FM 2 populations were well below the economic loss threshold.

CORRELATION BETWEEN HEAT SHOCK AND TREHALOSE SYNTHESIS IN ENTOMOPATHOGENIC NEMATODES. **Jagdale, G. B., and P. S. Grewal.** Department of Entomology, Ohio Agricultural Research and Development Center, Ohio State University, Wooster, OH 44691.

Trehalose accumulation has been correlated with the expression of heat-shock protein in yeast. We tested this phenomenon in an entomopathogenic nematode. Infective juveniles of *Heterorhabditis bacteriophora* (Hb 88) cultured at 25 °C were heat shocked at 10° and 35 °C separately for 3 hrs and the accumulation of trehalose was measured using anthrone reagent procedure. When infective juveniles were shifted from 25 °C to 35 °C and maintained for 24 hrs, trehalose started to increase within 3 hrs and remained significantly high until all nematodes were dead. In contrast, the infective juveniles, which were heat shocked at 35 °C for 3 hrs and then transferred to 25 °C, the trehalose levels were significantly declined within 3 hrs of latency period. In case of nematodes that were shifted from 25 °C to 10 °C, the levels of trehalose were significantly increased after 96 hrs of storage. Cold shock for 3 hrs at 10 °C following 3 hrs latency period at 25 °C had no significant effect on the synthesis and accumulation trehalose in infective stages of *H. bacteriophora*. Although it has been shown that the heat shock induces the heat shock proteins (hsp 70) in *H. bacteriophora*, there was no correlation between heat shock and trehalose production in the infective juveniles of these nematodes. However, the synthesis and accumulation of more trehalose at high temperatures may be playing an important protective role against denaturing of proteins.

NEMATICIDAL ACTIVITY OF MARIGOLD PLANT PARTS AGAINST ROOT-LESION NEMATODES (*PRATYLENCHUS PENETRANS*). **Jagdale, G. B., B. Reynolds, B. Ball–Coelho, and J. Potter.** Agriculture and Agri-Food Canada, SCPFRC, Vineland Station, L0R 2E0 Ontario, Canada.

The main aim of this study was to determine the nematicidal activity (if any) present in the leaves and roots of *Tagetes* marigold plants. Data from two greenhouse experiments showed that only marigold plants grown alone killed almost all root-lesion nematodes. Although marigold roots significantly reduced the population of *P. penetrans* as compared to the control (corn plant grown alone), there were no significant differences in root-lesion nematode populations when plant parts (leaves and roots) of corn or of marigolds were incorporated as soil treatments. In fact, incorporated corn leaves also numerically reduced nematode populations as compared to the control. Homogenized extracts of roots and leaves of marigold plants and various concentrations (from 0.5 ppm to

10.0 ppm) of synthetic alpha-Terthienyl solution showed no nematicidal activity against root-lesion nematodes in Petri dish bioassays. These results suggest that the alpha-Terthienyl synthesized by marigold plants may not be directly involved in controlling populations of root-lesion nematodes.

POTENTIAL OF SELECTED BACTERIA AS BIOCONTROLS OF *MELOIDOGYNE INCOGNITA ON* TOMATO AND BANANA. **Jonathan, E. I.,[1] K. R. Barker,[1] F. F. Abd-El-Aleem,[1] T. C. Vrain,[2] and D. W. Dickson.[3]** [1]Department of Plant Pathology, North Carolina State University, Raleigh, NC 27695, [2]Agriculture and Agri-Food Canada, Summerland, British Columbia V0H 1Z0, Canada, and [3]Entomology and Nematology Department, University of Florida, Gainesville, FL 32611.

Selected rhizobacteria, including *Agrobacterium radiobacter, Bacillus cereus, B. sphaericus, B. subtilis, Burkholderia cepacia, Pseudomonas chlororaphis, P. fluorescens,* two uncharacterized actinomycetes, and *Pasteuria penetrans* showed promise in suppressing *M. incognita* race 1 on tomato and banana in greenhouse tests, and on banana in a microplot test. All bacteria inhibited root-gall development and nematode reproduction, and enhanced plant growth. Root-gall indices on tomato inoculated with *M. incognita* and bacteria ranged from 25% to 43%, as compared to 86% and 94% for the nematode controls. The reproductive factor for *M. incognita* on bacteria-treated tomato ranged from 1.7 to 9.2 vs. 64.9 for the nematode control in test 1 and 0.9 to 2.4 vs. 14.3 for the control in test 2. Shoot growth of tomato was enhanced by all bacteria, but this was consistently significant in one of two tests. Growth of banana in microplots infested with *M. incognita* and *A. radiobacter* or *P. fluorescens* was enhanced by about two fold as compared to that of control plants.

IDENTIFICATION OF THE SEX PHEROMONE OF THE SUGAR BEET CYST NEMATODE, *HETERODERA SCHACHTII.* **Jonz, M. G.,[1] A. J. Mercier,[1] and E. Riga.[2]** [1]Department of Biological Science, Brock University, St. Catharines, and [2]Agriculture and Agri-Food Canada, SCPFRC, Vineland Station, L0R 2E0 Ontario, Canada.

Previous studies on the behaviour of the sugar beet cyst nematode, *Heterodera schachtii*, suggest that females of this amphimictic species emit a sex pheromone to attract homospecific males. However, water soluble pheromones of *H. schachtii* have not yet been isolated. We present the development of a reliable, sensitive means of identifying nematode sex attractants for use as an alternative to pesticides. Homospecific males displayed a dose dependent chemotactic response to water soluble chemicals produced by 1, 5, 10 and 20 virgin females of *H. schachtii*. Extracellular electrophysiological assays are being developed to investigate the underlying chemosensory phenomena of this response. This work may ultimately lead to characterization of a pheromone and the use of this substance in a pest management strategy.

RME IS REQUIRED FOR THE MI-1-DEPENDENT RESISTANCE TO ROOT-KNOT NEMATODES IN TOMATO. **Kaloshian, I., O. Martinez de Ilarduya, and A. Stromberg.** Department of Nematology, University of California, Riverside, CA 92521.

Root-knot nematodes (*Meloidogyne* sp.) are obligate plant endoparasites that cause major losses in many crops. Mi-1 is the only commercially available gene that confers resistance against several *Meloidogyne* species in tomato. It encodes a protein structurally related to a broader class of plant resistance proteins against different pathogens, but it is unusual in that it also confers resistance against aphids. As an approach to elucidate the pathway underlying the Mi-1-mediated resistance, we are searching for mutants showing susceptibility or reduced resistance to root-knot nematodes. Different bulks of Mi-1-containing tomato seeds were either treated with EMS or irradiated with fast neutrons. The mutagenized seeds were planted and the resulting plants (M1) allowed to self. Seeds from each plant were collected separately. From each M2 seed family, 25 seeds are planted and individually inoculated with nematodes. After six to eight weeks, plants are checked for nematode infection, measured as number of egg masses developed on the root system. After

screening of 298 seed families, we identified three mutants showing different degrees of infection. One mutant (rme, for resistance to *Meloidogyne*) showed complete susceptibility. RT-PCR and Southern data suggest that this mutant carries an intact Mi-1 gene. Moreover, crosses with both susceptible and resistant cultivars resulted in resistant F_1 progeny, indicating that the mutated gene is different from Mi-1. We have also identified two different mutants with reduced nematode resistance. Further genetic work is being done to determine if they are mutated in different genes. Our results confirm that a number of genes are involved in nematode resistance in tomato. Interestingly, mutation of a locus different than Mi-1 completely abolishes resistance.

APPLYING EVOLUTIONARY AND PHYLOGENETIC SPECIES CONCEPTS TO CLARIFY THE TAXONOMIC STATUS OF *RADOPHOLUS SIMILIS*. **Kaplan, D. T.** ARS, USDA, Orlando, FL 32803.

The taxonomic status of burrowing nematodes that are described best morphologically as *Radopholus similis*, but whose host range includes citrus, has been proposed to be races, sibling species, subspecies and most recently as a junior synonym of *R. similis*. Results of genetic, molecular and biological analyses of burrowing nematodes in this group indicate that, with the exception of citrus parasitism, basic criteria upon which citrus-parasitic burrowing nematodes can be described as an operational species are lacking. That is, there is no practical method to recognize burrowing nematodes that differ with respect to citrus parasitism. Citrus-parasitic burrowing nematodes have a shared ancestry with burrowing nematodes that can not parasitize citrus based upon comparison of karyotype (DAPI-stained polar bodies), sequence homology of ITS1 and D2/D3 of the large rDNA gene, RAPD, genetic analysis, and morphology. Citrus-parasitic burrowing nematodes may represent first steps that will lead to speciation, but results of a wide range of tests of their independence suggest they can only be considered a pathotype of *R. similis*.

TOWARD RESOLUTION OF THE *RADOPHOLUS* CONUNDRUM. **Kaplan, D. T.,[1] W. K. Thomas,[2] L. M. Frisse,[2] K. Morris,[2] J. L. Sarah,[3] J. M. Stanton,[4] P. R. Speijer,[5] D. H. Marin,[6] and C. H. Opperman.[7]** [1]ARS, USDA, Orlando, FL 32803, [2]University of Missouri, Kansas City, MO 64110, [3]IRFA, CIRAD, Montpelier, France, [4]DPI, Indooroopilly, Queensland, Australia, [5]IITA, Kampala, Uganda, [6]DelMonte, Costa Rica, and [7]North Carolina State University, Raleigh, NC 27695.

The taxonomy, identification and genetic basis of burrowing nematodes that emulate *Radopholus similis*, but which differ with respect to citrus parasitism, have been controversial. To further characterize burrowing nematodes, sequence homology of PCR-amplified rDNA ITS1 and the D2/D3 expansion segments of the large nuclear rDNA gene were compared for 58 burrowing nematode isolates collected from Africa, Australia, Central America, Cuba, Dominican Republic, Florida, Guadeloupe, Hawai'i, Indonesia, and Puerto Rico. Of the burrowing nematode isolates, 55 were morphologically similar to *R. similis*. Of these, all citrus-parasitic isolates were collected in Florida. The genetic sequences for ITS1 and the D2/D3 expansion segment were identical for all 55, further suggesting that the citrus parasites did not represent a distinct species. Sequence divergence for both the ITS1 and the D2/D3 were evident in morphologically distinct burrowing nematode *R. bridgei*, *R. citri* and *Radopholus* n. sp. In addition, karyotype analysis based on DAPI-stained polar bodies indicated that the karyotype for all 55 burrowing nematodes was $n = 5$. These findings suggest that burrowing nematodes that appear to be *R. similis*, but which differ with respect to their ability to parasitize citrus, are not distinct species.

RESISTANCE OF SELECTED LEGUME CULTIVARS TO *MELOIDOGYNE* SPP. AND *PRATYLENCHUS BRACHYURUS*. **King, P. S., R. Rodríguez–Kábana, and C. F. Weaver.** Department of Plant Pathology, Auburn University, Auburn, AL 36849.

Twenty-nine legume cultivars (*Trifolium* spp., *Medicago* spp., *Vicia* spp., *Clitoria* spp., *Crotalaria* spp. and *Pisum* spp.) were evaluated for resistance to *Meloidogyne arenaria*, *M. incognita* and

Pratylenchus brachyurus in the greenhouse. Twenty seed of each cultivar were planted in 1-L, 10-cm-diameter, cylindrical pots filled with a 50:50 mixture of soil from a soybean field and fine river sand. 'Young' soybean was included as a positive control. There were eight replications per cultivar and pots were arranged in a randomized complete block design. The plants were allowed to grow for eight weeks, after which soil and root nematode populations were determined and roots were evaluated for galling. A wide range of resistance to the nematode species was observed among the different lines. Most resistant to *Meloidogyne* spp. were tropical alfalfa, 'Tropic Sun' sunn hemp (each < 10 J2/g root) and several clover lines including 'Sweet White Annual,' 'Dutch White,' 'Sweet Yellow Biennial,' and 'AU Sunrise' (each < 100 J2/g root). Most clover lines showed high susceptibility to *Meloidogyne* spp. (150–500 J2/g root). A wide range of resistance and susceptibility was noted for *P. brachyurus* (0–300/g root) among the different cultivars, and in most instances an inverse relationship was observed between *Meloidogyne* spp. and *P. brachyurus*.

EVALUATION OF SYNTHETIC COMPOUNDS FOR CONTROL OF THE SOYBEAN CYST NEMATODE. **Knips, A. M.,[1] G. L. Tylka,[1] J. R. Coats,[2] and G. A. Kraus.[3]** [1]Department of Plant Pathology, [2]Department of Entomology, and [3]Department of Chemistry, Iowa State University, Ames, IA 50011.

Four novel compounds were assessed to determine effects on *H. glycines* population densities in laboratory and soil environments. The compounds tested were 2 hydroxymethylenecyclopentanone (referred to as compound AA); 2 1 (1-ethoxycarbonyl-1-hydroxymethylene) cyclopentanone (compound Va), synthetic analogs of glycinoeclepin A; 1 cyano-1 hydroxy-2 propene (compound CHP), a synthetic aglycone; and methyl pelargonate, a fatty acid derivative. Compounds AA, Va, and CHP inhibit hatching of *H. glycines* eggs; methyl pelargonate is an emulsion of a fatty acid that is toxic to nematodes. In laboratory experiments, *H. glycines* eggs were incubated in test solutions and transferred to trays with fresh solution every two days for 22 days. Hatched second-stage juveniles (J2) were counted after each transfer. Hatch of *H. glycines* eggs in water was greater than hatch in each of the four control compounds beginning on the fourth day and continuing for the duration of the experiment. A separate experiment assessed the volatility of the control compounds. Eggs were incubated in trays of treatment solution and in trays of water adjacent to the trays of treatment solution inside airtight boxes. Eggs incubated in water were inhibited from hatching in the presence of CHP and methyl pelargonate, suggesting volatile effects. In field studies, liquid treatments were applied to microplots and manually incorporated into the soil. Half of the plots were planted with Kenwood 94 soybeans (susceptible to *H. glycines*) and half remained unplanted. Soil samples were collected and processed to determine *H. glycines* egg and J2 population densities. There were no differences in population changes through the season within planted or unplanted microplots. The greatest soybean yields were obtained in the microplots treated with methyl pelargonate and compound AA. In four of the five planted microplots treated with CHP, a majority of the soybeans suffered phytotoxicity, resulting in significantly reduced yields.

ULTRASTRUCTURAL CYTOCHEMISTRY OF SECRETORY GRANULES FROM THE DORSAL ESOPHAGEAL GLAND OF *PRATYLENCHUS CRENATUS.* **Knösel, M., and U. Zunke.** University of Hamburg, Institute of Applied Botany, 20355 Hamburg, Germany.

Within the plant parasitic nematodes in agriculture, *Pratylenchus* is the second important genus attacking a wide range of different host plants, e.g., corn, coffee, bananas, vegetables and nursery plants. Using transmission (TEM) and scanning electron microscopy (SEM), the ultrastructure of the esophagus, the esophageal glands and their secretory granules was examined. Clearly separated by cell membranes, one dorsal and two subventral glands could be demonstrated. The secretory granules of the dorsal and subventral glands vary in size and number. Ultrastructural studies of the dorsal esophageal gland indicated that this organ is the site of synthesis of substances for export during nutrient uptake. Cytochemical tests for several enzymes, nucleis acids, carbohydrates, and

proteins of secretory granules from the dorsal gland of adult females of *Pratylenchus crenatus* were conducted for comparison to *Meloidogyne incognita*. Secretory granules stained positive for proteins. Incubation in sodium tungstate, one of three tested specific reagents, gave a positive reaction for nuclei acid. DNase, acid phosphatase, cellulase, carbohydrates, β-glucuronidase, and peroxidase could not be detected.

EXPRESSION OF AN R-GENE HOMOLOGUE IN GIANT CELLS: SIGNALING EVENTS AND THE COMPATIBLE *MELOIDOGYNE*–HOST INTERACTION. **Koltai, H., and D. Bird.** Plant Nematode Genetics Group, Department of Plant Pathology, North Carolina State University, Raleigh, NC 27695.

We are interested in the signaling necessary for compatible and incompatible host responses to root-knot nematode. One gene, originally defined by the tomato cDNA clone DB#131, is a likely candidate for participating in such events. Based on an RNA dot-blot survey of tomato tissues, we found this gene to be detectable only in giant cells, i.e., it is nematode-responsive; we are currently employing more sensitive techniques to confirm this observation. Using 3N and 5N RACE-PCR, we have cloned an apparently full-length transcript. Based on homology searches, the DB#131 gene encodes a receptor serine-threonine kinase, with a structure most similar to that encoded by the rice Xa-21 R-locus. Xa-21 confers resistance to the bacterial leaf pathogen *Xanthomonas oryzae*, and is composed an extracellular receptor, a short trans-membrane domain, and a cytoplasmic kinase. Additional analysis of the DB#131 kinase using the BLOCKS algorithm further suggests that it may be a member of the activin superfamily. The domain structure and cellular topology of the DB#131 protein and its kinase specificity will be confirmed experimentally. We also are interested in identifying additional components of the DB#131 signal cascade machinery. The potential role of DB#131 in compatible and resistance responses will be discussed.

SEASONAL POPULATION DYNAMICS OF LESION AND ROOT-KNOT NEMATODES IN STRAWBERRIES. **LaMondia, J. A.** Department of Plant Pathology and Ecology, The Connecticut Agricultural Experiment Station, Windsor, CT 06095.

Strawberry roots were sampled every three weeks throughout the season over two years to determine the population and distribution of *Pratylenchus penetrans* and *Meloidogyne hapla*. Three distinct root types were sampled; structural roots produced from crowns, feeder roots without secondary tissues, and suberized black perennial roots. Structural roots eventually become dark or black perennial roots due to the development of suberized tissues. Both lesion and root-knot nematodes primarily infected feeder roots branching from healthy black perennial or white structural roots. Few nematodes were recovered from soil, diseased roots or suberized roots. Lesion nematode recovery was correlated with percent healthy white roots ($R = 0.72$; $P = 0.01$). In both 1997 and 1998, *P. penetrans* populations peaked at about day 150 (end of May) and then declined. The decline in numbers corresponded to changes in total strawberry root weight and root type distribution. The loss of nematode habitat was a result of both loss of roots due to disease and the development of structural root to suberized perennial root. *M. hapla* juvenile recovery peaked at around 170 days (mid June) in 1997and at 85 days (late March), 147 days (late May), 229 days (mid August) and 308 (early November) in 1998. *M. hapla* survived the winter in field plots in 1997–98 as juveniles, adult females in roots and egg masses, and there appear to be at least four generations per year in Connecticut. The number of root-knot juveniles was related to the availability of feeder root for infection and development in the previous generation. Diagnostic nematode samples from an established strawberry bed may be most reliable and useful when they include feeder roots taken in late May.

NEMATODE COMMUNITY COMPOSITION AND DIVERSITY IN AN AGROECOSYSTEM. **Liang, W.,[1] I. Lavian,[2] and Y. Steinberger.[2]** [1]Faculty of Life Sciences, Bar-Ilan University,

Ramat-Gan, Israel, and Institute of Applied Ecology, Chinese Academy of Sciences, Shenyang, PR China, and [2]Faculty of Life Sciences, Bar-Ilan University, Ramat-Gan, Israel.

