

ABSTRACTS

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EFFECT OF GREEN MANURE CROPS ON YIELD OF POTATOES GROWN IN NEMATODE-INFESTED SOIL. **Al-Rehiyani, S., and S. L. Hafez.** Research and Extension Center, University of Idaho, Parma, ID 83660.

Green manure effects of barley, buckwheat, oil radish, and velvetbean on yield of Russet Burbank potato were tested in field microplots infested with *Meloidogyne chitwoodi* race 2 (125 second-stage juveniles/100 cm³ soil), *Pratylenchus neglectus* (65/100 cm³) or combinations of both nematode species, and an uninfested soil. Green manures were compared with aldicarb or no green manure (fallow). The experimental design was factorial with two factors: green manure (A) and nematode species (B). Factor A had a significant effect on yield and tuber size. There was no effect of factor B or AB on total yield, but there was a significant effect of factor B on tuber size. All green manure crops or aldicarb treatments produced significantly higher yield and larger tubers (> 100g) than fallow. Yields from barley and oil radish treatments were significantly higher than from the other crops and aldicarb. Buckwheat treatments yielded significantly less than aldicarb treatments. Numbers of small tubers (<50 g) were significantly fewer from barley, buckwheat, and oil radish treatments than from fallow and velvetbean. Microplots infested with *M. chitwoodi* alone produced significantly more small and fewer large tubers.

EFFECTS OF ANAEROBIC CHILLING AND DAY LENGTH OF MATERNAL HOST PLANT ON DIAPAUSE IN EGGS OF *MELOIDOGYNE NAASI*. **Al-Zubaidy, M. A. J., and A. A. F. Evans.** Imperial College at Silwood Park, Ascot, Berkshire SL5 7PY, United Kingdom.

Egg masses of a population of *Meloidogyne naasi* from Wales were chilled at 5 °C for 12 weeks under one of three regimes: (i) anaerobiosis; (ii) anaerobiosis interrupted by one week of aerobiosis; (iii) continuous aeration, before eggs were hatched at 20 °C. Control treatments were (iv) anaerobiosis at 20 °C and (v) interrupted anaerobiosis at 20 °C, both with hatching at 20 °C. Egg masses from *M. naasi* raised on host plants grown in 8-hour, 16-hour, or continuous light were chilled at 5 °C for 12 weeks, before hatching at 20 °C. Chilling under anaerobic conditions was as effective as aerobic conditions at breaking the diapause in eggs, but that day length under which host plants were grown had no effect on subsequent hatch of eggs after chilling.

EFFECT OF SOYBEAN ROOT LEACHATE ON *HETERODERA GLYCINES* EGG HATCH. **Anand, S. C.,¹ and Z. Handoo.²** University of Missouri, Delta Center, Portageville, MO 63873, and USDA ARS, Nematology Laboratory, Beltsville, MD 20705.

Leachate derived from roots of soybean is known to stimulate egg hatch in soybean cyst nematode (SCN), *Heterodera glycines*. Experiments were conducted to determine SCN egg hatch with leachate obtained from Essex, a SCN-susceptible cultivar, and Hartwig, a highly resistant cultivar. Approximately 100 SCN eggs were placed in a petri plate in 10 ml of water with or without leachate. Hatched eggs were counted each week for three weeks. Approximately 27% of the eggs hatched in water (control) in one week, whereas the egg hatch was 41.5% in leachate from Essex and 50.8% in leachate from Hartwig. By the end of three weeks, the egg hatch was significantly greater (76.4%) in leachate from Hartwig compared with 55.8% in leachate from

Essex. The egg hatch in water (without leachate) was significantly lower than hatch in leachate from either cultivar. Hartwig stimulated SCN egg hatch more than the leachate from Essex. It is interesting that a soybean cultivar that is almost immune to *H. glycines* would stimulate egg hatch of this pathogen.

A MITOCHONDRIAL DNA PERSPECTIVE ON DIVERSITY IN THE POTATO CYST NEMATODE, *GLOBODERA PALLIDA*. Armstrong, M. R., V. C. Blok, B. E. Harrower, M. S. Phillips, and D. L. Trudgill. Scottish Crop Research Institute, Invergowrie, Dundee DD2 5DA, Scotland.