In recent years, the need to understand the responses and dynamics of free-living nematode communities to a wide array of management practices has grown. The effects of agricultural inputs on the nematode community were investigated in a potato field in Israel. Soil samples from depths of 0–10 cm and 10–20 cm were collected between September 1997 and March 1998. Various ecological indices were assessed and compared between the managed (treatment plot) and unmanaged (control plot) field. Nineteen nematode families and 24 genera were observed. Rhabditidae, *Cephalobus*, *Eucephalobus*, *Aphelenchus*, *Tetylenchus*, *Tylenchus* and *Dorylaimus* were found to be the dominant families/genera in both plots at both depths. During the post-planting and mid-season periods, the total number of nematodes was less in the treatment plot than in the control plot at both depths, and was higher at the 0–10 cm depth than at the 10–20 cm depth; bacterivores and fungivores were found to be the most abundant trophic groups in both plots and both depths. During the harvesting and post-harvesting periods, plant parasites increased significantly, and the irrelative abundance in both plots averaged 16.7–20.4% and 20.7–25.9% of nematode community, respectively. Among the ecological indices tested, ratio of fungivores and bacterivores to plant parasites (WI), Shannon diversity index (H'), maturity index (MI) and plant parasite index (PPI) were effective in assessing the response of nematode communities to agricultural inputs in the Israeli agroecosystem.

USE OF ENTOMOPATHOGENIC NEMATODES IN THE BIOLOGICAL CONTROL OF THE ORIENTAL FRUIT MOTH, *GRAPHOLITA MOLESTA* (BUSCK). **Lickman, K. L.,[1] E. Riga,[1] W. H. Cade,[2] and D. Gray.**[2] [1]Agriculture and Agri-Food Canada, SCPFRC, Vineland Station, L0R 2E0 Ontario, Canada, and [2]Department of Biological Sciences, Brock University, St. Catharines, Ontario, Canada.

The Oriental fruit moth (OFM), *Grapholita molesta* (Busck), is the most important insect pest of peaches in Ontario. As OFM resistance to many insecticides has been observed, the potential use of entomopathogenic nematodes in the biological control of the OFM was investigated. OFM mortality was significantly influenced by the presence of various entomopathogenic nematode species at 50 nematodes per larva. These species include *Steinernema glaseri*, *S. feltiae*, *S. carpocapsae* and a field strain (FS3). Larval mortality rates appear to be dependant on nematode species. Greenhouse trials will clarify the applicability of this host-parasite interaction in the field.

DETECTION OF THE NEMATOPHAGOUS *HIRSUTELLA* SPECIES IN SOUTHERN MINNESOTA SOYBEAN FIELDS. **Liu, X. Z., and S. Y. Chen.** Southern Experiment Station, University of Minnesota, Waseca, MN 56093.

Hirsutella rhossiliensis is an endoparasite of vermiform nematodes. Parasitism of the soybean cyst nematode (SCN) second-stage juveniles (J2) by the fungus in natural soil was first observed in a Minnesota soybean field. Consequently, a survey was conducted in 1996–97 to determine infestation of the fungus in Minnesota soybean fields infested with the SCN. A total of 435 soil samples representing about 270 fields in 27 counties in southern Minnesota were examined. J2 were extracted from (i) 50 grams of soil 14 days after addition of 2,000 J2, (ii) 50 cm^3 of soil that had been added with 1,000 J2 weekly for 3 weeks, and (iii) 50 cm^3 of soil without addition of J2. The J2 were examined with an inverted microscope at magnifications of 40-200 for fungal infection. J2 colonized by the fungal mycelium or J2 with attached *Hirsutella* spores were considered as being parasitized. Fungi were isolated from colonized J2 for identification to species. Two species of *Hirsutella*—*H. rhossiliensis* and *Hirsutella* sp.—were frequently encountered on J2. When the data of the three soil treatments is combined, parasitism of SCN J2 by *Hirsutella* species was detected in 39% of the soil samples. *Hirsutella rhossiliensis* was observed in 36% and *Hirsutella* sp. in 7.4% of the soil samples. A high percentage (about 60%) of J2 parasitized by

Hirsutella spp. was observed in about 0.5% of the soil samples. Addition of J2 into the soil increased efficiency of detection of the fungal parasitism.

MORPHOLOGICAL, MOLECULAR AND BIOLOGICAL CHARACTERIZATION OF *MEHDINEMA ALII* (NEMATODA: DIPLOGASTERIDA) FROM THE DECORATED CRICKET (*GRYLLODES SIGILLATUS*). **Luong, L. T.,[1] E. G. Platzer,[2] P. De Ley,[3] and W. K. Thomas.[4]** [1]Departments of Biology and Nematology, [2]Department of Nematology, University of California, Riverside, CA 92521, [3]Vakgroep Biologie, Universiteit Gent, B-9000 Gent, Belgium, and [4]Division of Molecular Biology and Biochemistry, School of Biological Sciences, University of Missouri, Kansas City, MO 64110.

Mehdinema alii was recovered from the decorated cricket, *Gryllodes sigillatus*. The nematode is characterized by dense arrays of spines on the cuticle of the anterior half of the body, and a highly elongate, tubular stoma with a dorsal denticle in the glottoid region. Females have a protruding vulva. Young females are amphidelphic, but the anterior ovary disappears in older females bearing multiple developing juveniles. The male is monorchic with asymmetrically placed external genitalia, distally fused spicules and a highly complex gubernaculum bearing two sclerotized thorns that protrude through a separate, post-cloacal opening. Adult nematodes are located primarily in the hindgut, whereas juveniles or dauers occur mainly in the genital chamber of both male and female crickets. Males are significantly more likely to be infected than females. This male-biased infection may be linked to the complex venereal transmission mechanism of the dauers. Although morphologically unusual in many respects, placement of *M. alii* in *Diplogasterida* is supported by both the morphology of the anterior digestive tract as well as analysis of its 18S rDNA sequence. These sequence data suggest that *M. alii* groups most closely with members of the Cylindrocorporidae.

VENEREAL WORMS: SEXUALLY TRANSMITTED NEMATODES. **Luong, L. T.,[1] E. G. Platzer,[2] and M. Zuk.[1]** [1]Department of Biology, and [2]Department of Nematology, University of California, Riverside, CA 92521.

The causative agents of sexually transmitted diseases are commonly short-lived microparasites rather than long-lived macroparasites. Few studies have experimentally demonstrated venereal transmission in macroparasites. Here we present experimental evidence for the occurrence of a sexually transmitted macroparasite. The infective stages of a nematode, *Mehdinema alii*, are transferred from male to female crickets, *Gryllodes sigillatus*, during copulation. Adult female crickets harbouring infective stages subsequently transfer the nematode to their next mates. Since the reproductive stage of the nematode develops primarily in male hosts, females essentially serve as a vector. The reproductive success of a sexually transmitted parasite is directly linked to the host mating success.

SEASONAL HATCHING OF *HETERODERA GLYCINES* EGGS. **MacGuidwin, A. E., and A. Reid–Rice.** University of Wisconsin, Madison, WI.

The overwinter survival of *Heterodera glycines* eggs is often close to 100% in Wisconsin. Eggs collected from the field and incubated in water frozen by a controlled nucleating event have a high rate of survival, which is greater than the survival rate of hatched second-stage juveniles. The objective of our study was to determine whether *H. glycines* eggs collected in the fall hatch without host-related stimuli. Eggs were collected from the same soybean field in May, August, October and December. The eggs were extracted from cysts, counted and approximately 1,000 were delivered to hatching chambers. On each date, 10 hatching chambers were incubated in a 0.5 mM solution of zinc chloride or in water. The chambers were moved to a new dish containing fresh solution each week for eight weeks and the hatched juveniles were counted. Egg hatch in zinc chloride, a hatching stimulant, was used to estimate egg viability, which varied between 77% and 85% over the four dates. Egg hatch in water was greatest for the cohort collected in August and lowest for the cohort collected in October, with a cumulative hatch of 40% and 5%, respectively. The cohort

collected in December had a cumulative hatch rate of 5% for the first three weeks of incubation and then hatch increased at a rate greater than that of the cohort collected in May. The cumulative hatch for the December cohort was 27%. Our current estimate is that the majority of eggs that do not hatch in mid-summer have a time-mediated dormancy of approximately 20 weeks, after which time about 20% hatch even without appropriate host signals. These data will be used in a model predicting the decline of *H. glycines* populations in the absence of a host.

PHORETIC RELATIONSHIP BETWEEN A *BACILLUS* SP. AND THE ENTOMOPATHOGENIC NEMATODE, *HETERORHABDITIS* SP. **Marti, O. G., Jr., and P. Timper.** IBPMRL and NWCRU Research Units, ARS, USDA, Tifton, GA 31793.

During a survey to identify native species of entomopathogenic nematodes, we exposed *Galleria mellonella* larvae to soil samples collected from fields and pastures near Tifton, GA. Unidentified species of *Heterorhabditis* and *Steinernema* were found. In four of eight collection sites, we observed sporangia of an unidentified *Bacillus* sp. frequently attached to the sheath of infective juveniles of *Heterorhabditis* sp. that had emerged from *Galleria* exposed to soil. Sporangia were spindle-shaped, 9–11 μm in length, and contained a central refractile endospore. They differed from published descriptions of *B. popilliae*. Sporangia were observed either loosely attached by one end to the nematode or more securely attached in a manner resembling that of *Pasteuria*. Numbers of sporangia attached to individual nematodes varied from zero to more than 100. This *Bacillus* sp. has been maintained *in vivo* for several months in the laboratory by exposing *Galleria* larvae to sporangia-bearing *Heterorhabditis* juveniles. Large numbers of mature and developing sporangia occur in *Galleria* cadavers and become attached to infective juveniles of *Heterorhabditis* as these migrate out of the cadaver. No evidence of pathogenicity to the nematode has been found, although there may be competition between *Bacillus* sp. and *Photorhabdus* for resources in the insect cadaver. The relationship between *Bacillus* and *Heterorhabditis* seems to be phoretic and serves to spread the sporangia beyond the immediate vicinity of the insect cadaver.

INFLUENCE OF TEMPERATURE ON EXPRESSION OF RESISTANCE IN CARROT TO *MELOIDOGYNE JAVANICA.* **Matthews, W. C., Jr., [1] P. W. Simon,[2] and P. A. Roberts.[1]** [1]Department of Nematology, University of California, Riverside, CA 92521, and [2]Horticulture Department, ARS, USDA, University of Wisconsin, Madison, WI.

Resistance in carrot to *Meloidogyne javanica* was characterized by evaluating F1 to F4 and BC1 populations of a cross between Brasilia, (Bra1252), the resistance donor, and a susceptible USDA inbred. This high level of resistance is conferred by a single dominant gene, or possibly linked duplicate genes, and it is also partially effective against some isolates of *M. incognita*. Studies in temperature-controlled water baths of two Brasilia, lines (Bra1252, Bra1091) and a susceptible check (cv. Imperator 58), indicated that resistance in these lines to *M. javanica*, based on nematode egg production, was significantly high from temperatures of 22 °C to 28 °C, but was weakened at 31 °C. There were differences in the reactions of the two lines. Resistance in Bra1091 was consistently high up to 28 °C, but was lost at 31 °C. Loss of resistance in Bra1252 was gradual from 22 °C to 31 °C. These studies also demonstrated the influence of temperature on the dominance of resistance expression in Bra1252 to *M. javanica*. Evaluation of BC1 populations of the above cross, based on feeder root galling (0 to 4 scale), indicated that dominance was partial at 28 ± 1 °C, with heterozygous plants classified as intermediate (score of trace to 1) compared to homozygous resistant (score of 0) and susceptible (score of 3 to 4) plants. The heterozygous plants were highly resistant and indistinguishable from homozygous resistant plants at 23 ± 1 °C, indicating complete dominance of resistance at the lower temperature.

TWO NOVEL GENES EXHIBITING TRANSCRIPT ABUNDANCE CHANGES IN *HETERODERA GLYCINES*-INFECTED SOYBEAN ROOTS. **Mazarei, M., and T. J. Baum.** Department of Plant Pathology, Iowa State University, Ames, IA 50011.

Differential display of mRNA was used to isolate cDNA clones corresponding to mRNA species with altered abundances in *Heterodera glycines*-infected soybean roots (Hermsmeier et al. 1998). cDNA clones D10.1 and D17.1 were used as probes to identify larger cDNA fragments by screening a library prepared from *H. glycines*-infected susceptible soybean roots. Screening of approximately 350,000 plaques resulted in the isolation of putative full-length cDNA clones for both probes. Analyses of the nucleotide and deduced amino acid sequences of these clones revealed that they belong to previously undescribed genes because no significant similarities could be found in available databases. Transcripts corresponding to these two genes have previously been shown to increase in *H. glycines*-infected roots at 24 hrs after inoculation. Probing of total RNA-blots obtained from roots and shoots of susceptible soybean at one, three and six days after inoculation with *H. glycines* confirmed the transcript changes also at later stages during the *H. glycines*–soybean interaction. Transcripts corresponding to these genes were also present in shoots. However, their abundances were not altered by nematode infection. Furthermore, transcript abundances in shoots were substantially lower than in roots. These results suggest a root-specific gene regulation following *H. glycines* infection. Similar northern blot analyses using RNA from an incompatible interaction between *H. glycines* and soybean are currently being carried out.

COMPARISON OF PRE-PLANT TREATMENTS FOR TWO-YEAR NURSERY CROPS. **McKenry, M. V., T. Buzo, and S. Kaku.** Department of Nematology, University of California, Riverside, CA 92521.

Rainfall of 7.5-cm in the four months preceding pre-plant treatments resulted in drench applications of 1,3 dichloropropene (1,3-D) providing better nematode control than comparable shank applications. After removal of a nursery crop to this sandy loam soil, replicated plots were treated and planted to *Prunus* sp. the following spring. Over the next two years, measurements of plant growth and counts of *Pratylenchus vulnus* and *Paratylenchus hamatus* were collected. A stacked drench of 370 kg/ha 1,3-D in 11 cm water immediately followed by 5 cm of water containing 125 kg/ha metam sodium (MS) resulted in 99.90% nematode control. Shank injection of 370 kg/ha 1,3-D followed in one week by a surface drench of 125 kg/ha MS in 5 cm water resulted in 97% nematode control. A uniform drench of 370 kg/ha 1,3-D or MS in 16 cm water provided 99.92% nematode control and 99.65% control, respectively. Methyl bromide shanked at 268 kg/ha provided 83.5% nematode control. Plant growth was slightly better in sites receiving shank treatments compared to drench treatments. Shanked applications provided the familiar increased growth response associated with soil fumigation, whereas drench treatments did not.

CHANGES IN PEROXIDASE ACTIVITY IN RESISTANT AND SUSCEPTIBLE CLONES OF SUGAR CANE INOCULATED WITH *PRATYLENCHUS ZEAE*. **Mehta, U. K., and T. Kathiresan.** Nematology Section, Sugarcane Breeding Institute, Coimbatore, India.

Sequential development of peroxidase activity in leaves and roots of susceptible (CoC 671) and resistant (Co 7717) sugar cane clones following inoculation with *Pratylenchus zeae* was investigated. The enzyme activity in the leaves and roots of inoculated plants was less as compared to uninoculated plants in susceptible clone. In resistant clone, the enzyme activity in leaves and roots of inoculated plants was more as compared to uninoculated plants. In the case of susceptible clone, per cent reduction in enzyme activity in inoculated plants over uninoculated plants was maximum in roots and leaves at 14 and 35 days after inoculation (DAI), respectively. In the case of resistance clone, per cent increase of enzyme activity in inoculated plants over uninoculated was maximum in roots and leaves at 14 and 35 DAI. Native PAGE analysis of perxodiase activity in roots of resistant and susceptible clones showed qualitative differences in low-molecular-weight protein bands. No such qualitative difference could be observed in leaves. The results thus revealed that both the resistant and susceptible clones respond to parasitic invasion by bringing about qualitative and quantitative changes in peroxidase. Further analysis of two susceptible (CoC 85061 and CP 44-101) and four resistant (Co 89003, Co 86011, BO 99, Co 86010) clones showed a new

peroxidase isozyme with Rf value of 3.6 in both inoculated and uninoculated plants of resistant clones. However, the same was not found in susceptible clones. Hence, this peroxidase isozyme may be used as a selection marker in breeding for nematode resistance.

EVALUATION OF BIOTIC DIVERSITY FOR THE IMPROVEMENT OF BENEFICIAL TRAITS OF ENTOMOPATHOGENIC *NEMATODE HETERORHABDITIS* UNDER SUGAR CANE ECOSYSTEM. **Mehta, U. K.,[1] and P. Sundararaj.[2]** [1]Sugarcane Breeding Institute, Coimbatore, India, and [2]University of Idaho, Parma, ID.

Entomopathogenic nematode *Heterorhabditis indicus* is a biocontrol agent having potential to kill the white grub, *Holotricha serrata*, of sugar cane crop under natural habitat. In order to explore the biotic diversity of *Heterorhabditis*, a survey was carried out in the sugar cane growing tracts of subtropical India. A total of 10 isolates were identified from Andhra Pradesh (2 isolates), Karnataka (6 isolates) and Tamil Nadu (2 isolates). A wide range of diversity has been observed in host range, specificity, multiplication rate, infectivity and genetic variability. Major contributing factors for such diversity are biotic and abiotic factors, cropping sequences, and faunal diversity. Susceptibility to several stresses at field level is a major constraint observed in many species of *Heterorhabditis*. It is possible, by virtue of the natural diversity among *Heterorhabditis*, to develop a resistant isolate for the effective white grub management.

GREENHOUSE AND FIELD SCREENING PROCEDURES FOR *HETERODERA GLYCINES* TOLERANCE. **Melakeberhan, H., and G. W. Bird.** Department of Entomology, Michigan State University, East Lansing, MI 48824.

Three decision-criteria (considering nematode population increase, yield potential and tolerance index) were used to identify Maturity Group I–III soybean cultivars with tolerance to *Heterodera glycines* under greenhouse and field experiments. Eleven susceptible and one resistant (control) cultivars were selected for a two-season field study after two greenhouse screenings of 48 susceptible and two resistant cultivars. The two-season field studies were conducted on the same location and same plots under both high- and low-*H. glycines* infestations. A nematicide was applied to maintain low nematode population density. In addition to yield and nematode data, leaf nutrient status was determined in the second season. CX 252, a Dekalb cultivar, was the only *H. glycines* susceptible cultivar that met the three tolerance selection criteria in the two field studies, and along with Jack (resistant) exhibited the best tolerance to nutrient imbalance.

EFFECT OF *HETERODERA GLYCINES* ON *MELOIDOGYNE INCOGNITA* AND *PRATYLENCHUS PENETRANS* INFECTION. **Melakeberhan, H.,[1] and J. Dey.[2]** [1]Department of Entomology, and [2]Department of Statistics, Michigan State University, East Lansing, MI 48824.

The objective of this study was to test how *H. glycines* competes against *M. incognita* (Experiments I and II) and *P. penetrans* (Experiments III and IV) separately under greenhouse conditions (25 ± 2 °C). Forty-eight-hour-old *H. glycines* and *M. incognita* and mixed vermiform stages of *P. penetrans* were inoculated into a *H. glycines* susceptible soybean cultivar 'Tray M' at 1:0, 0.75:0.25, 0.50:0.50, and 0.25:0.75 ratio to give 1,000 individuals per treatment. Treatments were replicated five times, inoculated in 3 ml suspensions, each experiment repeated once, and all experiments were terminated 18 days after inoculation. Levels of competition were calculated by taking the ratio of the numbers of each nematode inoculated and dividing it by the numbers recovered in roots. Suitable linear and quadratic fits were obtained with proportion of the competing nematodes to test the fitness for competition. In Experiment I, the numbers of *M. incognita* significantly ($P \leq 0.05$) decreased with increasing *H. glycines* proportion of inoculum, while the level of significance was at $P \leq 0.08$ in Experiment II. Increasing *M. incognita* had a significant effect on *H. glycines* in Experiment II. The numbers of *H. glycines* in both experiments decreased with increasing proportion of *P. penetrans*, while increasing *H. glycines* had no effect on *P.*

penetrans. The results indicate that *P. penetrans* is more competitive with *H. glycines* than *M. incognita*.

NEMATODES IN OREGON AGRICULTURE: A WORLD WIDE WEB SITE. **Merrifield, K.,[1] B. Muir,[2] J. Hanus,[3] S. Pittam,[3] and R. Ingham.[3]** [1]Botany and Plant Pathology, Oregon State University, Corvallis, OR 97331, [2]Botany and Plant Pathology, Oregon State University, Corvallis, OR 97331, and Lewis and Clark College, Portland, OR, and [3]Botany and Plant Pathology, Oregon State University, Corvallis, OR 97331.

The Nematodes in Oregon Agriculture web site [http://mgd.nacse.org/hyperSQL/squiggles/] was initiated during 1997 as an Oregon Coalition of Interdisciplinary Databases project. Taxonomic character states in tables supporting a nematode identification key were used as a protypical small database to test and use HyperSQL, a language created by Mark Newsome of the Northwest Alliance for Computational Science and Engineering. HyperSQL enables users to easily search databases on the Internet. Nematodes in Oregon Agriculture provides an illustrated synoptic key to plant-parasitic nematode genera occurring in the Pacific Northwest. Additional pages provide general information about plant-parasitic and free-living nematodes and drafts of nematode host range and damage level literature surveys for Pacific Northwest crops. Updates will include more extensive literature surveys encompassing native plants and weeds in an easier-to-search format as well as species identification information for some genera treated in the synoptic key.

NEMATODE-ANTAGONISTIC ACTIVITY OF *GLIOCLADIUM VIRENS* AND *BURKHOLDERIA CEPACIA*. **Meyer, S. L. F.,[1] D. P. Roberts,[2] S. I. Massoud,[3] and D. J. Chitwood.[1]** [1]Nematology Laboratory, Beltsville Agricultural Research Center (BARC), ARS, USDA, Beltsville, MD 20705, [2]Biocontrol of Plant Diseases Laboratory, Beltsville Agricultural Research Center (BARC), ARS, USDA, Beltsville MD 20705, and [3]Botany Department, Faculty of Agriculture, Suez Canal University, Ismailia, Egypt.

The fungus *Gliocladium virens* and the bacterium *Burkholderia cepacia* were studied as potential microbial control agents for *Meloidogyne incognita* on tomato, and as producers of natural compounds that could be efficacious for decreasing nematode populations. In assays conducted in microwell tissue culture plates, filtered culture broth from *B. cepacia* grown in potato dextrose broth (PDB) reduced *M. incognita* egg hatch ca. 50%. Culture filtrate from *G. virens* grown in PDB did not reduce egg hatch, but did affect mobility of hatched second-stage juveniles (J2), tripling the number of immobile J2 compared with a PDB control. In an initial trial with *G. virens* applied as a root drench to growth chamber-grown tomato plants, nematode population numbers (eggs and J2) counted 35 days after addition of *M. incognita* were reduced ca. 40%. However, in a greenhouse trial with *G. virens* applied as a seed treatment and again as a root drench to young tomato plants, nematode population numbers at 35 days were not affected by application of *G. virens*. *Burkholderia cepacia* was also studied in the greenhouse trial (applied to tomato as a seed coat and as a root drench); application resulted in ca. 41% reduction in nematode population numbers.

SECRETION OF CELLULASE INTO PLANT TISSUE BY *HETERODERA GLYCINES*. **Meyers, D.,[1] X. Wang,[1] Y. Yan,[1] T. Baum,[2] G. Smant,[3] R. Hussey,[4] and E. L. Davis.[1]** [1]Department of Plant Pathology, North Carolina State University, Raleigh, NC 27695, [2]Department of Plant Pathology, Iowa State University, Ames, IA 50011, [3]Nematology, Wageningen Agricultural University, Wageningen, The Netherlands, and [4]Department of Plant Pathology, University of Georgia, Athens, GA 30602.

Two b-1,4-endoglucanase (cellulase) genes, Hg-eng-1 and Hg-eng-2, have been identified and shown to be expressed specifically within the subventral esophageal gland cells of the soybean cyst nematode, *Heterodera glycines*. Polyclonal sera specific to each endoglucanase was used to identify a nematode esophageal gland protein secreted into host plant tissue for the first time. Soybean roots were inoculated with second-stage juveniles of *H. glycines*. After 24 h, infected root tissue

was collected and frozen cross-sections were made. HG-ENG-1 was localized within the nematode's subventral gland cells and was not detected in root tissue. HG-ENG-2 was localized within the subventral gland cells and within the root tissue surrounding the nematode head, clearly showing that it is secreted from the juvenile's stylet into root cortical tissue at 24 h after inoculation. HG-ENG-2 was also localized along the juvenile's migratory path through the root cortex. Both sera generated to HG-ENG-1 and HG-ENG-2 did not cross-react with soybean root proteins on Western blots or within non-inoculated (control) soybean root sections visualized by immunofluorescence microscopy.

OCCURRENCE AND DISTRIBUTION OF *HETERODERA* SPP. IN MICHIGAN SUGAR BEET PRODUCTION. **Miller, A. M., F. W. Warner, and G. W. Bird.** Department of Entomology, Michigan State University, East Lansing, MI 48823.

Sugar beet acreage has increased, sugar beet yield per unit of land decreased and crop rotation lengths shortened in MI during the past 15 years. In an attempt to determine the current role of *Heterodera schachtii* in sugar beet yield declines, a survey for its detection was conducted in 1998, cooperatively with Michigan and Monitor Sugar Companies. Company fieldmen were each responsible for collecting ten root-soil samples for nematode analysis. Five of these samples were taken at random from fields not exhibiting symptoms associated with low sugar beet yields. The remaining five were taken using a stratified sampling method from fields exhibiting foliar symptoms similar to those caused by *H. schachtii*. A total of 214 samples were collected throughout 13 counties. *Heterodera* spp. were recovered from 54% of the sites. Cyst nematode population densities ranged from 0 to 49,352 eggs and juveniles per 100 cc of soil. Cyst nematodes were recovered from 51% of the 109 sites that did not exhibit symptoms, with a mean population density of 2,198 eggs and juveniles. In the 105 sites exhibiting symptoms, cyst nematodes were recovered from 58% of the samples, with a mean population density of 4,482 eggs and juveniles. High cyst nematode population densities were most frequently associated with short crop rotation intervals. *Heterodera* spp. were detected in more than 50% of the samples collected from Bay, Huron, Saginaw and Tuscola counties. *H. schachtii, H. glycines, H. avenae, H. trifolii* and *H. carotae* are all known to exist within the sugar beet-growing region of MI.

COMBINED USE OF FUMIGATION AND GRANULAR NEMATICIDES TO REDUCE YIELD LOSS CAUSED BY POTATO CYST NEMATODES. **Minnis, S.,[1] P. P. J. Haydock,[1] and K. Evans.[2]** [1]Crop and Environment Research Centre, Harper Adams University College, Newport, Shropshire, UK., and [2]IACR-Rothamsted, Harpenden, Hertfordshire AL5 2JQ, UK.

In the UK the most problematic pests of the potato crop are the potato cyst nematodes *Globodera pallida* and *G. rostochiensis*. Populations of *G. rostochiensis* can be managed by the integrated use of nematicides (granular and fumigant), crop rotation and resistant cultivars. However, there are no commercially available cultivars with full resistance to *G. pallida* and both crop rotation and granular nematicides are less effective at controlling this species. It is not known whether control with fumigant nematicides is equally efficient for both species of PCN. Fumigant nematicides such as 1,3-dichloropropene (Telone II) are able to kill up to 80% of a PCN infestation with one application. In situations of very high PCN levels, it may be possible to reduce populations and yield losses by using an autumn application of 1,3-dichloropropene followed by a spring application of a granular nematicide. A field experiment was done to compare the use of 1,3-dichloropropene with the granular nematicides aldicarb, oxamyl and fosthiazate applied at planting in the spring. The experimental site contained both species of PCN and had an initial population density of 66 eggs g^{-1} of soil. The experiment had a factorial design to compare 1,3-dichloropropene (applied in autumn and spring) and the granular nematicides both individually and in combination. The initial results of the experiment show that the combination of 1,3-dichloropropene with each of the granular nematicides gave higher yields than either treatment

alone. In addition, the use of 1,3-dichloropropene increased the overall number of tubers and improved the tuber size distribution compared to the granular nematicides alone.

MILKWEED PLANT PARTS CONTAIN NEMATICIDAL COMPOUNDS. **Mojtahedi, H.,[1] R. Fries,[2] G. S. Santo,[1] R. A. Holser,[3] R. E. Harry–O'Kuru,[3] S. F. Vaughn,[3] and T. P. Abbott.[3]** [1]Washington State University, Prosser, WA 99350, [2]New Fiber, Ogallala, NE 69153, and [3]ARS, USDA, Peoria, IL 61601.

Milkweed, *Asclepias syriaca*, is a new crop being produced for its fiber in pillows and comforts. As the volume of harvested seed and crop trash grows, new uses are sought for the crop to be fully successful economically. Green shoots, dried pod shells, a mixture of dried pod shell and crop trash, and seedmeal were evaluated as possible soil amendment to control *Meloidogyne chitwoodi*. Nematode-infested soil was amended with milkweed plant parts at 0.5 to 4% by weight and bioassayed on tomato seedlings for three weeks. Tomato roots were stained and numbers of infective nematodes were determined. All plant parts significantly ($P < 0.5$) reduced number of infective *M. chitwoodi* and injured the bioassay tomato plants. The most potent plant material was pod shell, followed by seedmeal, crop trash, and green shoots. Since the defatted seedmeal was still active, the oil component of milkweed seed was not involved in nematode kill.

APHELENCHID NEMATODES ASSOCIATED WITH CERAMBYCID INSECTS IN PINE TREES IN PORTUGAL: IMPORTANCE OF SURVEY AND STUDY. **Mota, M. M.,[1] H. Braasch,[2] M. A. Bravo,[3] A. C. Penas,[1] W. Bergermeister,[4] K. Metge,[4] and E. Sousa.[5]** [1]Departmento de Biologia/ICAM, Universidade de évora, 7000 évora, Portugal, [2]Federal Biological Research Center for Agriculture and Forestry, Department for National and International Plant Health, Kleinmachnow Branch, Stahnsdorfer Damm 81, D-14532 Kleinmachnow, Germany, [3]Departamento de Fitopatologia, Estação Agronómica Nacional, INIA, Quinta do Marquês, 2780 Oeiras, Portugal, [4]Biologische Budensanstalt fur Land- und Forstwirtschaft, Institut fur Biochemie und Pflanzenvirologie, Braunschweig, Germany, and [5]Departamento de Protecção Florestal, Estação Florestal Nacional, INIA, Quinta do Marquês, 2780 Oeiras.

The nematode *Bursaphelenchus xylophilus* (Nemata: Aphelenchoididae) is present in North America (from where it is native) as well as parts of East Asia (Japan, China, Korea); it has been classified, according to the European Organization for Plant Protection (EPPO), as an A1 quarantine organism, thus causing concern about its possible entry or presence in the EU countries. The devastation and economic damage caused in trees of the above-mentioned countries is well known and has caused considerable reservations to importing pine timber from the USA and Canada. The Mediterranean-type climate conditions in our country–dry hot summers–provide the ideal setting for this nematode, particularly when pine trees are subject to water stress. In Portugal, a general survey has been initiated to search for *B. xylophilus*, as well as other aphelenchid nematodes associated with pine. The genera *Laimaphelenchus* and *Bursaphelenchus* are reported for the first time in the Iberian Peninsula. High numbers of *Bursaphelenchus xylophilus* specimens have been found associated with declining trees at 2 sites southeast of Lisbon. Morphological and morphometrical aspects, as well as DNA (ITS-RFLP) profiles are shown that unequivocally confirm this species. This is the first report of *Bursaphelenchus xylophilus* in Europe.

POTENTIAL USE OF *VERTICILLIUM CHLAMYDOSPORIUM* AS A BIOLOGICAL CONTROL AGENT AGAINST ROOT KNOT NEMATODES: ALTERNATIVE TO THE USE OF METHYLBROMIDE. **Mota, M. M.,[1] Mª I. Clara,[2] L. Alho,[1] C. Franco,[2] J. Cravo,[1] I. Brito,[1] Mª J. Martins,[1] C. Pombo,[2] M. Laranjo,[1] C. Mira,[1] and T. Louro.[2]** [1]Dept. de Biologia, Universidade de évora, 7000 évora, Portugal, and [2]Dept. De Sanidade Animal e Vegetal, Universidade de évora, 7000 évora, Portugal.

Plant-parasitic nematodes are responsible for huge crop losses worldwide. Certain *Meloidogyne* species are particularly damaging to horticultural crops in southern Europe. To reduce these losses,

a variety of methods have been employed, namely crop rotation, resistant varieties and the use of nematicides. In order to obtain alternatives to the use of certain nematicides such as methyl bromide, a very efficient but ozone-depleting fumigant to be banned within the European Union in 2010 in accordance with the Montreal Agreement, biological control agents with potential efficacy may be used. The facultative parasite *Verticillium chlamidosporium*, present in the soil, has proven to be a potential antagonist against nematodes by parasitizing the exposed egg masses produced by *Meloidogyne* females. Preliminary results include the identification of *V. chlamydosporium* isolates in Portugal, as part of an ongoing European research project. Results will also include data on interaction with non-target organisms, such as AMF and *Rhizobium* species, as well as egg colonization and histopathology.

TAXONOMIC STUDIES OF GREAT PLAINS CRICONEMATINAE. **Mullin, P. G., T. O. Powers, T. S. Harris, and B. Higgins.** Department of Plant Pathology, University of Nebraska, Lincoln, NE 68583.

The identity of some of the ring nematodes (Criconematinae) occupying the Great Plains region of the United States has long been in question. In particular, where some authors have considered *Mesocriconema xenoplax* (Raski, 1952) Loof and de Grisse, 1989 to be widespread in this region, others have found only *M. curvatum* (Raski, 1952) Loof and de Grisse, 1989. Likewise, some authors have noted the presence of *Ogma cobbi* (Micoletzky, 1925) Siddiqi, 1986, while others have not recorded this species. In the course of our studies of nematode diversity and host associations in tall-grass prairie ecosystems, we have thus far recovered *Ogma decalineatum* (Chitwood, 1957) Andrássy, 1979, *O. fimbriatum* (Cobb in Taylor, 1936) Raski and Luc, 1987, *M. curvatum* and *Criconemella annulata* (Cobb in Taylor, 1936) Luc and Raski, 1981. *M. curvatum* and *O. decalineatum* have been more frequently encountered than have other species in Criconematinae. Comparison of tall-grass prairie *M. curvatum* with *M. xenoplax* specimens from the type culture maintained at the University of California–Davis has revealed significant morphological differences and sequence disparity in the internal transcribed spacer (ITS1) region between the two species. It is likely that many of the reports of *M. xenoplax* from the Great Plains actually refer to *M. curvatum*.

EFFECT OF MATRIC POTENTIAL ON NEMATODE COMMUNITY COMPOSITION. **Neher, D. A.,[1] T. R. Weicht,[1] M. Savin,[2] J. Görres,[2] and J. Amador.[2]** [1]Department of Biology, University of Toledo, Toledo, OH 43606, and [2]Department of Natural Resources, University of Rhode Island, Kingston, RI.

Impacts of contrasting equilibrium moisture regimes on nematode community composition were quantified. Intact cores (5 cm dia., 10 cm deep) were collected from an old field with perennial grasses near Kingston, Rhode Island, in May, August and November 1997 and March 1998. For each treatment and season, 10 pairs of cores were saturated and incubated at field temperatures and matric potentials of −3, −10, −20 or −50 kPa for 21–28 or 42–58 days. Nematodes were extracted using Cobb's sieving followed by sucrose flotation and identified to taxonomic family or genus. *Coomansus*, Monhysteridae, *Prismatolaimus*, and *Tylenchorhynchus* were more abundant at −3 kPa than −10, −20 or −50 kPa, suggesting their tolerance of wet soils. *Dorylaimellus*, *Dorylamoides*, and *Paraxonchium* were more abundant at −10 and −20 kPa than −3 and −50 kPa, suggesting their sensitivity to both extreme dry and moist soils. *Aphelenchoides*, Cephalobidae, *Ditylenchus*, Panagrolaimidae, *Paratylenchus*, and Rhabditidae were relatively abundant at −50 kPa, which may reflect their ability to tolerate drought through an hydrobiosis. Seasonal differences mainly affected numbers of plant-parasitic nematodes. For example, *Xiphinema* and *Coslenchus* were more abundant in March and May than August and November. *Hirschmanniella* and *Axonichium* were progressively more abundant in May, August and March and absent in November. *Hoplolaimus* was present in August but no other season. *Ditylenchus* and *Paratylenchus* were increasingly more abundant in May, March, August and November. It is well established that nematode populations

are affected by seasonal change and plant phenology. The next step is to validate associations between nematode abundance and activity within dynamic environmental conditions that more closely resemble nature.

OPTIMAL SAMPLING OF NEMATODE POPULATIONS IN COLORADO USING A GEOGRAPHIC INFORMATION SYSTEM. **Niles, R. K., J. E. Cipra, R. M. Reich, and D. H. Wall.** Natural Resource Ecology Laboratory, Colorado State University, Ft. Collins, CO 80523.

Geographic information systems (GIS) can aid in making decisions about land use. To promote the improved management of alfalfa in Colorado, our project plans to use GIS to determine the relative risk posed by the alfalfa stem nematode to agricultural sites in the South Platte River Basin. A GIS database was constructed containing information about the soil characteristics for the southern portion of Weld County, Colorado. We used the database to devise a sampling plan that allowed us to capture, with a low number of samples, the wide variation in site characteristics of fields grown to alfalfa. Selected GIS data layers conformed to variables representing the determinants of alfalfa stem nematode disease: nematode distribution was represented by the clay content of soil, alfalfa growth was represented by the expected average yield of alfalfa, and a moist environment was represented by irrigated cropland. Using these data layers, we identified nine classes of alfalfa sites. Our collection of 109 samples from among the classes of sites was proportional to the alfalfa hectacreage in each class. At the time of sampling, geographic coordinates were acquired, along with the plant samples for nematode analysis and a soil sample for physical and chemical analysis. We enumerated the abundances of four nematodes, which all inhabited alfalfa crowns and stems: *Ditylenchus dipsaci* (causal agent of alfalfa stem nematode disease), *Aphelenchoides* spp., *Pratylenchus* sp. and *Panagrolaimus* sp. The probability density surface for each nematode showed large-scale patterns of distribution that varied among the four nematodes.

FUNGAL COMPOUNDS TOXIC TO ROOT-KNOT NEMATODE (*MELOIDOGYNE INCOGNITA*). **Nitao, J. K., S. L. F. Meyer, and D. J. Chitwood.** Nematology Laboratory, Plant Sciences Institute, ARS, USDA, Beltsville, MD 20705.

Fungal natural products that reduce root-knot nematode (*Meloidogyne incognita*) egg hatch and larval mobility *in vitro* are being isolated. Amberlite XAD gel was used to extract culture broths of *Fusarium equiseti* and a second fungal species. Nematode-antagonistic compounds in the *Fusarium* extract were separated from polar constituents by partitioning between water-methanol and ethyl acetate. Fractionation of the ethyl acetate extract on a series of silica gel chromatography columns produced fractions that reduced egg hatch by 66% when tested at 50 μg/ml. Components of the active *Fusarium* fractions are being purified with reversed-phase HPLC. The XAD extract of the second fungal species yielded a methanol-soluble fraction that reduced egg hatch by 55% when tested at 250 μg/ml. Further bioassay-directed separation of the methanol- soluble fraction with reversed-phase column chromatography is expected to yield a more potent fraction.