Diversity in mitochondrial DNA was examined in populations of *Globodera pallida* from Britain, continental Europe, and South America, along with two populations of *G. rostochiensis*. Total genomic DNA was digested and hybridized with fragments of mtDNA either from an mtDNA library or generated by PCR. The patterns of restriction fragments observed suggest that there can be mitochondrial variants within populations. Populations from South America were distinct both from each other and the European populations. The divergence was often so large that probes failed to hybridize, raising questions of speciation or sub-speciation within *G. pallida*. It is clear that a considerable genetic diversity exists within South America and that European *G. pallida* isolates are a subsample of this diversity. The data indicate that two atypical British populations are separate introductions. The majority of the continental European *G. pallida* populations shared mitochondrial characteristics with British populations, with some evidence of additional haplotypes.

LIFE CYCLE AND HOST SPECIFICITY OF *PASTEURIA* SP. PARASITIZING *HETERODERA GLYCINES*. Atibalentja, N.,¹ and G. R. Noel.^{1,2} ¹Department of Crop Sciences, University of Illinois, and ²USDA ARS, Urbana, IL 61801.

The life-cycle of an undescribed *Pasteuria* sp., first reported in 1994 as a parasite of *Heterodera glycines* in North America, was investigated by examining *H. glycines* adults and cysts extracted from naturally infested soil, and juvenile stages excised from roots of soybean grown in this soil. Germ tubes originating from *Pasteuria* endospores penetrated into the body of late second-stage juveniles (J2). Mycelial microcolonies formed in J2, then fragmented and proliferated throughout the body cavity of third-stage juveniles (J3). Sporulation began in J3 with the formation of grape-like clusters of sporangia and continued in fourth-stage female juveniles, adult females, and cysts, which became filled with various developmental stages of *Pasteuria*, including octets, quartets, triplets, doublets, and mature endospores. Numbers of endospores per cyst ranged from 30,000 to 820,000 ($\bar{x} = 313,500 \pm 233,700$). Endospores from race 4 of *H. glycines* attached to J2 of *H. glycines* races 1, 2, 3, 5, and 14, and to J2 of *H. schachtii*, *H. trifolii*, and *H. lespedezae*. *Meloidogyne arenaria* race 1, *Tylenchorhynchus nudus*, and *Labronema* sp. were not hosts for this isolate of *Pasteuria* sp.

IS *CAENORHABDITIS ELEGANS* A GOOD NEMATODE MODEL? Baillie, D. L. Institute of Molecular Biology and Biochemistry, Department of Biological Sciences, Simon Fraser University, Burnaby, B. C., Canada.

The *Caenorhabditis elegans* Genome Sequencing Labs in Hinxton, Cambridge, UK, and St. Louis, Missouri, USA, have currently sequenced and made available more than 70Mb of genomic sequence from *Caenorhabditis elegans*. This represents 70% of the genetic material contained in this free-living nematode, and makes *C. elegans* the metazoan closest to having its genome completely sequenced. It is estimated that the entire genomic sequence of *C. elegans* will be available by 1998. This sequence opens new paths for the investigation of other nematodes. What is the biological function of genes revealed by the sequence data? We are collaborating with the *C. elegans* genome sequencing labs to create a biological resource that will enhance the rate at which this information can be determined. Utilizing microinjection transformation, we are creating a library of transgenic animals that carry specific, sequenced cosmids. This cosmid DNA is maintained in the transgenic animals within arrays, and can be moved between strains by means of standard genetic techniques.

STRUCTURE AND DEVELOPMENT IN RELATION TO A MOLECULAR EVOLUTIONARY FRAMEWORK FOR RHABDITINA. Baldwin, J. G.,¹ L. M. Frisse,² J. T. Vida,² C. D. Eddleman,¹ and W. K. Thomas.² ¹Department of Nematology, University of California, Riverside, CA 92521, and ²Division of Molecular Biology and Biochemistry, University of Missouri, Kansas City, MO 64110.