IMPACT OF PRE-PLANT SOIL SOLARIZATION ON *CRICONEMELLA XENOPLAX* IN A PEACH TREE SHORT LIFE SITE. **Nyczepir, A. P.,[1] D. A. Kluepfel,[2] J. L. Lawrence,[2] and E. I. Zehr.[2]** [1]Southeastern Fruit and Tree Nut Research Laboratory, ARS, USDA, Byron, GA 31008, and [2]Department of Plant Pathology and Physiology, Clemson University, Clemson, SC 29634.

In 1995, a field experiment was initiated at the USDA–ARS laboratory in Byron, Georgia to determine if the pairing of soil solarization with the subsequent introduction of the nematode biocontrol agent, *Pseudomonas aureofaciens* (BG33), increases the potential for control of *Criconemella xenoplax* under orchard conditions. Plots consisted of four treatments: 1) solarized soil alone; 2) solarized soil + BG33; 3) non-solarized soil; and 4) non-solarized soil + BG33. Treatment plots were replicated nine times in a randomized, complete block design. Bacteria were applied to peach roots and soil at planting in February 1996, following solarization the previous summer. Bacteria were applied again to the rhizosphere orchard soil in October 1996. The native soil-borne

microbial community was dramatically altered quantitatively and qualitatively by solarization, thus diminishing the competitive stress on the introduced BG33. Furthermore, soil solarization significantly reduced the *C. xenoplax* population density for approximately two years post-solarization as compared to non-solarized soil. During this period of time, the nematode population was below or near the economic nematicide treatment threshold of 50 *C. xenoplax* per 100 cm^3 soil.

INDUCED RESISTANCE TO PLANT-PARASITIC NEMATODES IN PLANTS BY BETA-AMINOBUTYRIC ACID. **Oka, Y.,[1] Y. Cohen,[2] and Y. Spiegel.[1]** [1]Department of Nematology, ARO, The Volcani Center, Bet Dagan, Israel, and [2]Faculty of Life Sciences, Bar-Ilan University, Ramat Gan, Israel.

Beta-aminobutyric acid was found to induce resistance in plants to several plant-parasitic nematodes by soil drench or foliar spray. In experiments with *Meloidogyne javanica*, foliar spray of tomato plants with 2,000 ppm BABA reduced root-galling by 76% seven days after inoculation, as well as the number of eggs by 60% thirty days after inoculation. Soil drench with 500 ppm BABA caused reduction in the number of eggs by 64%. Nematodes invading BABA-treated tomato roots induced small and vacuous giant cells. Post-infection treatment of tomato plants with BABA inhibited nematode development. Soil drench with 500 ppm BABA caused reduction in the number of *Heterodera avenae* and *H. latipons* cysts on wheat and barley roots by more than 99%. Foliar spray with higher concentrations (< 8,000 ppm) of BABA controlled these cereal cyst nematodes. Although syncytia were initiated in BABA-treated wheat roots by *H. avenae* juveniles, nematode development was inhibited. Radio-labeled BABA applied to foliage of tomato, cucumber and wheat plants, translocated to younger shoots and leaves, and to the root systems. In roots of tomato and cucumber plants, higher radioactivity was observed in root-galls and female bodies of *M. javanica*, while BABA accumulation was not observed in the infection sites of *H. avenae* on wheat roots. BABA was also effective to *Rotylenchulus reniformis* on tomato and cotton plants. The results suggest that BABA may serve as a potential nematicide.

INTERACTIONS OF ROOT-KNOT NEMATODE EGG MASSES AND SEPARATED EGGS WITH SOIL MICROORGANISMS. **Orion, D., S. L. F. Meyer, and D. J. Chitwood**. Nematology Laboratory, USDA ARS, Beltsville Agricultural Research Center, Beltsville, MD 20705.

Petri dishes containing 0.8% water-phytagel medium were seeded with root-knot nematode (*Meloidogyne incognita*) egg masses obtained from monoxenic nematode cultures on excised tomato roots or with suspensions of separated eggs obtained from similar egg masses by dissolving the gelatinous matrix with sodium hypochlorite solution. Natural garden soil (500 mg) was placed around each egg mass and the separated eggs. Each treatment was replicated 12 times; the Petri dishes were kept in the dark at 25 °C, and microscopic observations were made daily for a period of three weeks. Forty-eight hours following the onset of the experiment, various species of bacteria, fungi, nematodes, and mites were near both the egg masses and the separated eggs. Within 7 to 10 days after experimental set-up, all the separated eggs were destroyed by the soil microorganisms, whereas, the egg masses remained intact. When separated eggs were placed on pieces of a gelatinous matrix exposed to soil as described above, the eggs survived, possibly because of protection by the gelatinous matrix. Light microscopic observations of egg masses placed in Petri dishes and exposed to various bacteria and fungi typical of soils showed that although the microorganisms were in close contact with the egg mass surface, they could not penetrate the egg masses. It is concluded that the gelatinous matrix has antimicrobial properties that may enable root-knot nematode eggs to survive in the soil in spite of hostile microbial flora.

INCIDENCE AND OCCURRENCE ABOVE THRESHOLDS OF *ROTYLENCHULUS RENIFORMIS* AND *MELOIDOGYNE INCOGNITA* IN LOUISIANA DURING 1997–98. **Overstreet, C.,[1] and E. C. McGawley.[2]** [1]Louisiana Cooperative Extension Service, and [2]Department of Plant Pathology and Crop Physiology, Louisiana State University, Baton Rouge, LA 70803.

Previous studies have indicated that reniform nematode *Rotylenchulus reniformis* and root-knot nematode *Meloidogyne incognita* are two of the most important nematodes in the cotton production areas of Louisiana. The Nematode Advisory Service processed 4,670 nematode samples from 14 cotton-producing parishes in 1997–1998. Cotton was the predominant crop (77%) represented by the nematode samples, with soybean (18%) and corn (5%) comprising the remainder. Reniform nematode was present in 48% of the samples. This nematode occurred above a threshold level (1,000 per 500 cm^3 of soil) in 1,531 samples. Root-knot nematode was found only in 8% of the samples, but was above threshold level (150 per 500 cm^3 of soil) in 75% of the samples where it was present. Incidence of reniform nematode within individual parishes ranged from 8–78%, with 4 of the 14 parishes having reniform in greater than 50% of the soil samples. Root-knot nematode was present in 2–30% of the samples within individual parishes, with 11 of 15 having incidence by this nematode of less than 15%. These findings continue to substantiate the importance of reniform nematode as the premier nematode pest in Louisiana.

CONTROLLED RELEASE OF HIRSUTELLA RHOSSILIENSIS FROM HOLLOW BEADS FOR BIOLOGICAL CONTROL OF PLANT-PARASITIC NEMATODES. **Patel, A. V.,[1] T. Rose,[1] V. Gutberlet,[2] J. Muller,[2] and K. D. Vorlop.[1]** [1]Institute of Technology, Federal Agricultural Research Centre (FAL), Braunschweig, Germany, and [2]Institute of Nematology and Vertebrate Research, Federal Biological Research Centre for Agriculture and Forestry (BBA), Munster, Germany.

Nematophagous fungi are antagonists of plant-parasitic nematodes, but up to now no successful biological control agent was available. This is mainly due to missing formulation techniques. The aim of our work is to develop a formulation method for the nematophagous fungus *Hirsutella rhossiliensis* that attacks the plant-parasitic nematode *Heterodera schachtii*. *H. rhossiliensis* mycel was formulated in a new type of hollow beads. These hollow beads are made by dropping an aqueous solution of the polyanion sulfoethylcellulose into a stirred aqueous precipitation bath containing a polycation. The membrane is formed in a fast reaction between the polyanion and the polycation on the surface of the droplet (polyelectrolyte-polyelectrolyte-complex). The nematophagous fungus was raised in liquid culture, microencapsulated and then investigated in vitality and pathogenicity assays. Investigations on the influence of nutrient and biomass content of the capsules on the radial growth of fungus led to a capsule containing 20% corn gluten, 0.5% yeast extract and biomass down to 0.1%. The fungus grows in the core of the hollow bead like in a minifermenter and then out of the capsule still using the nutrient reserve. Further experiments on the influence of capsule diameter, soil moisture and a comparison to calcium alginate beads will be shown. Pathogenicity assays were done where free and encapsulated fungus were added to boxes containing 100 mL non-sterile field soil and a sugar beet seed. After one week of incubation, 1,000 infective *Heterodera schachtii* juveniles were added, and after another week the infection of sugar beet plantlets was determined. It was found that encapsulated *Hirsutella rhossiliensis* suppressed the invasion of seedling roots by about 80%. Free fungus showed very small suppression.

MUTANTS AND GIGANTISM IN *CAENORHABDITIS ELEGANS*. **Patel, M. N., and A. M. Leroi.** Department of Biology, Imperial College of Science, Technology and Medicine, Silwood Park, Ascot, Berkshire SL5 7PY, UK.

Egg-laying-defective (*egl*) mutants of *Caenorhabditis elegans* have been well characterised in terms of egg laying. However, our interest is not so much in this primary effect *per se* but in one of the secondary, lesser known, phenotypic traits which is found in some of the *egl* mutants, namely gigantism. We looked at about 50 known *egl* mutants and determined their body size at 36 and 48 hours after hatching, and in some cases more detailed growth curves have been produced. A few of these mutants were found to be significantly larger than the wild-type Bristol N2 strain. For example, adult worms of *egl-19* and *egl-25* are about 50% longer in length and about 70% larger by volume compared with wild-type worms. Based on the size variation already observed in

mutants of *C. elegans*, these particular mutants certainly warrant the classification "giant." In addition, from the onset of hatching, *egl-19* has a faster growth rate, which suggests that the larger body size is not entirely a consequence of bloating. To date, only five of the *egl* mutants have been cloned and one of these is *egl-19*, which may encode a calcium channel subunit. This would suggest that the *egl-19* mutation is in some way associated with muscular activity. Two obvious processes that involve muscle activity are egg laying and feeding, and so we have also examined their impact on body size.

THE CONTROL OF *MELOIDOGYNE* SPECIES IN GREENHOUSE AND FIELD EXPERIMENTS USING SELECTED MARIGOLD VARIETIES. **Ploeg, A. T.** Department of Nematology, University of California, Riverside, CA 92521.

The continued concern and restrictions on nematicide use requires that alternative nematode control strategies are developed. Marigolds (*Tagetes* spp.) have long been known to suppress soil and root population levels of *Pratylenchus* and *Meloidogyne* nematode species. However, several reports showed conflicting results on the efficacy of control of different root-knot nematode species by different marigold varieties or species. In greenhouse studies, a range of marigold varieties was tested for suppression of four *Meloidogyne* species (*M. arenaria, M. hapla, M. incognita, M. javanica*). Marigolds were grown in plastic cones filled with *Meloidogyne*-infested soil for 60 days. After determining nematode infestation levels in marigold roots and the soil, tomatoes were transplanted into the cones. After six weeks, plant growth (fresh top and root weight) and infestation and galling of the tomato roots was assessed. Results showed that large differences occurred between marigold varieties and between species of *Meloidogyne*, with some combinations resulting in near complete nematode control and other combinations resulting in an increase in nematode numbers and tomato root-infestation compared to fallow controls. Two marigold varieties, *T. patula* "Single Gold" and the *Tagetes* hybrid "Polynema," were subsequently tested in a field trial in southern California on a *M. incognita*-infested site. Marigold *T. patula* "Single Gold," grown for three months, suppressed galling and root infestation of subsequently grown tomato to levels similar to those achieved with soil fumigation. Furthermore, tomato fruit yields after both marigold varieties were c. 1.6 times those after fallow controls and not significantly different from those after soil fumigation. It is concluded that these marigold varieties are of potential use to manage *M. incognita* under field conditions. Studies evaluating the efficacy of marigolds for root-knot nematode control under field conditions in different crops and under different soil and climatic conditions are continuing.

THERMAL–TIME RELATIONSHIPS FOR LIFE CYCLE COMPLETION OF THE ROOT-KNOT NEMATODE *MELOIDOGYNE INCOGNITA*. **Ploeg, A. T.,[1] and P. C. Maris.[2]** [1]Department of Nematology, University of California, Riverside, CA 92521, and [2]Department of Nematology, Wageningen Agricultural University, Wageningen, The Netherlands.

In spite of the agricultural importance of *Meloidogyne incognita*, few studies have focused on the effects of temperature on the life cycle duration of this nematode. An understanding of the relationship between temperature and nematode development rates is necessary to predict geographical distributions, nematode population dynamics and resulting crop yield losses. We examined the relationship between soil temperature and the time required for life cycle completion for a *M. incognita* race 3 population. Second-stage juveniles (J2s) were inoculated onto tomato plants. Plants were grown for two weeks at average soil temperatures of 16.2°, 19.5°, 25.0° 30.0° and 35.4 °C. Four days prior to the expected emergence of newly developed J2s, the plants were transferred to new pots containing coarse gravel. J2s were then collected daily by percolating water through the pots and counting of J2s in the percolated water. Analysis of the data showed that a positive linear relationship existed between the rate of development (day 1) and temperature. Estimates for the base temperature (Tb) and for the required heat sum (S) were 10.1 °C and 400 °C day, respectively. Total reproduction at 16.2 °C was lower than at 19.5°, 25.0° and 30.0°. Reproduction

did not occur at 35.4 °C. Comparing our results with those published for *M. hapla* and *M. javanica* suggests that development of *M. incognita* will be slower than that of *M. hapla* at soil temperatures below 14°, but faster than that of *M. javanica* at all temperatures. The hypothesis that, for biologically similar species, Tb is inversely proportional to S was supported by our data.

IMPACT OF THREE WEED SPECIES ON REPRODUCTION OF *ROTYLENCHULUS RENIFORMIS* ON COTTON. **Pontif, M. J., and E. C. McGawley.** Department of Plant Pathology and Crop Physiology, Louisiana State University Agricultural Center, Baton Rouge, LA 70803.

In the spring of 1998, a microplot study was conducted to determine the effects of reniform nematode (Louisiana isolate 930437) and three endemic weed species [morning glory (*Ipomoea purpurea*–MG)], hempses bania (*Sesbania exaltata*–HS) and johnson grass (*Sorghum halepense*–JG)] on cotton (LA. 887). Treatments were arranged as a RCB design with seven replications of seven treatments: 1) cotton; 2) MG; 3) JG; 4) HS; 5) cotton + MG; 6) cotton + JG; and 7) cotton + HS. Seeds were sown in the greenhouse in flats of fumigated soil and seedlings were transplanted after two weeks into microplots containing 15 kg fumigated soil. The test was established on 18 May 1998, inoculated on 11 June with a suspension containing 1,300 reniform nematode juveniles, and harvested on 11 August (60 days after inoculation). At harvest, plant matter was cut, dried and weighed, and a soil sample was collected from each pot. A 150 g subsample of soil was then used to extract nematodes using the sugar flotation/centrifugation procedure. Numbers of juveniles per microplot and reproductive values (R, where R = Pf/Pi and Pf and Pi are final and initial inoculum levels, respectively) were calculated and data were analyzed using ANOVA and Tukey's HSD test (SAS version 6.12 for Macintosh). Reproductive values in decreasing order were: 60.5 (MG), 45.0 (cotton + MG), 41.7 (cotton), 35.8 (cotton + HS), 21.4 (HS), 17.3 (cotton + JG) and 13.2 (JG). This study was re-established during the period 15 September–7 December 1998. Results from both tests suggest an allelopathic effect of these weed species on reproduction by reniform nematode.

TAXONOMIC STUDIES OF GREAT PLAINS BELONOLAIMIDAE. **Powers, T. O., P. G. Mullin, T. S. Harris, and R. Higgins.** Department of Plant Pathology, University of Nebraska, Lincoln, NE 68583.

The family Belonolaimidae Whitehead, 1960 was redefined by Fortuner and Luc (1987) based primarily on a reconsideration of the taxonomic significance of overlapping esophageal glands. The redefined family included two subfamilies and 13 genera of plant-parasitic nematodes. Five of these genera are encountered in the northern Great Plains, several only in native, uncultivated soils. *Geocenamus* Thorne and Malek, 1968, *Merlinius* Siddiqi, 1970, and *Nagelus* Thorne and Malek, 1968 can be found in remnant short-grass prairie, mountain slopes, and alpine meadows. Their biogeographic patterns suggest that they survived and diversified during the Wisconsin glacial maximum in the *Beringia refugium*, which includes, in part, Alaska. *Belonolaimus* Steiner 1949 and *Tylenchorhynchus* Cobb, 1913 have dramatically different biogeographic affinities. Phylogenetic studies of these genera are helping to provide a historical explanation of present-day nematode distribution.

SHORT-TERM STUDY OF *PRATYLENCHUS SCRIBNERI* POPULATION DYNAMICS IN ENDOPHYTE- INFECTED AND ENDOPHYTE-FREE TALL FESCUE ROOTS. **Reiss, J., and E. C. Bernard.** Department of Entomology and Plant Pathology, University of Tennessee, Knoxville, TN 37996.

Previous studies have indicated that the reduction in *Pratylenchus scribneri* populations in endophyte-infected (E+) tall fescue roots, when compared to endophyte-free (E+) tall fescue roots, may be due to suppressed reproduction; no eggs were present in E+ roots at 60 days after inoculation (dai), and life-stage distribution of the vermiform nematodes showed a lack of juvenile recruitment. In this short-term study, the population dynamics of *P. scribneri* in E+/E− tall fescue roots were examined with particular attention given to reproductive capability. E+ and E− clonal

pair tillers of tall fescue were planted in containers and inoculated with 1,000 vermiform *P. scribneri* per container. Roots were harvested at 20, 40 and 60 dai, and the number of eggs and vermiform *P. scribneri* in each root system was counted. The first 50 vermiform *P. scribneri*, or as many as possible up to 50, were dissected from each root system, mounted and traced. The life stage of traced nematodes was determined based on length and presence of a vulva. Live adult females were extracted from two E+/E− root systems per harvest, surface-sterilized, and added singly to alfalfa callus culture to determine reproductive capability. At all harvest dates, the mean number of vermiform *P. scribneri* in E+ roots was significantly lower than in E− roots. Significantly fewer eggs were present in the E+ roots, but the number of eggs per adult was not significantly different. There was no significant difference in the life stage distribution of *P. scribneri* in the E+ and E− roots. Females from both E+ and E− root systems reproduced in callus culture. The reduced population of *P. scribneri* in these E+ tall fescue roots cannot be attributed to suppressed reproduction. Since all life stages were affected, it is more likely that toxins or plant physiological changes are detrimental to part of the nematode population.

SWINE MANURE AFFECTS *HETERODERA GLYCINES* SOIL POPULATION DENSITIES. **Reynolds, D. A., G. L. Tylka, and C. A. Martinson.** Department of Plant Pathology, Iowa State University, Ames, IA 50011.

Swine manure traditionally has been applied to corn production fields in the midwestern United States as a source of plant nutrients. Because of the increased concentration of swine production in Iowa, farmers also are applying swine manure to soybean production fields. The effect of swine manure application on the soybean cyst nematode, *Heterodera glycines*, is not known. In 1997, swine manure at 9,194 kg/ha and inorganic fertilizer with NPK analysis similar to the swine manure were applied to 4 rows by 7.5 m field plots infested with *H. glycines* and planted to corn. Similar rates of swine manure and inorganic fertilizer were applied in-furrow, between rows, and as a broadcast application to plots prior to planting soybean in 1997. Some plots were left untreated in both the experiments. Experiments were randomized, complete blocks designs with eight replications in corn and ten replications in soybean. Soil cores were collected prior to nutrient application, at midseason, and at the end of the season from the two center rows and from between and on either side of the center two rows. Eggs and second-stage juveniles of *H. glycines* were extracted from soil samples and counted. Swine manure applied in-furrow prior to planting corn apparently reduced egg hatch relative to the untreated check, as indicated by mid-season and final egg population densities. End-of-season egg densities were greater in soybean plots treated with manure in-furrow than in untreated soybean plots. Additionally, soybean yields were increased by in-furrow and broadcast application of swine manure relative to untreated plots. Application of swine manure in corn may increase survival of *H. glycines* populations in the soil and may increase *H. glycines* population densities in manure-amended soybean fields due to increased plant growth from the additional nutrients provided by the manure.

EFFICACY OF FOUR RATES OF 1,3-DICHLOROPROPENE (1,3-D) APPLIED WITH PARACHISELS IN FLORIDA DEEP SAND SOILS. **Riegel, C.,[1] D. W. Dickson,[1] and L. N. Shaw.[2]** [1]Entomology and Nematology Department, and [2]Agricultural and Biological Engineering, University of Florida, Gainesville, FL 32611.

1,3-Dichloropropene has been used for many years in Florida for control of plant-parasitic nematodes on many crops; however, its performance has not been consistent on some crops, e.g., peanut and tobacco. The rate used for fumigation is a factor that affects efficacy. Our objective was to evaluate four rates of 1,3-D in a field heavily infested with *Meloidogyne incognita* and *M. javanica*. The chemical was applied with two parachisels (designed with a 45° bend in order to eliminate the chisel opening that allows for rapid release of 1,3-D from the soil) per row spaced 25 cm apart. Rates tested were 56, 84, 112 and 168 liters/ha. The treatments were disked after fumigation to enhance sealing of the 1,3-D. Tomato cv. Solarset seedlings were transplanted seven

days after fumigation. The number of second-stage juveniles (J2) that penetrated the root systems of 10 plants per plot and the number of J2 per 100 cm^3 soil were counted. The number of J2 in the roots and in soil decreased with increasing rates of 1,3-D, $Y = 4.7–0.2X$, $R2 = 0.7$, $P < 0.001$, and $Y = 3.0–0.1X$, $R2 = 0.4$, $P < 0.001$, respectively. Juveniles survived even at the highest rate. However, at the rate of 168 liters/ha, there was a 98% reduction in the number of J2 per root system and a 123% reduction in the number of J2 per 100 cm^3 of soil, compared with the untreated control.

EVALUATION OF SEVERAL METHODS OF 1,3-DICHLOROPROPENE (1,3-D) APPLICATION IN FLORIDA SAND SOILS. **Riegel, C.,[1] D. W. Dickson,[1] and L. N. Shaw.[2]** [1]Entomology and Nematology Department, and [2]Agricultural and Biological Engineering, University of Florida, Gainesville, FL 32611.

The performance of 1,3-dichloropropene has not been consistent on crops such as peanut and tobacco in Florida. Application depth, method of application, equipment, and sealing of the fumigant are factors that contribute to its efficacy. Our objective was to evaluate the effect of these factors on the efficacy of 1,3-D. The rate used was 84 liters/ha. Treatments included broadcasting 1,3-D with standard chisels at injection depths of 23 to 25 cm deep and 15 to 20 cm (with and without disking), row application with four parachisels vs. standard chisels, and two (in row) and three (broadcast) subsurface winged chisels. Peanut was used as the indicator crop and the site selected was infested with *Meloidogyne arenaria* race 1. Plots were 12.2 m long with four rows spaced 0.9 m apart. The two center rows were treated and the two outer rows were untreated and served as a border. Parameters tested were yield, incidence of root-knot nematode galls on pods, and second-stage juvenile (J2) densities in soil. The incidence of galls on the pods and J2 per 100 cm^3 soil were lower and yield was higher in fumigated plots compared to the untreated plots ($P < 0.1$). Broadcast fumigation with standard chisels at 23 to 25 cm deep with and without disking was the most effective treatment for reducing galls on peanut pods. In row fumigation with parachisels, standard chisels and subsurface winged chisels were not as effective as the broadcast treatment for all parameters tested.

THE USE OF ENTOMOPHILIC NEMATODES AS BIOLOGICAL CONTROL AGENTS AGAINST INSECT PESTS OF CORN IN ONTARIO. **Riga, E.,[1] J. Potter,[1] and J. Whistlecraft.[2]** [1]Agriculture and Agri-Food Canada, SCPFRC, Vineland Station, L0R 2E0 Ontario, Canada, and [2]Agriculture and Agri-Food Canada, SCPFRC, London, Ontario, Canada.

The purpose of our project is to assist the Ontario corn growers manage insect pests, primarily the European Corn Borer and secondarily the Fall Armyworm, Corn Rootworm and Seedcorn Maggot Fly with the use of entomophilic nematodes. The *in vitro* studies showed that *Steinernema feltiae*, *S. glasseri*, *S. carpocapsae*, *Heterorhabditis megidis* and *H. bacteriophora* killed, on average, 60% to 80% of the European Corn Borer, Fall Armyworm, Seedcorn Maggot Fly and Corn Rootworm, while only 5–10% of the controls died. During the *in vivo* studies, *S. feltiae* provided higher corn plant protection against the Seedcorn Maggot Fly, the Corn Rootworm and the Fall Armyworm, while *S. glasseri* provided higher protection to corn plants against the European Corn Borer. The number of corn plants protected in the field with entomophilic nematodes was significantly higher than the control group.

COMPARATIVE REPRODUCTION BY *ROTYLENCHULUS RENIFORMIS* AND *MELOIDOGYNE INCOGNITA* ON 300 GENOTYPES OF UPLAND COTTON. **Robinson, A. F.,[1] A. C. Bridges,[1] C. G. Cook,[2] M. J. Oliver,[3] A. E. Percival,[1] and J. P. Velten.[3]** [1]ARS, USDA, College Station TX 77843, [2]United Ag Products, Santa Rosa, TX, and [3]ARS, USDA, Lubbock, TX.

Rotylenchulus reniformis and *Meloidogyne incognita* race 3 are the most damaging nematodes of Upland cotton (*Gossypium hirsutum*). Only a few genotypes of *G. hirsutum* are highly resistant to *M. incognita* and none are highly resistant to *R. reniformis*. We conducted a series of studies in which reproduction by *R. reniformis* and *M. incognita* were directly compared under standardized

growth chamber conditions on 6–12 replicate pairs of more than 300 cultivars, breeding lines, and primitive accessions of Upland cotton. We measured plant height, foliar and root weights, root length, main stem nodes, fruiting, gall index for *M. incognita*, number of vermiform stages of *R. reniformis* extractable by Baermann funnel, and eggs per plant for both nematodes. Gall index was highly correlated with extractable eggs of *M. incognita* and was the less variable and cheaper indicator of resistance. Vermiform stages of *R. reniformis* extracted from soil gave more efficient data than eggs from roots. Reproduction by *R. reniformis* and galling induced by *M. incognita* were weakly correlated; complete suppression of galling was associated with a 40% average decrease in *R. reniformis* egg production per plant. Reproduction by *M. incognita* commonly differed 100-fold, whilst reproduction by *R. reniformis* seldom differed more than 5-fold among cotton genotypes. The results suggest that cultivars with strong resistance to *M. incognita* and moderate resistance to *R. reniformis* can be developed from known genotypes of *G. hirsutum.*

ELECTROPHYSIOLOGICAL ANALYSIS OF THE CONCENTRATION-DEPENDENT RESPONSES OF *GLOBODERA ROSTOCHIENSIS* J2S TO TEST COMPOUNDS. **Rolfe, R.,[1] J. Barrett,[2] and R. N. Perry.[1]** [1]Entomology and Nematology Department, IACR-Rothamsted, Harpenden, Hertfordshire AL5 2JQ, UK, and [2]Institute of Biological Sciences, University of Wales, Aberystwyth SY23 3EB, UK.

Electrophysiological techniques enable responses in nematodes to be analysed in detail and have the additional advantage over agar plate assays of enabling concentration-dependent effects to be defined. Modification of the electrophysiological recording apparatus has enabled the determination of extracellular responses of live second-stage juveniles (J2s) of *Globodera rostochiensis* to set concentrations of acetylcholine. Analysis of responses to acetylcholine established the delay in response after stimulus application and changes in spike activity before, during and after exposure to the stimulus. The occurrence and onset of adaptation was also determined. For example, the delays in response to 10 mM and 100 mM concentrations were 7.0+/−1.03 s and 5.7+/−1.38 s, respectively, which were not significantly different. In contrast, the number of spikes per second after perfusion with 100 mM concentration was more than 300% greater than that recorded in response to 10 mM solutions. Data will be compared to the responses of J2s to potato root diffusate and to set concentrations of putative phagostimulatory compounds such as glycine and L- and D-glutamicacid.

INFLUENCE OF ENTOMOPATHOGENIC NEMATODES ON *PARATYLENCHUS PENETRANS* AND *MELOIDOGYNE CHITWOODI.* **Santo, G. S.,[1] H. Mojtahedi,[1] and L. A. Lacey.[2]** [1]Washington State University, Prosser, WA 99350, and [2]USDA-ARS-YARL, Wapato, WA 98951.

Five entomopathogenic nematodes were evaluated for control of *Pratylenchus penetrans* and *Meloidogyne chitwoodi* in greenhouse tests. *Pratylenchus penetrans* and *M. chitwoodi* were added alone (1,500/500 g soil) and in combination with *Steinernema carpocapse* All and Sal strains, *S. feltiae*, *S. kraussei*, and *Heterorhabditis bacteriophora* (4.9 billion/ha) on 3-week-old alfalfa and tomato seedlings, respectively. The roots were harvested, stained with acid fuchsin, and infective nematodes were counted 3 weeks after inoculation with *P. penetrans* or *M. chitwoodi*. The number of *P. penetrans* and *M. chitwoodi* was decreased significantly ($P < 0.05$) when entomopathogenic nematodes preceded them by 2 weeks compared to simultaneous inoculations. Multiple applications of entomopathogenic nematodes further decreased ($P < 0.05$) *P. penetrans* and *M. chitwoodi*.

NUMBERS OF *HETERODERA SCHACHTII* EGGS/CYST OVER TIME UNDER NON-HOST CONDITIONS IN HAWAI'I. **Schmitt, D. P.,[1] B. S. Sipes,[1] D. Meyer,[1] and R. Shimabuku.[2]** [1]Department of Plant Pathology, University of Hawaii, Honolulu, HI 96822, and [2]Maui County–CES, Kahului Extension Office, Kahului, Maui, HI 96732.

Heterodera schachtii causes significant yield losses on cabbage in the Kula area on the island of Maui, Hawai'i. Nematode damage remains high because producers grow at least one crop of

cabbage per year in the same field. A management option is avoiding a host crop until the population of the nematode decreases below a damaging level. An experiment was established to determine the rate of decline over time in the numbers of cysts and eggs per cyst. Six plots were established along a transect across a commercial cabbage field from which cabbage had just been harvested. Two 5-cm diameter cores were collected from each plot at 0–15, 15–30, and 30–45 cm depths in the soil profile. Cysts were extracted by elutriation and centrifugal flotation. Cysts were counted and then each cyst was crushed to determine the number of eggs per cyst. There was considerable variation in numbers of cysts over eight months, but no evidence of a significant change in numbers. Eggs/cyst averaged 169 at the 0–15-cm level with little difference over time. Egg numbers decreased sharply from 280 eggs/cyst to 153 in the first month after cabbage in the 15–30-cm level, and gradually decreased to 111 during the following seven months. The change in population density in the 30–45-cm depth was similar to that of the 15–30-cm level. In conclusion, decline in egg numbers will likely require a very long absence of a host to reduce numbers below a damaging level.

INITIAL CHARACTERIZATION OF ENDOCHITINASE GENES OF THE PLANT-PARASITIC NEMATODE *HETERODERA GLYCINES.* **Schwekendiek, A.,[1] T. R. Maier,[1] C. R. Womack,[1] D. H. Byrne,[1] J. M. de Boer,[1] E. L. Davis,[2] and T. J. Baum.[1]** [1]Department of Plant Pathology, Iowa State University, Ames, IA 50011, and [2]Department of Plant Pathology, North Carolina State University, Raleigh, NC 27695.

In plant-parasitic nematodes, chitin, a β-1,4-linked N-acetylglucosamine, has been found only in the eggshell. Therefore, it is believed that chitinases may function during the hatching process. Here we report the identification of putative endochitinase genes and one corresponding cDNA, as well as the detection of endochitinase transcripts in various stages of the soybean cyst nematode (SCN) *Heterodera glycines*. Using a PCR-based approach, two distinct gene fragments were cloned from *H. glycines* genomic DNA. BLAST database searches revealed significant similarities to endochitinases of *Caenorhabditis elegans* and filarial nematodes. We named these putative endochitinase genes Hg-cht-1 and Hg-cht-2. Screening of a SCN second-stage juvenile (J2) cDNA library with probes derived from these genomic fragments lead to the identificaton of two cDNA clones of different lengths, but with identical nucleotide sequences in overlapping regions. The deduced amino acid sequence showed 50% identity to a putative endochitinase from *C. elegans* and 39% identity to an endochitinase precursor from *Brugia malayi*. Both of these known sequences are members of family 18 of glycosyl hydrolases. The best conserved consenus pattern around the active site glutamate-residue is common to all enzymes in this family and is also present in the SCN sequence. Hybridizations of endochitinase probes to blots of total *H. glycines* RNA from eggs, pre-parasitic J2, parasitic stages, and adult females did not detect endochitinase mRNA. Nevertheless, the apparently rare endochitinase messenger could be detected by RT-PCR analyses throughout the SCN life cycle. Furthermore, as shown by PCR experiments with SL1-specific primers, endochitinase transcripts appear to be trans-spliced. Taking all these findings together, there is strong evidence that endochitinases are expressed in *H. glycines* after hatching. Efforts are now made to identify the site of expression and to characterize these endochitinases on the enzymatic level.

SEASONAL PATTERNS OF *MELOIDOGYNE KONAENSIS* POPULATION DENSITIES IN COFFEE GENOTYPES AND IRRIGATION REGIMES IN HAWAI'I. **Serracin, M., D. P. Schmitt, and B. S. Sipes.** Department of Plant Pathology, University of Hawai'i, Honolulu, HI 96822.

Soil population densities of *M. konaensis* subjected to two irrigation regimes (continuous irrigation, natural rainfall), two cultivars of *Coffea arabica* ('Guatemala' and 'Catuai') and 'Guatemala' grafted on a *C. dewevri* rootstock were determined at selected intervals from July 1997 to February 1999. Nematode numbers differed among cultivars ($P < 0.001$) and seasons ($P < 0.001$),

with second-stage juveniles (J2) densities increasing when plants were maintained under continuous irrigation. However, total soil and root nematode population densities (J2 and eggs) measured in February 1999 were higher under natural rainfall ($P < 0.001$). More nematodes were found in the coffee roots than in soil ($P < 0.001$). Growth and flowering patterns of coffee genotypes were also influenced by nematode population densities. Flowering patterns, plant height, and nematode population densities were correlated among coffee cultivars, and irrigation regimes. This experiment examined the extremes on the effects of soil moisture on nematode behavior and plant response. The results demonstrated the need to refine timing and quantity of irrigation to reduce or suppress population development of the nematode and induce a regular flowering pattern of coffee trees.

FACTORS AFFECTING PATHOGENICITY OF AN EGYPTIAN STRAIN OF HETERORHABDITIS INDICUS (NEMATODA: HETERORHABDITIDAE) INFECTING THE EGYPTIAN COTTON LEAFWORM SPODOPTERA LITTORALIS (LEPIDOPTERA: NOCTUIDAE). **Shamseldean, M. M.,[1] M. M. Abd-Elgawad,[2] and A. A. Atwa.[1]** [1]Department of Agriculture, Zoology and Nematology, Faculty of Agriculture, Cairo University, and [2]Department of Plant Pathology, National Research Center.

An entomopathogenic nematode strain of *Heterorhabditis indicus* (EAS59) isolated from Southern Egypt was tested for single and combined effects of soil temperature, exposure time and host introduction. Differences among the three exposure times, 5, 18 and 60 hours, concerning the amount of infective juveniles produced at 25° and 35 °C were more pronounced than those produced at 10° and 30 °C. It was concluded that *H. indicus* (EAS59) reported herein, in terms of their reproductive efficiency, possesses better persistence at high temperature than other entomopathogenic nematodes. The significant interaction among the studied factors suggested that it is necessary to obtain information on a single factor over a wide range of other environmental factors.

TOMATO ROOT DIFFUSATE INDUCES PHYSIOLOGICAL CHANGES IN *GLOBODERA ROSTOCHIENSIS* J2S THAT ARE NOT INDUCED BY POTATO ROOT DIFFUSATE. **Sheridan, J.,[1] R. N. Perry,[1] J. Barrett,[2] D. Pattison,[3] and J. T. Jones.[4]** [1]Entomology and Nematology, IACR-Rothamsted, Harpenden, Hertfordshire AL5 2JQ, UK, [2]Institute of Biological Sciences, University of Wales, Aberystwyth SY23 3EB, UK, [3]Maxicrop International Ltd., Corby, Northants, UK, and [4]Nematology Department, Scottish Crop Research Institute, Invergowrie, Dundee DD2 5DA, Scotland.

Both tomato root diffusate (TRD) and potato root diffusate (PRD) stimulate hatch of second stage juveniles (J2s) of *G. rostochiensis*. Recent work has also shown that TRD induces physiological changes in J2s of *G. rostochiensis*, including an increase in transcriptional activity in the nucleolus of the dorsal oesophageal gland (DOG) cell. The effects of TRD and PRD on hatched and unhatched J2s of *G. rostochiensis* have been compared. Using acridine orange staining, it was possible to detect increased transcriptional activity in the DOG nucleolus in J2s hatched in TRD and unhatched J2s exposed to TRD for four days. By contrast, these effects were not detectable in J2s hatched in PRD or unhatched J2s exposed to PRD for four days. Molecular biological experiments, using a modified differential display technique, revealed that TRD induced many changes in gene expression when comparing hatched and unhatched J2s. Similar experiments comparing unhatched nematodes and those hatched in PRD revealed far fewer changes in gene expression. A factor in TRD, distinct from that which induces hatch, may be responsible for causing the physiological and molecular changes observed. In support of this hypothesis, a known inhibitor of hatch did not inhibit the physiological changes in unhatched J2s exposed to TRD for four days. It is feasible that these changes in parasite physiology are not induced in potato until invasion and/or feeding have occurred.

MICROBE–GRAZER–PREDATOR COMMUNITY DYNAMICS DURING ORGANIC MATTER DECOMPOSITION. **Shouse, B. N., and H. Ferris.** Department of Nematology, University of California, Davis, CA 95616.

Despite extensive knowledge of the physiological, population and trophic ecology of microbivorous nematodes and other soil fauna, the impact of these fauna on community and nutrient dynamics remains unpredictable. We used a novel "field incubator" technique to study populations of soil fauna and their effect on decomposing organic matter *in situ*. This technique combines the experimental control of the litter bag approach with more realistic contact between soil and decaying litter. Microbivorous nematodes were more abundant early in the decomposition process and fungal feeders (e.g., *Aphelenchus avenae*) were more abundant in the presence of high C/N ratio organic matter. Experimental exclusion of microarthropods (mostly mites and Collembola) led to reduced mass loss of leaf litter; it did not affect fungi, the primary prey of microarthropods, or populations of fungal feeding nematodes, their potential competitors. However, microarthropod exclusion was associated with elevated populations of predator/omnivore members of the Dorylaimida. Furthermore, in incubators from which microarthropods were not excluded, they were more abundant under the high C/N conditions that favor fungal decomposition. This suggests that microarthropods and predatory nematodes may be linked either directly via predation or indirectly via other members of the food web. The increase in the Dorylaimida is also interesting because it represents colonization by these supposedly sensitive nematodes relatively early in the decomposition process.

TURMOIL IN THE TROPICS: AN INTERDISCIPLINARY COURSE FOR THE NEXT CENTURY. **Sipes, B. S.,[1] C. Evensen,[2] and J. Uchida.[3]** [1]Department of Plant Pathology, [2]Department of Agronomy and Soil Science, and [3]Department of Plant Pathology, University of Hawaii, Honolulu, HI 96822.