Conflicts in characters that are poorly understood or convergent in Rhabditida, including overall shape, collar, and teeth of the buccal region, are being resolved and provide a basis for comparison with separate studies on vulval induction and male tail development. A molecular phylogenetic analysis including three different genes allows for independent interpretation of these morphological changes. The phylogenetic analysis does not support monophyly of Mesorhabditinae and identifies an undescribed species with a distinctive buccal capsule as a sister taxon of *C. elegans*. The inferred phylogeny provides a framework to interpret evolution of morphological and developmental change.

RELATIVE HOST SUITABILITY OF THREE SUNFLOWER HYBRIDS TO SELECTED MELOIDOGYNE SPECIES AND RACES. Barker, K. R., and C. S. Echerd. Plant Pathology Department, North Carolina State University, Raleigh, NC 27695-7616.

The host status of three sunflower oil-seed hybrids (ISO-003, ISO-894, and Sigoc-954) to the four common *Meloidogyne* species and races was determined. Single plants were established in 20-cm-diam. clay pots filled with a 1:1 sandy soil-sand mix, inoculated with 10,000 eggs of a given nematode, and grown in a greenhouse for 3 months. Nematodes included races 1 to 4 of *M. incognita*, races 1 and 2 of *M. arenaria*, *M. javanica*, and *M. hapla*. All three sunflower hybrids were susceptible to these parasites, with average root-gall ratings ranging from 41 to 54 on a 0 to 100 scale. Mean reproductive factors ($R_f = P_f/P_i$) for these cultivars ranged from 30 to 64. *Meloidogyne arenaria* race 2 had the highest reproductive factor (89) and root-gall index (64). In contrast, *M. hapla* was the least aggressive with a R_f of 5 and a root-gall index of 28. The gall index and R_f for the other nematode populations ranged from 49 to 58 and 36 to 61, respectively.

INTERACTIONS OF MELOIDOGYNE INCOGNITA POPULATIONS WITH SELECTED COTTON CULTIVARS. Barker, K. R., and S. R. Koenning. Plant Pathology Department, Box 7616, North Carolina State University, Raleigh, NC 27695-7616.

The relative reproductive and parasitic fitness of races 3 and 4 of *Meloidogyne incognita*, as well as that of a variant population of *Meloidogyne* isolated from cotton in North Carolina, were evaluated on selected cotton cultivars in greenhouse and microplot experiments. The level of resistance of La-887, Auburn 634, and Nemx cotton cultivars was assessed in the greenhouse. Race 4 was the most aggressive population. Based on root-galling and egg development, all three cultivars had high levels of resistance to the three populations evaluated. On a 0–100 scale, the gall indices for the resistant cultivars ranged from 2 to 10 in contrast to 49 to 61 for Deltapine 16. Cotton cultivar Deltapine 20 supported somewhat more reproduction and tended to have higher gall indices than did Deltapine 50 in microplot experiments. The cultivar by population interaction was not significant. Although the race 4 population generally induced extensive root galling, it did not persist overwinter as well as two other North Carolina populations.

NEMATODE COMMUNITY STRUCTURE UNDER SECONDARY SUCCESSION AND ALTERNATIVE FARMING SYSTEMS. Berney, M. F., and G. W. Bird. Department of Entomology, Michigan State University, East Lansing, MI 48824-1115.

Nematode community structure was studied during 1995 and 1996 in seven research sites with different land use histories: four farming systems (conventional, integrated fertilizer, integrated compost, and organic) and three secondary successions (0, 10, and 30 years since cultivation), in replicated plots. Sites were sampled three times per year, and nematodes were identified to genus. Community analysis included total number, plant parasite genera and number, non-plant parasite

genera and number, maturity index, and plant parasite index. Lower population densities were found in all four of the farming systems, compared with the successions. The proportion of plant parasites associated with the organic system was similar to those of the successions, and different from the other farming systems. Nematode community structure in the conventional system most closely resembled the earliest stage of succession (0 years).

PARADIGMS FOR NEMATODE PARASITISM. Bird, D., and C. Opperman. Department of Plant Pathology, North Carolina State University, Raleigh, NC, 27695-7616.

Plant and animal-parasitic nematodes face similar challenges to successful reproduction in their respective hosts. Given the striking morphological conservation of nematodes, mechanisms for parasitism might also be conserved cross-phylum. The imminent completion of the *Caenorhabditis elegans* genome sequencing project enables homologs of genes from parasitic nematodes to be identified and manipulated in this tractable system. We are using this approach to study root-knot and soybean cyst nematode (SCN) homologs of *asp-1* and *asp-2*, which were previously isolated as encoding hookworm secretion products. To test the function of parasitism-gene candidates experimentally (e.g., by genetic ablation) we developed a transformation system for SCN. Concurrently, we constructed a genetic map of SCN to unambiguously identify genes necessary for parasitism. We also have begun to isolate genes for pathways that are well characterized in *C. elegans* (including sex determination and the dauer pathway) and that are likely to be employed by parasitic nematodes. In *C. elegans*, the dauer pathway is a mediator between nutritional status and developmental decisions made by the nematode. Analogous functions in parasitic nematodes might include diapause entry and exit, sex determination, and switching from pre-parasitic to feeding behaviors. These events are obvious targets for disruption to effect control.

ROLE OF PLANT-PARASITIC NEMATODES AND OTHER FACTORS IN MICHIGAN SWEET CHERRY PRODUCTION. Bird, G. W., and H. Melakeberhan. Department of Entomology, Michigan State University, East Lansing, MI 48824.

A research initiative has been ongoing since 1989 to investigate the role of plant-parasitic nematodes and associated factors in sweet cherry (*Prunus avium*) production in Michigan. In a survey of 26 orchards in three cherry-growing regions, *Pratylenchus*, *Criconebella*, *Xiphinema*, *Meloidogyne*, and *Paratylenchus* were the most prominent plant-parasitic genera, in descending order. There were, however, no community structure or population density differences discernible between declining and healthy orchards. Bacterial canker, Ca and N deficiency, low soil pH, high soil Al concentrations, and winter injury were among other factors frequently associated with declining orchards. Research with cherry seedlings indicated that low soil pH could result in seedling death, in part by increasing absorption of Al to toxic levels. In the field, the impact of *P. penetrans* varied among cherry rootstocks. In optimal and deficient nutrient regimes under greenhouse conditions, final populations of *P. penetrans* were greater in the nutrient deficient regime, compared to the optimal one. Plant growth response to *P. penetrans* under the regimes varied with rootstock type.

DIFFERENTIATION OF MELOIDOGYNE CHITWOODI AND M. FALLAX FROM OTHER ROOT-KNOT NEMATODE SPECIES. Blok, V. C., J. Wishart, and M. S. Phillips. Department of Nematology, Scottish Crop Research Institute, Invergowrie, Dundee, Scotland DD2 5DA.

Meloidogyne chitwoodi and *M. fallax* recently have been identified in northwestern Europe and pose a threat to potato production. Sensitive methods for both detecting and identifying these species are required. Variation in the sizes of the intergenic spacer (IGS) regions are proving useful for differentiating *M. chitwoodi* and *M. fallax* from other *Meloidogyne* spp.. The regions between the 5S and 18S, and 5S and 28S genes can be amplified separately with PCR and both regions yield products of different sizes for *M. chitwoodi* and *M. fallax*. The sizes of the products from these

species differed from those from *M. hapla*, *M. incognita*, *M. javanica*, *M. arenaria*, and *M. mayaguensis*. These IGS regions can be amplified from individual juveniles, females, and males of *M. chitwoodi* and *M. fallax*. Isolates of *M. chitwoodi* can be differentiated following restriction enzyme digestion of the PCR product from the IGS region between the 5S to 28S genes.

PHYTOPARASITIC NEMATODE OCCURRENCE AND EFFECT ON SUGARCANE GROWTH IN LOUISIANA. Bond, J. P., E. C. McGawley, and J. W. Hoy. Department of Plant Pathology, Louisiana State University Agricultural Center, Baton Rouge, LA 70803.