Students have great difficulty in understanding the complex interactions between agricultural production systems, population growth, urbanization, and environmental quality. The political and popular discourse on these subjects are often polarized and contentious, rather than thoughtful and illuminating. We are developing a scientifically based course that provides pre-baccalaureate students with the foundation for making critical judgements concerning agriculture and the environment. The course is integrated with the World Wide Web (http://www.hawaii.edu/webct/) and employs electronic discussion groups to increase effectiveness and efficiency of instruction, as well as teaching students current technology. The objectives of the course are: to create a science-based, environmental issues course highlighting the interactions among agricultural production systems, human demands and environmental quality; to introduce and expose students to the many aspects of research, product development, environmental management and scientific activity that occur in land-grant colleges; and to provide students with a holistic perspective and appreciation for agriculture and environmental quality required by a growing population, for use as enlightened citizens. Case studies, discussion questions, and laboratory/field exercises assist students in learning and evaluating environmental and agricultural issues. The course, in its content and structure, can serve as a prototype for similar courses.

HOT WATER DISINFECTION FOR BURROWING NEMATODES. **Sipes, B. S.,[1] A. Hara,[2] C. Jacobsen,[2] and M. Tsang.[3]** [1]Department of Plant Pathology, University of Hawaii, Honolulu, HI 96822, [2]Department of Entomology, University of Hawaii, Honolulu, HI 96822, and [3]College of Agriculture, University of Hawaii, Hilo, HI.

The palms *Chamaedorea elegans* and *C. seifrizii* and anthurium *Anthurium andraeanum* previously infected with *Radopholus similis* were subjected to several hot-water treatments replicated six times. Bare rooted palms were placed in 50 °C water for 15 min and cooled 15 min in ambient water or placed in ambient water for 30 min. The anthurium treatments were 1) bare roots in a 22-min ambient water dip, 2) bare roots in a 15-min 50 °C water dip with a 7-min ambient dip, 3)

potted plants in a 15-min 50 °C water dip with a 7-min ambient dip, 4) potted plants drenched for with 50 °C water for 15 min, or 5) potted plants drenched for with 50 °C water for 20 min. The bare rooted plants were repotted. All plants survived the treatments. Four months later, all plants were assayed for burrowing nematodes. Two 12-g palm root samples, a 20-g anthurium root and 50-g anthurium shoot sample for each replication was placed in a mist chamber for five days. In the palms, *R. similis* was eliminated in four out of six plants receiving the hot-water treatment. In anthurium, *R. similis* was eliminated in all of the hot-water treated plants. Untreated *C. elegans* and *C. seifrizii* had 12 and 14 nematode/g fresh root, respectively. The treated palms averaged less than 1 nematode/g fresh root. Untreated anthurium had 34 and 5 nematodes/g dry root and dry shoot, respectively. Hot-water treatment provides a management tool for eliminating *R. similis* in infected plants with applicability for quarantine and clean planting material.

EVALUATION OF NON-CHEMICAL APPROACHES FOR MANAGEMENT OF PHYTONEMATODES IN SUGAR CANE ECOSYSTEM. **Somasekhar, N., and U. K. Mehta.** Nematology Section, Sugarcane Breeding Institute, Coimbatore, India.

Phytonematodes are one of the major biotic constraints for sustainable sugar cane production in many parts of world. Conventional nematode management using synthetic nematicides has become environmentally and economically unsound in the sugar cane ecosystem due to removal of most efficacious nematicides from world market on environmental grounds and the need for heavy doses and/or multiple applications of available nematicides in sugar cane crop owing to its long duration and multiple ratooning features. This forced us to look for sustainable alternatives to nematicides. Hence, non-chemical approaches viz. recycling of sugar cane wastes (pressmud @ 25t/ha, cane trash @ 5t/ha), green-manuring with sunnhemp, *Crotalaria juncia*, application of oil cakes (neem cake @ 2t/ha, caster cake @ 2t/ha) and fungal biocontrol agent, *Trichoderma viride* were evaluated for suppression of phytonematodes infecting sugar cane in comparison to synthetic nematicide, carbofuran3G (3 kg a.i./ha) under field conditions in randomized block design for two years. All the treatments gave significantly high cane yield, sugar yield and reduction in nematode population as compared to the untreated control in both the years. Maximum increase in cane yield, sugar yield and reduction in nematode population was recorded with pressmud followed by carbofuran in both the years.

LIFE CYCLE OF *NACOBBUS ABERRANS* (NEMATA: PRATYLENCHIDAE) UNDER CONTROLLED GROWTH CONDITIONS. **Souza, R. M., and J. G. Baldwin.** Department of Nematology, University of California, Riverside, CA 92521.

The biology of *Nacobbus aberrans* is poorly understood, including the behavior of third- (J3) and fourth- (J4) stage juveniles under suboptimal conditions. The life cycle of *N. aberrans* was followed in tomato plants grown in soil-less pouches under continuous, optimal conditions (illumination of 14,000 or 11,000 LUX for 16 hours/day, temperatures of 25–26 °C in the light period, and 17–22 °C in the dark period). Fuchsin-stained roots were examined every five days until 50 days after inoculation. To assess the survival of J3 and J4, the optimal conditions were interspersed with suboptimal conditions (14,000 LUX for 10 hours/day, temperatures of 15 °C in the light period and 10 °C in the dark period), while those life stages predominated in the population. The results suggested that even under optimal conditions a fraction of the J4 undergoes obligate quiescence. Both J3 and J4 withstood the suboptimal conditions tested, but differences in behavior suggested that J4, relative to J3, is the preferential survival stage of *N. aberrans*.

TESTING THE GOLGI COMPLEX-SPECIFIC PROBE BODIPY-FL C5-CERAMIDE AS A TOOL TO STUDY THE ESOPHAGEAL GLANDS OF *NACOBBUS ABERRANS* (NEMATA: PRATYLENCHIDAE). **Souza, R. M., and J. G. Baldwin.** University of California, Department of Nematology, Riverside, CA 92521.

To date, *Meloidogyne* and *Heterodera* (Heteroderidae) are the only genera of plant-parasitic nematodes in which molecular approaches have been incorporated into the study of esophageal

glands (EG). Previous results on the developmental biology of the EG of several developmental phases of *Nacobbus aberrans*, obtained with light and electron microscopy, were the reference to test the Golgi complex-specific fluorescent probe, Bodipy-FL C5-ceramide, as a more dynamic tool to assess EG activity in *N. aberrans*, and to extend assessment to other Tylenchida. Eleven developmental phases of *N. aberrans* life history were stained with Bodipy-FL C5-ceramide, using a variety of protocols, and examined with fluorescence and light microscopes. Only the developmental phases in which the EG were known to be active were stained by Bodipy-FL C5-ceramide, with the exception of active EG of males and sedentary females. Such failure seems to disqualify Bodipy-FL C5-ceramide as a reliable probe for studies on EG in nematodes.

FIELD EVALUATION OF *MUSA* ACCESSIONS TO NEMATODE DAMAGE IN UGANDA. **Speijer, P. R., and F. Ssango.** Eastern and Southern Africa Regional Center (ESARC), International Institute of Tropical Agriculture (IITA) Kampala, Uganda.

Twenty-five *Musa* accessions were evaluated in the field for their host plant response to *Radopholus similis* and *Helicotylenchus multicinctus* at Namulonge, Uganda. Suckers detached from first- and second-crop cycles of harvested plants, grown in nematode infested and non-infested plots, were assessed for both root and rhizome damage and nematodes extracted from roots. High correlation coefficients were observed between nematode counts and damage indices ($r > 0.74$, $P < 0.001$). Principle component analysis (PCA) was used to establish the accessions, susceptibility and sensitivity using nematode densities and damage indices. A mixed model analysis of principle score1, which explained 67% of the total variation in the data set, was used to separate the respective accessions using Prin1 score. Three major groups were displayed using zero score as the reference point. Eight accessions including Valery were in the most susceptible and damaged group, while 14 accessions, including the group of highland bananas, were intermediate, and three were least susceptible and damaged, which included Pisang Awak, Sukali Ndizi and Gros Michel, respectively.

EVALUATION OF *MUSA* LANDRACES AND HYBRIDS FOR NEMATODE RESISTANCE AND TOLERANCE IN SOUTHEASTERN NIGERIA. **Speijer, P. R.,[1] A. Tenkouano,[1] T. Dubois,[2] B. De Schutter,[3] and D. De Waele.[4]** [1]International Institute of Tropical Agriculture (IITA), Croydon, UK, [2]IITA, Croydon, UK, and Field of Entomology, Cornell University, Ithaca, NY 14853, [3]IITA, Croydon, UK, and [4]Laboratory of Tropical Crop Improvement, Catholic University of Leuven, Heverlee, Belgium.

Fifteen *Musa* accessions were evaluated in the field for their host–plant response to a species mixture of *Radopholus similis, Helicotylenchus multicinctus, Helicotylenchus dihystera, Hoplolaimus pararobustus* and *Meloidogyne incognita*. The plants were grown in nematode infested and non-infested (nematicide-treated) plots. Production loss comparing infested and non-infested plots was estimated using bunch weight reduction and loss of bunches as a result of plant toppling. Production of the *Musa* genotypes Cardaba, SH3640, Pisang Ceylan, Yangambi km5, FHIA-3 and FHIA-1 was reduced up to 26% in the infested plots compared to the non-infested plots. FHIA-23, TMPx-548-9, Bluggoe, SH3436-9, FHIA-22, TMPx 2796-5, Valery, Obino l'Ewai and Mimi Abue suffered losses ranging from 46% to 86%. Suckers were detached from first cycle, harvested plants and assessed for root and rhizome damage. Nematodes were extracted from the assessed roots. Relationships between root damage parameters and nematode population densities were examined using correlation and principal component analysis. Damage indices were constructed by means of the communalites of the performed factor analysis. Major contribution to the damage index was from percentage dead roots, percentage root necrosis, percentage root bases with lesions and *R. similis* densities. A low damage score was related to a low production loss, with the exception of the genotypes Cardaba and SH3640. For these two genotypes, possibly a hypersensitive resistance response occurs.

PATH ANALYSIS OF NEMATODE DENSITIES, NEMATODE AND WEEVIL DAMAGE INDICES IN RELATION TO BUNCH WEIGHT OF EAST AFRICA HIGHLAND BANANA (*MUSA* AAA-EA) UNDER TWO-FIELD MANAGEMENT REGIMES. **Ssango, F., and P. R. Speijer.** Eastern and Southern Africa Regional Centre (IITA–ESARC), International Institute of Tropical Agriculture, Kampala, Uganda.

Path analysis was used to establish the relative influence of *Radopholus similis* and *Helicotylenchus multicinctus* densities, nematode damage (percentages dead roots, root necrosis and root bases with necrotic lesions) and banana weevil-related damage (percentages outer and inner corm damage) on bunch weight of highland banana (cv Mbwazirume, *Musa* AAA). The highland banana was grown under two management regimes. Either the plots were kept weed-free and heavily mulched or plots were inter-cropped with finger millet. Nematode densities ($r > -0.59$, $P < 0.05$) and damage parameters ($r > -0.76$, $P < 0.01$), independent of crop management, were negatively correlated to bunch weight. Under good management, banana weevil damage was negatively correlated with nematode densities and damage ($r > 0.70$, $P < 0.05$). However, no such association was observed under poor management. Path analysis revealed that root necrosis, followed by dead roots, are the major factors negatively affecting bunch weight in well-managed plots. While in the finger millet inter-cropped plots, *R. similis* densities, followed by *H. multicinctus* densities, directly affected the bunch weight.

PHYLOGENY OF THE GENUS *STEINERNEMA* (NEMATODA: STEINERNEMATIDAE): MOLECULES AND MORPHOLOGY. **Stock, S. P.,[1] J. F. Campbell,[2] and S. A. Nadler.[1]** [1]Department of Nematology, University of California, Davis, CA 95616, and [2]GMPRC, ARS, USDA, Manhattan, KS.

Phylogenetic relationships of 21 of the 24 known species of the genus *Steinernema* were inferred based on morphological data and 28S rDNA sequences. Morphological analysis was based on 28 characters from first generation adults and third-stage infective juveniles. Molecular analysis was based on 867 nucleotides of nuclear large subunit sequence (including the D2 and D3 domains). The morphological characters were analyzed by parsimony, which yielded seven trees of length 127 (C.I. 1.0, although strict consensus had minimal resolution). The sequence characters were analyzed by parsimony and likelihood methods; molecular trees were generally consistent with traditional taxonomic expectations. Morphological and molecular data were also combined in a parsimony analysis, and this tree was used to develop hypotheses for the evolution of morphological characters. The value of molecular and morphological data for inferring relationships among *Steinernema* species is evaluated.

SUITABILITY OF SELECTED CROP CULTIVARS AS HOSTS FOR *HOPLOLAIMUS COLUMBUS* SHER IN THE GREENHOUSE. **Supramana, S., and S. A. Lewis.** Department of Plant Pathology and Physiology, Clemson University, Clemson, SC 29634.

Hoplolaimus columbus Sher is one of the most important pathogens of soybean in the Coastal Plains of North Carolina, South Carolina and Georgia. Previous experiments indicated a wide variety of hosts, so that the potential of rotation for managing the nematode was limited. The success in culturing this nematode species both in excised root cultures and in the greenhouse, however, will lead to more precise and effective experiments to assess host status of crops, cultivars and genotypes. Ten crops and seven soybean cultivars were assessed for suitability as hosts of this nematode species. These were 'Wrangler' alfalfa, 'Jackson' lima bean, common bermudagrass, 'Pioneer 3163' corn, 'Delta Pine 90' cotton, 'Hutcheson' soybean, 'pioneer XS 530' sorghum, 'Coker 9835' wheat, and 'Rutgers' tomato. They were arranged in a completely randomized design with six replications. The experiment was conducted by using 1-liter plastic pots with pasteurized river bottom sand and gravel (7.5:2.5 v/v) that were kept at 30 °C in a Wisconsin water bath in the greenhouse. Nematodes from soybean excised root culture were used as inoculum and the final population was assessed after 90 days by extraction of the nematodes in a mist chamber for five

days. A similar experiment was conducted using seven soybean cultivars, including Braxton, Bryan, Centennial, Coker 368, Hagood, Hutcheson, and Perrin. 'Georgia Green' peanut was used as a negative control. Soybean and lima bean were the best hosts, with 680 and 619 Columbia lance nematodes per gram root dry weight, respectively. Cotton, alfalfa and sorghum were good hosts. Corn, wheat and bermudagrass were poor hosts. Soybean cultivars varied in host suitability, with 'Braxton' being more susceptible than the other cultivars. Final populations after 40 days were 1,843 on Braxton, 1,250 on Hagood, 1,188 on Hutcheson, 1,060 on Perrin, 983 on Coker 368, 887 on Bryan, 797 on Centennial, and 23 on peanut. Tomato and peanut were non-hosts, which was consistent with field reports. These results indicate relative host status of important regionally grown crops and indicate differences in host status among cultivars of soybean.

CULTURING *HOPLOLAIMUS COLUMBUS* SHER ON SOYBEAN EXCISED ROOT CULTURE. **Supramana, S., and S. A. Lewis.** Department of Plant Pathology and Physiology, Clemson University, Clemson, SC 29634.

Hoplolaimus columbus Sher is a serious pathogen of soybean in the Coastal Plains of North Carolina, South Carolina and Georgia. Difficulty in producing large quantities of this species has become the major impediment to conducting research on it. Experiments were established to evaluate the effect of temperature and initial population on final population numbers and to compare the pathogenicity of the cultured nematodes to field populations. 'Hutcheson' soybean seed were sterilized by soaking in 95% ethanol, followed by 1.3% sodium hypochlorite each for five minutes. The seed were germinated in 1.0 % water agar. Root sections 30 mm in length were transferred to solidified Gamborg's B5 medium (without auxin and cytokinin, pH 5.8) in 1.3% water agar. The nematodes were sterilized in 0.5 % streptomycin sulfate and 0.5% chlorhexidine diacetate each for five minutes, respectively. Ten nematodes were transferred onto 7-day-old cultures in 9-cm plastic petri plates and kept inverted in an incubator. The nematode's final population was recovered 90 days after inoculation. In the first experiment, with seven replications, nematode cultures were grown at 23°, 25°, 27°, 29°, 31° and 33 °C. The optimal temperature was 31 °C. Five initial populations of 10, 20, 30, 40, and 50 nematodes per plate were grown at 30 °C, with five replications. Ten nematodes per plate resulted in the highest reproduction factor. The final set of experiments was conducted in 30° C Wisconsin water tanks in the greenhouse. Nematodes from soybean excised culture and fresh nematodes extracted from field soil were used as inoculum sources. One hundred nematodes were infested into a 1-liter plastic pot containing sterile river bottom sand with 'Hutcheson' soybean. After 90 days, the average final population of nematodes from culture was 3,150 compared with 3,416 for field soil inoculum. This indicates that cultured nematodes are comparable in reproductive fitness to nematode inoculum derived from field soil. Moreover, nematodes from sterile culture are less likely to be contaminated by other species.

CHARACTERIZATION OF RESISTANCE TO *MELOIDOGYNE ARENARIA* RACES 1 AND 2, *M. HAPLA* AND *M. JAVANICA* CONFERRED BY THE N GENE IN PEPPER (*CAPSICUM ANNUUM*). **Thies, J. A., and R. L. Fery.** U.S. Vegetable Laboratory, ARS, USDA, Charleston, SC 29414.

Root-knot nematodes (*Meloidogyne incognita*, *M. arenaria races* 1 and 2, *M. hapla* and *M. javanica*) are major pests of bell peppers in the USA and world-wide. The N gene conditions resistance to *M. incognita* in the recently released bell pepper cultivars Charleston Belle and Carolina Wonder (USDA, ARS, 1997). However, it is unknown whether the N gene also confers resistance to the other major root-knot nematode species. We characterized Charleston Belle and Carolina Wonder (both NN) and their respective recurrent backcross parents, Keystone Resistant Giant and Yolo Wonder (both nn), for resistance to *Meloidogyne arenaria* races 1 and 2, *M. hapla* and *M. javanica* in greenhouse and growth chamber tests. Charleston Belle and Carolina Wonder exhibited high resistance to *M. arenaria* race 1, and Keystone Resistant Giant and Yolo Wonder B were susceptible. Although *M. arenaria* race 2 and *M. javanica* are not highly pathogenic to

pepper, Charleston Belle and Carolina Wonder both exhibited higher ($P < 0.05$) resistance to *M. arenaria* race 2 and *M. javanica* than Keystone Resistant Giant and Yolo Wonder B. All four cultivars were susceptible to *M. hapla*. We concluded that the N gene conditions resistance to *M. arenaria* races 1 and 2 and *M. javanica*, but not to *M. hapla*.

EFFICIENCY OF CHITINOLYTIC BACTERIA WITH DIFFERENT LEVELS OF CHITIN SUBSTRATE FOR CONTROL OF *HETERODERA GLYCINES* ICHINOHE. **Tian, H., R. D. Riggs, and D. L. Crippen.** Department of Plant Pathology, University of Arkansas, Fayetteville, AR 72701.

Our previous study revealed that five chitinolytic bacterial isolates consistently reduced the population level of soybean cyst nematode (SCN), *Heterodera glycines*, when pasteurized, sandy soil was amended with 0.6% (w/w) chitin substrate. In this research, the same isolates were tested with lower levels of chitin substrate for their effects on the reproduction of SCN and plant growth in a greenhouse. When no chitin or 0.01% chitin was added into the soil, the five isolates had no effect on either SCN reproduction or plant growth. At 0.3% chitin level, none of the isolates affected SCN population level, but two of them increased plant height compared with the control, which had nothing added to the soil (blank control). When chitin level was increased to 0.4% or 0.5%, all isolates significantly decreased SCN reproduction compared with either blank control or with chitin addition only ($p = 0.05$). The effects of different isolates on plant growth varied from positive to negative. The results indicated that the five bacterial isolates function as effective biocontrol agents only when relatively high levels of chitin are incorporated into the soil.

EFFECT OF CROP ROTATION AND NEMATICIDE USE ON ABUNDANCE OF *PASTEURIA PENETRANS*. **Timper, P.** ARS, USDA, Coastal Plain Experimental Station, Tifton, GA 31793.

The objective of this study was to determine the effect of rotation and nematicide use on abundance of *Pasteuria penetrans*, an obligate parasite of nematodes. An experiment was initiated in 1991 with the main plot treatments being peanut following two years of either bahia grass, corn or cotton, and continuous peanut, corn and cotton. The subplots were either treated at-plant with nematicide (aldicarb or ethoprop) or untreated (control). There were 4 and 12 replicate plots for the continuous and rotated crop sequences, respectively. The field site contained an unknown level and distribution of *P. penetrans* prior to the start of the experiment. Because spores of *P. penetrans* were frequently found on juveniles of *Meloidogyne arenaria*, this nematode was considered the primary host for the parasite. In July 1998, soil was collected from each plot and a subsample (100 cm^3) was assayed for *P. penetrans* spores. Greenhouse-cultured *M. arenaria* juveniles were utilized as assay nematodes. The mean number of spores acquired by assay nematodes after being shaken in a soil:water slurry for 24 h was used to estimate abundance of *P. penetrans*. The number of spores per assay nematode was highest in the continuous peanut sequence (22), intermediate in the peanut–bahia grass sequence (13), low in the peanut–corn sequence (2), and very low in all other sequences (< 1). Abundance of spores was similar in the nematicide-treated and untreated plots. When the soil from each cropping sequence was tested in the greenhouse for reproduction of *M. arenaria* on peanut, there was a negative correlation between the number of nematode eggs produced and the abundance of *P. penetrans* spores detected [log(eggs) = 4.2-0.048 (spores), $P = 0.006$, $R = 0.59$].

SAPROPHYTIC GROWTH OF A STERILE FUNGUS (ARF) UNDER DIFFERENT CONDITIONS. **Timper, P.,[1] R. D. Riggs,[2] and D. L. Crippen.[2]** [1]Coastal Plain Experimental Station, ARS, USDA, Tifton, GA 31793, and [2]Plant Pathology Department, University of Arkansas, Fayetteville, AR 72701.

A sterile fungus designated ARF is a facultative parasite of the soybean cyst nematode, *Heterodera glycines*. The objectives of this study were to compare saprophytic growth among isolates of ARF, and to compare growth in the presence and absence of soybean plants and soil microor-

ganisms. ARF produces mycelial mats in soil, which can be used as an indicator of saprophytic growth. Two experiments were conducted, both in the absence of *H. glycines:* in the first, biomass of ARF isolates was compared in heat-treated soil with and without Lee 74 soybean, and in the second, biomass of isolates was compared in heat-treated and native (non-heated) soil. The dry weight of mats extracted from soil by wet sieving was compared in the different treatments 10 days after mixing homogenized mycelium into soil. In both experiments, the isolates that were previously shown to be more effective in reducing *H. glycines* numbers in soil usually produced a greater biomass of mats than did isolates that were less effective. Soybean roots had no consistent effect on the biomass of mycelial mats. Mats were sometimes associated with the rhizosphere, but most were recovered from the bulk soil. There was a positive correlation ($P = 0.0001$, $R = 0.66$) between the total biomass of mats and the number associated with roots indicating that isolates producing more or larger mats in soil have a greater probability of contacting roots. In the second experiment, biomass of mycelial mats was 2× greater in heat-treated than in native soil in one trial and similar in another trial. There were no isolate by plant or soil treatment interactions.

INDUCTION AND REPRESSION OF RESPECTIVELY DORSAL AND SUBVENTRAL PHARYNGEAL GLANDS DURING ONSET OF PARASITISM IN HETERODERA SCHACHTII. **Tytgat, T.,[1] J. De Meutter,[2] M. Claeys,[1] G. Gheysen,[3] and A. Coomans.[1]** [1]Institute of Zoology, [2]Department of Genetics, and [3]Department of Genetics, and Faculty of Agricultural and Applied Biological Sciences, University of Ghent, Belgium.

According to the current hypothesis, syncytium induction in plant roots by cyst nematodes is caused by secretions from the pharyngeal glands. We investigated the pharyngeal gland activities in *H. schachtii* during several stages of J2 juvenile development with DIC-microscopy and TEM. In freshly hatched, second-stage juveniles, the two subventral as well as the dorsal pharyngeal gland had a large cell body, with a clearly active nucleus and well-developed, rough endoplasmic reticulum and Golgi apparatus. A hugh number of secretory granules was present in the cytoplasm of the subventral glands. Secretory granules in the dorsal gland were smaller and not so numerous. The first day after root penetration, the dorsal gland had noticeably increased in size, and secretory granules were more numerous. In contrast, the subventral glands became smaller, but secretory granules could still be observed all over the cytoplasm. At day 3 after root penetration, the subventral glands were remarkably decreased in size, and no or very few small secretory granules could be found. The dorsal gland was further increased in size. These observations indicate that the subventral gland secretions only have a function during the first two or three days of the parasitic J2 juvenile stage. Probably, they are involved in root penetration. It is not clear whether the induction of the syncytium is caused by secretions from the dorsal or the subventral pharyngeal glands, but maintenance of the syncytium and formation of the feeding tubes is almost surely done by dorsal gland secretions. The fact that the dorsal pharyngeal gland was already active immediately after hatching is in contrast with published observations in root-knot nematodes.

RECOMBINATION BETWEEN GENES CONTROLLING HEAT-STABLE RESISTANCE TO *MELOIDOGYNE* IN *LYCOPERSICON PERUVIANUM* CHOTANO-HUMIFUSUM RACE. **Veremis, J. C., and P. A. Roberts.** Department of Nematology, University of California, Riverside, CA 92521.

Heat-stable resistance to root-knot nematodes (*Meloidogyne* spp.) was identified in ancestral races of wild tomato (*Lycopersicon peruvianum*). Accessions of *Lycopersicon peruvianum* Chotano-humifusum race accessions LA2157 and LA2334 and their F_1 and F^2 progenies were screened for genotype specific resistance to *Meloidogyne* spp. All individuals of accessions LA2157 and LA2334 and their F_1 hybrids were resistant to Mi-avirulent *Meloidogyne* spp. at 32 °C, indicating that the accessions were inbred homozygous for the heat-stable resistance. The inheritance of this heat-stable resistance was evaluated in F_2 progenies derived from hybrids of LA2334 × LA2157. The F_2 progenies segregated 15:1 (R:S) with *M. incognita* at 32 °C, indicating the presence of two

independent dominant genes. Thus, within the *L. peruvianum* Chotano-humifusum race, the heat-stable resistance in accession LA2157 is different from the heat-stable resistance in accession LA2334 even though both originate from the same geographical region. The inheritance of the heat-stable resistance from LA2157 was evaluated in a segregating F_2 progeny derived from a hybrid of LA392 (homozygous susceptible) × LA2157. The position of the novel heat-stable resistance of LA2157 was localized in the resistance genes cluster close to the location of gene Mi-1. Characterization of the heat-stable resistance locus from LA2157 will clarify its relationship to the Mi-1 gene and to the other novel resistance genes in accession LA2334 and in *L. peruvianum* Chamaya-Cuvita accessions LA1708 and LA2172.

CHANGES IN DIVERSITY AND POPULATION SIZE OF BACTERIAL SPECIES IN *GALLERIA MELLONELLA* LARVAE INFECTED WITH THE ENTOMOPATHOGENIC NEMATODES, *STEINERNEMA FELTIAE* AND *S. GLASERI.* **Walsh, K., and J. Webster.** Centre for Pest Management, Department of Biological Sciences, Simon Fraser University, Burnaby, British Columbia, Canada.

It has been widely assumed that insect cadavers infected with the entomopathogenic nematodes, *Steinernema* spp., contain a monoxenic culture of the symbiotic bacterium (i.e., *Xenorhabdus* spp.) specific to the nematode species. Experiments that we have done provide evidence that this is not always the case. Extracts (1.0 ml) from surface sterilized, macerated larval cadavers of the Greater Waxmoth, *Galleria mellonella*, infected with either *Steinernema feltiae* A21 strain (associated with *Xenorhabdus nematophilus*) or *S. glaseri* (associated with *X. poinarii*), were collected at successive time intervals up to 200 h post- infection. Each larval extract sample was serially diluted and 150 mL was spread over the surface of tryptic soy agar plates containing bromothymol blue. After incubating at 25 °C for 72 h, the number and type of bacterial colonies were recorded. The species diversity and population size of the bacteria from the plated extracts differed depending on the species of entomopathogenic nematode infecting the *G. mellonella* larvae. In larvae infected with *S. feltiae* low numbers of a bacterium other than *X. nematophilus* persist in the insect cadaver for up to 54 h post-exposure to the nematode. At 140 h, a different non-*Xenorhabdus* species appeared and persisted in the presence of *Xenorhabdus* bacteria for at least 60 h. In Galleria infected with *S. glaseri*, a bacterium other than *Xenorhabdus poinarii* persisted for up to 29 h post-nematode infection, but the population then crashed as the *Xenorhabdus poinarii* population increased. The biological significance of these population changes is not fully understood.

INTERACTIONS BETWEEN NEAR-ISOGENIC SOYBEAN LINES AND SOYBEAN CYST NEMATODE IN FIELD MICROPLOTS. **Wang, J.,[1] J. Mudge,[2] R. Denny,[3] N. D. Young,[1] and T. L. Niblack.[1]** [1]Department of Plant Pathology, University of Missouri, Columbia, MO 65211, [2]Plant Breeding Graduate Program, and [3]Department of Plant Pathology, University of Minnesota, St. Paul, MN.

Assessment of the performance of soybean lines that are resistant or susceptible to *Heterodera glycines*, the soybean cyst nematode, may be confounded by inherent cultivar differences. We report herein the first field assessment of two near-isogenic soybean lines (NILs) that differ for one gene for resistance to *H. glycines*, the rhg1 locus on linkage group G, which is associated with resistance to race 3 populations. The two NILs were developed in an 'Evans' background: with (+) and without (−) rhg1 derived from 'Peking.' These lines were grown in field microplots infested with a range (0 to 31,000 eggs/100 cm^3 soil) of initial population densities (Pi) of *H. glycines* race 3. Equivalent ranges were assigned to each of the two NILs. The plants were assessed by destructive sampling three times during the growing season for leaf, stem, pod and seed weights, and plant height. The experiment was conducted for two years. Data were analyzed by regression. The + and – NILs did not differ consistently in any of the measurements over the entire range of Pi; however, at low Pi, the + NIL had higher seed weight at harvest. These data were consistent with the observation that significant reduction in seed weight due to *H. glycines* parasitism is not

necessarily associated with a symptom such as stunting. In greenhouse experiments, no differences were observed in the numbers of second-stage juveniles or later developmental stages that infected the + and – NILs. Analysis of final population densities (Pf) of *H. glycines* in microplots revealed that population changes (Pf/Pi) were dependent on Pi and were unaffected by soybean line. The observation that *H. glycines* development and reproduction was similar on both lines, coupled with the observation that the effect of rhg1 in the field is measurable only at low Pi, is consistent with previous genetic analysis that showed that rhg1 by itself accounts for only a portion of the resistance expressed by cultivars containing this gene for resistance.

SUPPRESSION OF *ROTYLENCHULUS RENIFORMIS* IN TROPICAL COVER CROP–PINEAPPLE INTERCROPPING SYSTEM. **Wang, K.–H., and B. S. Sipes.** Department of Plant Pathology, University of Hawaii, HI.

The effects of intercropping pineapple *Ananas comosus* with cover crops to manage reniform nematode, *Rotylenchulus reniformis*, was investigated in a commercial plantation field on Oahu, Hawai'i. The suppressive effects of the following cover crops on *R. reniformis* were compared to weedy fallow and pineapple alone: Sunn hemp (*Crotalaria juncea*), rapeseed (*Brassica napus*), and marigold (*Tagetes erecta*). Population densities and mobility of *R. reniformis* in the soil were lower in cover crops and weedy fallow plots as compared to pineapple alone 11 months after planting ($P = 0.05$ and 0.01, respectively). When cowpea (*Vigna unguiculata*), a good host for *R. reniformis*, was planted in soil collected from plots with different teatments, the fewest *R. reniformis* vermiforms and eggs were recovered from soil previously planted with *C. juncea*. Although *C. juncea, B. napus* and weedy fallow had similar effects on suppressing *R. reniformis* densities, *Meloidogyne* spp. gradually increased to a higher level on *B. napus* and weedy fallow than on pineapple and other cover crops tested ($P = 0.05$). *C. juncea* suppressed *R. reniformis* reproduction and enhanced bacteriovorous nematode population densities as compared to pineapple ($P = 0.01$ and 0.05, respectively). Network-forming nematode trapping fungi were detected, but their presence was not different among the soil treatments.

DIVERGENCE OF RACE NUMBERS OF SINGLE FEMALE ISOLATES DERIVED FROM POPULATIONS OF SEVEN RACES OF *HETERODERA GLYCINES.* **Wang, S., and R. D. Riggs.** Department of Plant Pathology, University of Arkansas, Fayetteville, AR 72701.

Race shifts in population of soybean cyst nematode (SCN) are challenging control system with cultivars resistant to specific races in the field. Race shifts apparently result from selection pressure of host plants. To probe any genetic diversity within a race population, which may be responsible for race shifts, numerous single female isolates (SFIs) were derived from a population of each race of SCN, and SFIs were race tested. Only SFIs derived from a population of race 3 constantly tested to be the same race as the parent population. SFIs derived from populations of races 1, 4, 5, 6, 9 and 14 were identified as races other than that of the parent. Of 10 SFIs derived from a population of race 4, one was identified as race 9, one as race 10, two as race 6, and six as race 14. Of nine SFIs derived from a population of race 6, three were identified as race 9 and six as race 14. However, the majority of SFIs derived from populations of races 1, 5, 9 and 14 were identified as the same race as their parent populations. These results indicate a significant diversity of reproduction preference on the four differentials among individuals of the same race populations. This diversity may provide a good explanation of SCN race shifts and the variability of race tests.

HETERORHABDITIS BACTERIOPHORA: IS INFECTIVE JUVENILE LONGEVITY POSITIVELY CORRELATED WITH ABILITY TO TOLERATE ENVIRONMENTAL STRESS? **Wang, X., and P. S. Grewal.** Entomology Department, Ohio Agricultural Research and Development Center, Ohio State University, Wooster, OH 44691.

Infective juvenile longevity and tolerance to heat, hypoxicconditions, UV, and desiccation was compared among sixteen wild-type isolates of the entomopathogenic nematode *Heterorhabditis*

bacteriophora. Significant longevity differences were found among these isolates and they could be separated into long-, medium- and short-lived groups. Virulence of short-lived nematode isolates dropped quickly during advancing storage (aging). Considerable variation among these wild-type populations in heat, desiccation, hypoxia, and UV tolerance was detected. The long-lived group showed higher heat and UV tolerance, but greater tolerance to desiccation and hypoxia were not correlated with longer longevity. This may be due to different genetic mechanisms controlling longevity and tolerance to certain environmental stresses.

EXTRACTION OF CONTENTS FROM INDIVIDUAL GIANT CELLS AND SINGLE CELL RT-PCR ANALYSIS. **Wang, Z., R. Potter, and M. Jones.** W.A. State Agricultural Biotechnology Centre (SABC), Murdoch University, Perth, Western Australia.

Root-knot nematodes of the genus *Meloidogyne* have a very specific parasitic relationship with the plants they infect. The infection of nematodes induces the formation of giant cells in vascular tissues of roots. Giant cells have no central vacuole and are filled with metabolically active cytoplasm. They have characteristics of transfer cells, but are multinucleate. Based on a modified pressure probe system, we have successfully developed a rapid and direct method to extract cytoplasm from individual giant cells. Using a glass micropipette attached to the modified pressure probe, nanoliter quantities of giant cell cytoplasm can be extracted. The multinucleate nature of the cytoplasm has been confirmed by fluorescent staining of nuclei. The extracted giant cell cytoplasm has been used to carry out RT-PCR studies on gene expression of individual giant cells. The expression of two housekeeping genes, beta-actin and glyceraldehyde-3-phosphate dehydrogenase (GAPDH), and one cell membrane channel protein gene, RB7, were detected in giant cells contents by RT-PCR analysis. The expression of a series of other genes in giant cells is being studied. This approach provides a significant advance in studying metabolism and function of giant cells.

LONG-TERM STUDY TO ASSESS THE VALUE OF SWITCHGRASS FOR THE MANAGEMENT OF *MELOIDOGYNE ARENARIA* IN PEANUT. **Weaver, C. F., R. Rodríguez–Kábana, and D. G. Robertson.** Department of Plant Pathology, Auburn University, Auburn, AL 36849.

The value of switchgrass (*Panicum virgatum*) as a rotation crop for the management of *Meloidogyne arenaria* in Florunner' peanut (*Arachis hypogaea*) was assessed in a six-year (1992–97) field study in Southeast Alabama. Cropping systems included monoculture peanut untreated and treated with the nematicide aldicarb (3 lbs a.i./A at-plant), one and two years of switchgrass followed by peanut treated and untreated, and one and two years of 'EDPL-90' cotton (*Gossypium hirsutum*) followed by untreated peanut. All of the above cropping systems were also with and without treatment of the fungicide Folicur 3.6F applied four times at two-week intervals during the peanut growing season. Switchgrass and cotton were non-hosts for *M. arenaria*. Nematicide had no significant effect on juvenile populations of *M. arenaria*. One and two years of switchgrass did not suppress *M. arenaria* in peanut. One and two years of cotton did suppress *M. arenaria* in peanut by 30% and 50%, respectively. Nematicide had no significant effect on yield. In peanut without Folicur, one and two years of switchgrass did not increase yield. Folicur-treated peanut resulted in a 25% increase in yield in monoculture. Folicur-treated peanut preceded by one and two years of switchgrass increased yield 47% and 56%, respectively, when compared to untreated monoculture. Peanut preceded by two years of cotton provided a 63% increase in yield compared to untreated monoculture.

POPULATION FLUCTUATIONS OF RING NEMATODE *CRICONEMELLA XENOPLAX* IN CALIFORNIA PRUNE ORCHARDS. **Westerdahl, B. B.,[1] C. A. Anderson,[2] R. Buchner,[3] J. Edstrom,[4] B. Krueger,[5] B. Olson,[6] S. Southwick,[1] and J. T. Yeager.[2]** [1]Department of Nematology, University of California, Davis, CA 95616, [2] Department of Pomology, University of California, Davis, CA 95616, [3]University of California Cooperative Extension (UCCE), Red Bluff, CA, [4]UCCE, Colusa, CA, [5]UCCE, Orland, CA, and [6]UCCE, Oroville CA.

Sampling of ring nematode for three years in five California prune orchards indicated that peak nematode numbers occurred during the summer at or shortly before harvest with a rapid decline thereafter. Additional smaller peaks occurred in the winter and fall. Samples were taken from 0 to 31 cm and 31 to 62 cm deep. The number of nematodes appears to be greater at the 0 to 31 cm depth in the summer months, with greater numbers being present at the 31 to 62 cm depth in the fall and winter. Nematode numbers were lowest before an irrigation and sharply increased after an irrigation. No differences in nematode recovery were evident between sampling with a shovel, a 5-cm diameter auger, or a 2.5-cm diameter tube. The optimum strategy for sampling to determine if ring nematode is present in a prune orchard would be: 1) at a depth of 0 to 31 cm, 2) shortly after an irrigation, 3) between June and August, and 4) with either a shovel, an auger or a sampling tube. Samples taken in the fall and winter are more likely to detect the presence of ring nematode if they are taken from 31 to 62 cm deep than at shallower depths.