Plant-parasitic nematode populations were estimated in plant and ratoon sugarcane crops at 12 locations. Nematode populations were progressively higher in each successive crop cycle year. Average densities per 150 g of soil were 288, 438, 743, and 1,219 for plant cane, first, second, and third ratoon, respectively. Two, 6-month microplot experiments were conducted with cultivars CP 70-321 and LCP 82-89. Three infestation levels (0, 1,200 and 12,000 individuals) were used. Nematodes were incorporated into 35 kg of methyl bromide-treated field soil in each 45.7-cm-diam. pot. Inoculum consisted of approximately 35% *Paratrichodorus* spp., 35% *Tylenchorhynchus* sp., and 30% *Criconebella* spp. In 1995, reductions in shoot and root weights of LCP 82-89 resulted from nematode damage. Final nematode populations were 358,000 and 196,000 individuals per pot at the 1x level and 312,800 and 236,900 at the 10x level for LCP 82-89 and CP 70-321, respectively. In 1996, greater nematode reproduction was accompanied by growth reductions in CP 70-321. LCP 82-89 also supported higher nematode populations; however, growth reductions were not observed.

RESISTANCE IN POTATO TO *GLOBODERA ROSTOCHIENSIS* PATHOTYPE Ro2. Brodie, B. B.,¹ and R. L. Plaisted.² ¹USDA ARS, Department of Plant Pathology and ²Department of Plant Breeding, Cornell University, Ithaca, NY 14853.

Potato clones with *Solanum tuberosum* ssp. *andigena*, *S. tuberosum* ssp. *tuberosum*, and *S. vernei* parentage were evaluated for resistance to *Globodera rostochiensis* pathotype Ro2. Host reaction was measured 12 weeks after plants were inoculated with 5,000 eggs/7.5-cm-diam. pot. Host status was based on the number of cysts that developed on the roots of each plant. Plants with 30 or fewer cysts were considered to be resistant. Eighteen clones with different levels of resistance were identified in the initial evaluation, and reactions of these clones were confirmed in two later tests. Four clones were highly resistant (<5 cysts/plant). These clones were N42-49 with an average of 2 cysts/plant, Q237-25 with 1cyst/plant, R3-6 with 2 cysts/plant, and R6-4 with 1 cyst/plant. After an evaluation of horticultural characters and marketing qualities, clones Q237-25 and R6-4 were judged to be of sufficient quality to introduce them to the Foundation Seed Farm for future consideration as advanced clones for potential cultivar release.

FIELD TRIALS FOR METHYL BROMIDE AND ALTERNATIVES IN COMMERCIAL CARROT PRODUCTION. Butterfield, A. ID Services Nematode Laboratory, 12419 Lytle Ave., McFarland, CA 93250.

Seven different materials or combinations of nematicidal materials were applied preplant and post-plant to carrot plots during the Kern County fall carrot season for control of *Meloidogyne incognita* (Mi). Preplant second-stage juvenile numbers ranged from 726 to 9,048/300 cm³ soil. Combinations of preplant or pre- and post-plant materials resulted in significantly higher packable tons of carrots per acre than untreated checks. Methyl bromide, metham sodium, and 1,3-dichloropropene gave similar amounts of packable carrots. There was no significant difference between any of the treatments in reducing yield (in kg) of non-packable carrots, and the fumigants and metham sodium significantly increased the number of undersized carrots. Carrots treated with Telone plus metham sodium had significantly less Mi damage than those treated by means of sprinkler application of metham sodium.

HATCHING OF ENCYSTED EGGS IN *HETERODERA GLYCINES*. Casta, L., R. Poupard, and P. M. Tefft. Biology Department, Saint Joseph's University, Philadelphia PA 19131.

Egg hatching in *Heterodera glycines* increases in response to hatching stimuli but exhibits a wide variation in response. In order to determine cyst-to-cyst variation in the response, individual cysts were exposed to a hatching stimulant, zinc chloride (3.0 mM). The number of second-stage juveniles (J2) hatching from each cyst increased significantly in the zinc-treated group compared to the water controls. However, when freed eggs from individual cysts were treated in a similar manner, the number of zinc-treated eggs that had high numbers of hatched J2 (>60 J2/cyst) was the largest group and few cysts yielded low numbers of hatched J2 (<10/cyst). This difference in hatching response between encysted and freed eggs suggests that the cyst environment may influence the egg's response to hatching stimuli. Significantly fewer eggs treated with fluid from cysts and zinc hatched than did eggs treated with zinc alone. Additionally, cyst fluid obtained from cysts pretreated with zinc or water caused a significant reduction in hatching. The cyst fluid effects were unaltered by dialysis or chelation with EDTA.

NEMATODES: POSSIBLE BIOCONTROL AGENTS AGAINST HELICID SNAILS IN SOUTH AUSTRALIA? Charwat, S. M., and K. A. Davies. Department of Crop Protection, Waite Campus, University of Adelaide, Glen Osmond 5064, SA, Australia.

The introduced helicid snails *Cernuella virgata*, *Theba pisana*, and *Cochlicella acuta* are serious problems in the South Australian grains industry and a developing problem in the citrus, wine, and dried fruit industries. Snail cadavers and soil from snail-infested areas were surveyed for nematodes attacking snails. Soil samples were baited with *C. virgata* for 6 days and nematodes were extracted from snail cadavers with modified White traps. Fifteen nematode isolates were obtained. They included panagrolaimids, cephalobids, rhabditids, and a diplogasterid. These were cultured and their pathogenicity to snails was determined in a soil-based bioassay. Snails were kept for 3 or 6 days in 500 g of soil infested with four nematode densities (0 [control], 1×10^4 , 5×10^4 , 1×10^5 , and $2 \times 10^5/100$ cm soil) and number of dead snails was recorded. Diplogasterid, panagrolaimid, and most rhabditid isolates had low pathogenicity to snails, but the rhabditid isolate XM13 caused high levels of mortality in all three snail species.

NITROGEN MINERALIZATION BY *APHELENCHUS AVENAE* ASSOCIATED WITH *RHIZOCTONIA* SP. AND BARLEY STRAW. Chen, J., and H. Ferris. Department of Nematology, University of California, Davis, CA 95616.

Two microcosm experiments were conducted to assess mineralization of nitrogen in the presence of fungal-feeding nematodes. The microcosms contained ground barley straw, *Rhizoctonia* sp., and *Aphelenchus avenae* with or without sand. Organisms were introduced into 3 g straw or a 20 g straw-sand mixture (2% straw, w/w). Samples were incubated with water for 1 hr, added to an equal amount of 4 M KCl, and shaken for 1 hr. The principal nitrogen mineralized by *A. avenae* was in the form of ammonia ($\text{NH}_4^+\text{-N}$). *Rhizoctonia* sp. immobilized ca. 85–99% of $\text{NH}_4^+\text{-N}$ available from the barley straw. There were significantly fewer active hyphae in the microcosms containing *A. avenae* than in those without nematodes. When barley straw was colonized by *Rhizoctonia* sp., 25.9, 39.5, and 1.9 g $\text{NH}_4^+\text{-N}$ were extracted from the microcosms, whereas in the presence of *A. avenae*, 26.1, 50.5, and 19.9 μg were extracted on days 7, 14, and 21, respectively. When the straw-sand substrate was colonized by the fungus, 1.1, 11.2, and 9.0 μg $\text{NH}_4^+\text{-N}$ were extracted, compared with 2.1, 15.9, and 14.6 μg in the presence of the nematode. There were 486, 945, and 840 *A. avenae*/g soil on days 7, 14, and 21, respectively. A C:N budget was developed for *A. avenae*.

INFECTION OF *HETERODERA GLYCINES* BY *HIRSUTELLA RHOSSILIENSIS* IN A MINNESOTA SOYBEAN FIELD. **Chen, S.** University of Minnesota, Southern Experiment Station, 35838 120th Street, Waseca, MN 56093.

Hirsutella rhossiliensis, an endoparasitic fungus, was observed on second-stage juveniles (J2) of *Heterodera glycines* in a soybean field at Waseca, Minnesota. The fungus parasitized 11% to 53% of the J2 during the soybean growing season in 1996. The percentage of J2 parasitized by the fungus was positively correlated with J2 density. In plots infested with the fungus, nematode egg density did not increase when planted to the susceptible soybean cultivars, Sturdy and Parker. In *H. rhossiliensis*-infested and uninfested sites, each with similar initial nematode population densities, the average Rf ($Rf = Pf/Pi$) of the nematode on 10 SCN-resistant cultivars was 0.3 and 1.5, respectively. The site infested with the fungus appeared to be suppressive to the nematode.