CROPPING SEQUENCES IN *HETERODERA SCHACHTII*-SUPPRESSIVE SOIL. **Westphal, A., and J. O. Becker.** Department of Nematology, University of California, Riverside, CA 92521.

Cropping of nematode resistant cover crops to reduce *Heterodera schachtii* population densities is frequently used in Central Europe. This project examined the effect of *H. schachtii*-resistant crops on soil suppressiveness against the beet cyst nematode. In lathhouse and field trials, *H. schachtii*-suppressive soil was cropped to non-host wheat (*Triticum aestivum*), a resistant or susceptible cultivar of each sugar beet (*Beta vulgaris*) or oilseed radish (*Raphanus sativus* var. *oleiformis*) or was left fallow. Population densities of *H. schachtii* were monitored for two cropping periods in the lathhouse and for one season in the field. After termination of the lathhouse trial, the soil from each treatment was split into two parts. One half was additionally infested with *H. schachtii*, while the other one did not receive additional inoculum. Both parts were potted and cropped for two nematode generations with Swiss chard (*Beta vulgaris*). Nematode population densities remained low in all soils that were not additionally infested. After two consecutive crops of wheat and additional nematode infestation, population densities of *H. schachtii* increased significantly when cropped to Swiss chard, indicating a significant loss of soil suppressiveness. All other crops had no obvious effect on soil suppressiveness and beet cyst nematode population densities remained low. In a six-month field trial, *H. schachtii*-resistant sugar beets caused a significant beet cyst nematode population decline. Resistant cultivars may be useful in crop rotations in *H. schachtii*-infested fields to manage nematode populations without upsetting soil suppressiveness.

INDIRECT METHODS OF ASSESSING POTENTIAL FOR DAMAGE ON COTTON BY ROOT-KNOT NEMATODE. **Wheeler, T. A.,[1] H. W. Kaufman,[2] K. Siders,[3] P. Kidd,[4] and S. Searcy.[5]** [1]Texas Agricultural Experiment Station, Lubbock, TX 79408, [2]Texas Agricultural Extension Service, Lubbock, TX 79401, [3]Texas Agricultural Extension Service, IPM, Levelland, TX 79336, [4]Texas Agricultural Extension Service, Brownfield, TX 79316, and [5]Texas A&M University, Department of Agricultural Engineering, College Station, TX 77843.

Variable rate application of nematicides requires information on the spatial variability of nematodes. Intensive soil sampling is costly, so alternative techniques were assessed. A cover crop (corn) was planted one month prior to a cotton crop at two sites, and corn height was measured before planting cotton into the corn. Height was assessed by: hand measurements; a height sensor (developed by S. Searcy); and by infrared photography. Root-knot nematode mid-season population density was positively related to corn height at site 1, and negatively related to cotton yield at site 2. Corn height was negatively related with cotton yield at site 1, but had no relationship with cotton at site 2. Infrared photographs detected where soil texture changed from 80–89% sandy to 50% sand, which was also correlated with a decrease in root-knot nematode density. However, changes in infrared color shading were not always indicative of a change in corn height, since soil

texture changes or field slopes also affected the infrared coloring. A height sensor was designed based on two light bars, placed on a tool bar, behind a tractor, one row apart. A series of lightbeams at different heights were either stopped or passed to the second light bar, based on height of the plant. This strategy was successful on plants > 20 cm in height. The cover crop/height method did not replace the need for intensive soil assays.

FOSTHIAZATE AFFECTS HATCHING AND MOVEMENT OF THE POTATO CYST NEMATODE *GLOBODERA PALLIDA*. **Woods, S. R., and P. P. J. Haydock.** Crop and Environment Research Centre, Harper Adams University College, Newport, Shropshire, UK.

Granular formulations of oxime carbamates and organophosphates play an important role in the management of potato cyst nematodes (PCN), which cause annually an estimated 9% loss in UK potato production. A new non-fumigant organophosphate nematicide, fosthiazate (Nemathorin 10G™), has been approved for use in the UK for the control of PCN in potatoes. However, the method by which fosthiazate improves potato yield and reduces PCN multiplication is unknown. It has been widely reported that organophosphate and oxime carbamate nematicides applied at field rates do not kill nematodes directly in the soil, but act as nematostats effectively paralysing nematodes and disorientating them to such an extent that their ability to locate and invade a suitable host plant is impaired. This may not be the only mechanism involved, however, as suppression of nematode emergence from cysts (hatch) may also contribute to the overall efficacy achieved by a nematicide. This has been reported for other granular nematicides such as aldicarb and oxamyl, where concentrations above 1 ppm caused temporary inhibition of hatch. It is likely that the two mechanisms work together; suppression of hatch, the initial mechanism, followed by paralysis or disorientation of the juveniles that hatch when the nematicide soil concentration falls below that required to suppress hatch. Most work on nematicide mode of action has been done *in vitro*. However, poor estimates of nematode kill are achieved by such experiments and the only true estimate of nematode mortality (with non-fumigant nematicides) is to use the ratio of initial to final nematode population density. A pot experiment, where sequential sampling was used, measured the response of nematode hatch and root invasion to different soil concentrations of fosthiazate. Results showed that fosthiazate works initially by suppressing nematode emergence from cysts in the soil. The nematicide suppresses hatch until the concentration falls below 0.5mg kg^{-1} soil. Below this concentration hatching resumes. Other factors, such as the paralysis of hatched nematodes in the soil solution, are also involved.

PLANTING DEPTH AND NEMATICIDE INCORPORATION DEPTH AFFECT CONTROL OF THE POTATO CYST NEMATODE AND YIELD OF POTATOES. **Woods, S. R., and P. P. J. Haydock.** Crop and Environment Research Centre, Harper Adams University College, Newport, Shropshire, UK.

The potato cyst nematode is a serious pest of the UK potato crop causing an estimated 8–10% loss in yield each year. High population densities can reduce the economic viability of even the best potato growing soils. Whilst many growers have adopted an integrated management approach to this pest, granular nematicides such as fosthiazate (Nemathorin 10G™) are still important for maintaining potato yield and reducing nematode multiplication. Granular nematicides need to be thoroughly incorporated into the top 15 cm of the potato seed-bed to give economic returns on their use. However, the widespread use of stone and clod separators have caused a shift away from the traditional potato cultivation methods that achieved this depth of incorporation. Potato seed-beds are now cultivated to a greater depth, and seed tubers are planted and ridged up in one pass. These two factors may reduce nematicide efficacy as deep incorporation during soil tillage may dilute a nematicide in the soil to such an extent that it is below its minimum effective concentration. Planting and ridging up in one pass can lead to seed being placed at a depth of 25 cm in the ridge and potentially below nematicide-treated soil. A field experiment was done at Harper Adams to

investigate the effect of nematicide incorporation and seed tuber planting depth on the yield of the potato cultivar Estima and the population control of the potato cyst nematode *Globodera rostochiensis*. Nematicide was applied at commercial field rate and incorporated to three depths: shallow, which involved the broadcast of the nematicide on to the soil surface prior to planting, medium incorporation to 20 cm, and a deep incorporation down to 35 cm. Potatoes were mechanically planted to three depths: shallow (approx. 10 cm), medium (approx. 15 cm) and deep (approx. 25 cm). Results showed that the medium-depth nematicide incorporation, when tubers were planted at a shallow or medium depth, reduced root invasion compared with the other treatments. Medium-depth nematicide incorporation also gave consistently larger ware yields and better nematode control than the other incorporation methods, which were not significantly different from the control. However, yield and Pf/Pi ratios were not significantly affected by planting depth.

CHARACTERIZATION OF *ARABIDOPSIS THALIANA* MUTANTS WITH ALTERED SUSCEPTIBILITY TO *HETERODERA SCHACHTII*. **Wubben, II, M. J. E.,[1] K. A. Hardy,[1] H. Su,[2] S. R. Rodermel,[3] and T. J. Baum.[1]** [1]Department of Plant Pathology, [2]Interdepartmental Genetics Program, and [3]Department of Botany, Iowa State University, Ames, IA 50011.

The formation of syncytia in host plants by cyst nematodes *Heterodera* spp. presumably requires the presence of host signal transduction pathways that convey signals elicited by the nematode to the host nucleus. Mutant analysis is one strategy that can be used to identify host genes involved in these signaling pathways. An *in vitro* screening protocol based on the model system of *Arabidopsis thaliana* and *Heterodera schachtii*, the sugar beet cyst nematode, was used to identify three *A. thaliana* mutants from a total of 5,200 M2 plants that were obtained from selfed progeny of EMS-treated parents. These mutants exhibit altered susceptibility to *H. schachtii* and were designated cst for altered susceptibility to cyst nematodes. The phenotypes of these mutants are resistance cst1, hypersusceptibility cst2, and decreased susceptibility cst3. Genetic analyses of these mutants determined that they are the result of mutations in three independent genes, each inherited in a recessive manner. Phenotypic characterization of cst1 revealed that a lower number of second-stage juveniles (J2) become sedentary relative to wild type. Typically, the few J2 that are able to become sedentary on cst1 fail to develop past the J2 stage. The cst2 mutant allows more J2 to become sedentary than wild type, resulting in the development of a greater number of males and females. Also, cst2 exhibits an altered root morphology phenotype. Both phenotypes associated with cst2 are the result of a mutation in a single gene that we have shown to play a role also in ethylene signal transduction. cst2 has been mapped to the bottom of chromosome 1, and a chromosome walk is underway in an effort to clone the gene. The decreased susceptibility seen in the cst3 mutant is due to a lack of development of sedentary J2. This phenotype is similar to that of cst1; however, it is not as dramatic because cst3 does not inhibit J2 from becoming sedentary. Further characterization of these mutants will increase our understanding of the compatible interaction between *A. thaliana* and *H. schachtii*.

MORPHOGENESIS OF THE MALE TAIL IN *C. ELEGANS* AND OTHER RHABDITIDAE. **Yang, Y.,[1] C. Q. Nguyen,[1] D. H. Hall,[2] and D. H. A. Fitch.[1]** [1]Department of Biology, New York University, New York, NY, and [2]Center for *C. elegans* Anatomy, Albert Einstein College of Medicine, Bronx, NY.

How cells are coordinated to change their form remains a fundamental question in developmental biology. To understand the mechanism and components underlying multicellular morphogenesis, we have begun a comprehensive study of the four-celled male tail tip of *Caenorhabditis elegans*. During late post-embryonic development, the male tail is reshaped to form a copulatory structure. The most posterior hypodermal cells in the tail define a specialized, sexually dimorphic compartment in which cells fuse and retract in the male, changing their shape from pointed to round. Cell fusion, changes in cell shape and position, fluid displacement, nuclear migration and

changes in cell–cell associations are all important features of male tail tip morphogenesis, as they are in other systems. Using EM and immunofluorescent staining of adherens junctions, we have reconstructed the changes in cell architecture and intercellular associations that occur during morphogenesis of the tail tip. Cell fusions are initiated at or adjacent to adherens junctions. Anterior portions of the cells show the first evidence of fusions and retractions, consistent with an anterior trigger for these events. Mutations recovered in our morphological screens interfere with morphogenesis and implicate particular regulatory pathways. Comparing these mutants with related species in a phylogenetic context suggests loci at which evolutionary changes could have produced morphological diversity.

MORPHOLOGY OF SOME SPECIES OF *HEMICRICONEMOIDES* (NEMATODA: CRICONEMATIDAE) IN THE USA. **Ye, W., and R. T. Robbins.** Nematology Laboratory, Department of Plant Pathology, University of Arkansas, Fayetteville, AR 72701.

Six species of *Hemicriconemoides* were found from the USA in the second author's collection. They are *H. annulatus* Pinochet and Raski, 1975 from turf in Florida; *H. wessoni* Chitwood and Birchfield, 1957 from bentgrass in South Carolina; *H. brachyurus* (Loos, 1949) Chitwood and Birchfield, 1957 from California native plants; *H. strictathecatus* Esser, 1960 from California native plants; *H. chitwoodi* Esser, 1960 from soil in North Carolina; and thirteen populations of *H. californianus* Pinochet and Raski, 1975 from rose, loquat, turf, cabbage, grape, willow, citrus, and philodendron in California. Emended measurements, descriptions, illustrations, ultrastructure and new records of distribution are presented for these species. The comparative study of morphometric and morphological variability between *H. wessoni* and *H. annulatus* shows the great similarity. Information about the occurrence of all *Hemicriconemoides* species in the USA is provided.

SOYBEANS RESISTANT TO *HETERODERA GLYCINES* POPULATIONS ATTACKING HARTWIG CULTIVAR. **Young, L. D.**, USDA ARS, 605 Airways Blvd., Jackson, TN 38301-3201.

Heterodera glycines may be the most important pathogen of soybean in the United States. Several races of the nematode have been identified. The Hartwig cultivar has resistance derived from Plant Introduction (PI) 437654 and is resistant to most races of the nematode. Hartwig is attacked by a few race 4 populations. One hundred and eleven soybean PIs previously identified as resistant to one or more races of *H. glycines* plus soybean cultivar Lee 68, Bedford, and Hartwig were tested in the greenhouse for resistance to LY1, a *H. glycines* population resulting from a mass mating of race 2 with race 5 followed by selection for reproduction on Hartwig. PIs 79693, 548349 and 567516C each had female indices (number of females on the PI expressed as percentage of females on Lee 68) less than 10 in two tests, indicating resistance to LY1. When 11 PIs with the lowest female indices in tests with LY1 were tested with another population (LY2) selected for reproduction on Hartwig, mean female indices for PIs 79693, 548349 and 567516C ranged from 29 to 41. The LY2 population originated from a field infested with race 14 before selection for reproduction on Hartwig began. PI 87631-1 had female indices less than 10, and females indices for PI 458520 were 9 and 14 in two tests infested with LY2. When tested with LY1, PIs 87631-1 and 458520 had mean female indices of 21 and 15, respectively. Thus, none of the plant introductions could be classified as resistant (female index less than 10) to both LY1 and LY2.

COMPARATIVE ULTRASTRUCTURE OF BASAL BULB OF REPRESENTATIVES OF CEPHALOBINA, DIPLOGASTERIDA AND RHABDITINA WITH PHYLOGENETIC IMPLICATIONS. **Zhang, Y., and J. Baldwin.** Department of Nematology, University of California, Riverside, CA 92521.

The ultrastructure of the isthmus and basal bulb (post-corpus) of *Zeldia punctata* (Cephalobina) and *Diplenteron* sp. (Diplogasterida) was compared with previous observation of *Caenorhabditis*

elegans (Rhabditida). The post-corpus of *Z. punctata* consists of 31 cells, including 6 marginal, 13 muscle, possibly 5 gland, and 7 nerve cells. The post-corpus of *Diplenteron* sp. is glandular and muscular and is composed of 26 cells, including 6 marginal, 6 muscle, 3 gland, and 11 nerve cells. All six marginal cells in *Z. punctata* and *Dipleneron* sp. are homologous with those in *Caenorhabditis elegans*. Both *Z. punctata* and *C. elegans* have a grinder in their basal bulb, which is absent in *Diplenteron* sp. *Zeldia punctata* and *C. elegans* have four sets of muscle cells, but only two sets were found in *Diplenteron* sp. The anterior set of V-shaped muscle cells (set 1) of *Diplenteron* sp. consists of three cells each with two nuclei. This set apparently is homologous to m5 muscle cells in *C. elegans*. In contrast, set 1 muscle cell in *Z. punctata* is composed of three groups of two muscle cells each with one nucleus. Set 2 muscle cells of *Z. punctata* and *Diplenteron* sp. are homologous to m6 muscle cells in *C. elegans*. Set 3 muscle cells in *Z. punctata* contains three cells that are homologous to m7 cells associated with the grinder in *C. elegans*. *Diplenteron* sp. does not have muscle cells corresponding to the m7 cells. A single saucer-shaped muscle cell, m8, covering the posterior wall of the basal bulb in *C. elegans* is present in *Z. punctata*, but not in *Diplenteron* sp. We have confirmed that the post-corpus of *Diplenteron* sp. has three gland cells. Conversely, *C. elegans* has five glands and in *Z. punctata* we have confirmed three gland cells plus perhaps two additional. Although the post-corpus of *Z. punctata* shares a wider range of similarities with *C. elegans* than with *Diplenteron* sp., the polarity of these characters remains to be assessed and evaluated in the context of additional morphological and molecular data.

APPLICATION OF CHINESE HERBAL REMEDIES FOR PLANT-PARASITIC NEMATODE MANAGEMENT. **Zheng, L., I. A. Zasada, and H. Ferris.** Department of Nematology, University of California, Davis, CA 95616.

More than 500 plant species are documented in Chinese traditional medicine to have activity against helminth and micro-invertebrate pests of humans. In a progression of experiments, 164 of these plant sources were evaluated to determine their efficacy in reducing plant-parasitic nematode populations. Initial evaluations were by direct observation and measures of effects on nematode motility. Studies provide evidence that modes of action differ; sometimes effects are lethal (57% of plant sources tested); in the other remedies, no effect was observed or nematodes recovered. A majority of the species tested from the plant families *Asteraceae, Apiaceae, Cucurbitaceae, Euphorbiaceae, Liliaceae, Polygonaceae* and *Solanaceae* were effective. The Lethal Concentration50 and LC90 were determined for 43 plant sources; of these, 19 were evaluated under greenhouse conditions for effectiveness against *Meloidogyne incognita* in soil and phytotoxicity to tomato. High concentrations of *Hedera helix* and *Allium sativum* extracts suppressed nematodes but were phytotoxic to plants. All concentrations of *Allium cepa* suppressed nematodes and had no effect on plant growth. While *Ginkgo biloba* was effective in the laboratory, it did not reduce nematode damage to plants in the soil. Thirteen of the 164 sources have consistently demonstrated the ability to suppress nematodes, and four are available for use in agricultural systems (*A. cepa, A. sativum, H. helix* and *Cucurbita pepo*). Methods for implementing Chinese herbal remedies will vary with the nature of the plant source. Different methods of plant material delivery and the fate of plant-derived chemicals in the soil are being evaluated.

INHERITANCE OF RESISTANCE TO *MELOIDOGYNE INCOGNITA* IN THE COTTON CULTIVAR ACALA NEMX. **Zhou, E.,[1] J. L. Starr,[1] and C. W. Smith.[2]** [1]Department of Plant Pathology and Microbiology, and [2]Department of Soil and Crop Sciences, Texas A&M University, College Station, TX 77843.

Acala NemX was released in 1995 as root-knot resistance cotton cultivar. To determine the inheritance of resistance, crosses were made between Acala NemX and the susceptible cultivar Deltapine 90. Ten F1 plants had 4,226 eggs/g root, with a root-gall index of 3.7 (0–5 scale), which were not different from the susceptible Deltapine 90 ($P = 0.05$). Segregation of resistance in 43

F2 individuals was determined based on nematode reproduction and root-gall index, with individuals having 10% less of the susceptible parent or a root-gall index of ≤ 2 being classified as resistant. F2 individuals segregated 1 resistant:3 susceptible (chi-sq = 0.63; non-significant for P = 0.05), consistent with resistance being governed by a single recessive gene. If the F2 population was divided into three classes, with resistant defined as <10%, moderate resistant as 10%–50%, and susceptible as > 50% of nematode reproduction of the susceptible parent, segregation also could be categorized by a ratio of 1 resistant:2 moderate resistant:1 susceptible (chi-sq = 0.63; non-significant for P = 0.05), which was consistent with resistance being conditioned by a single gene with an additive effect.