A CASE STUDY OF NEMATODE BIOCONTROL USING *PASTEURIA PENETRANS*. **Chen, Z. X., and D. W. Dickson.** Entomology and Nematology Department, University of Florida, Gainesville, FL 32611.

Pasteuria penetrans has shown great potential as a biocontrol agent of *Meloidogyne* spp. Our objectives were to determine numbers of endospores required to control *M. arenaria* on peanut, to determine the quantitative suppression of *M. arenaria* on peanut by *P. penetrans*, to estimate incidence of attachment of endospores to *Meloidogyne* spp. with tally thresholds, and to study the ultrastructure, morphology, and sporogenesis of four isolates of *P. penetrans*. When various levels of endospores were inoculated in microplots, *P. penetrans* suppressed root and pod galls and increased pod yields. *Pasteuria penetrans* was established and amplified in field microplots and reached an equilibrium density of 133,000 endospores/g of soil in 3 years. The quantitative suppression of *M. arenaria* on peanut by *P. penetrans* was evaluated in a 6×6 factorial experiment in field microplots over 2 years. Nematode numbers and damage to peanut decreased with increasing *P. penetrans* levels and increased with increasing nematode inoculum levels. When *P. penetrans* levels were increased by 10%, the number of eggs per root system, root galls, pod galls, and juveniles per 100 cm³ of soil at harvest decreased by 8.6%, 7.8%, 8.4%, and 8.8%, respectively. Estimating numbers of endospores attached per juvenile using various tally thresholds can obviate counting every endospore on a juvenile. Electron microscopy studies showed that sporogenesis of *P. penetrans* was similar to that of other gram positive bacteria, especially the seven-stage scheme reported for *Bacillus thuringiensis*.

A NEW SPECIES OF *HEMICYCLIOPHORA* FROM BIOSPHERE 2 IN ARIZONA. **Chitambar, J. J.,¹ T. R. Mahato,² M. A. McClure,³ and B. D. V. Marino.⁴** ¹Nematology Laboratory, California Department of Food and Agriculture, Sacramento CA 95832; ²Biosphere 2 Center, Inc., of Columbia University, Oracle AZ 85623; ³Department of Plant Pathology, University of Arizona, Tucson, AZ 85721; ⁴Department of Earth and Planetary Sciences, Harvard University, Cambridge, MA 02138.

A new species of *Hemicycliophora* (Nemata: Criconematidae) was found in soil from a fallow field plot within the Biosphere 2 Center, Oracle, Arizona. This nematode closely resembles *H. armandae* Al Banna & Gardner, 1993 and *H. iranica* Loof, 1984, and is characterized by continuous and irregular breaks in transverse striae in the lateral field, smooth body annules, a rounded-truncate lip region with anterior margins, three lip annules, first labial annule elevated and widened laterally, dome-shaped, elevated labial disc, stylet length (76–97 µm), 234–273 body annules, and a tail terminus with an offset, cylindrical to slightly conoid digit.

COMPARISON OF INOCULATION METHODS AND DIFFERENT HOST PLANTS FOR PRODUCTION OF *PASTEURIA*. **Cho, M. R.,¹ D. W. Dickson,² and T. E. Hewlett.²** ¹National

Horticulture Research Institute, Suwon 441-440, Korea, and ²Department of Entomology and Nematology, University of Florida, Gainesville, FL 32611.

Pasteuria penetrans is an effective biocontrol agent of root-knot nematodes, but its practical use is limited by the lack of efficient methods for producing endospores. Our objective was to compare production levels of *P. penetrans* endospores in plants grown in soil vs. soilless media, in plants receiving single vs. double inoculations, and in different susceptible host plants. Second-stage juveniles (J2) of *Meloidogyne javanica* and *M. arenaria* with attached endospores were used as inocula. Number of J2 that entered tomato roots 6 days following inoculation was higher in soil than in a soilless medium. The number of root galls produced was 6-fold greater following double inoculation at 3-day intervals than following a single inoculation. Of six host plants tested, root galling was higher on cucumber followed by pumpkin and tomato, than on squash, radish, or okra. Endospore numbers per female were higher in cucumber than in tomato, pumpkin, or okra. Of the methods tested, inoculation of plant roots twice had the greatest impact on the production of endospores.

CHARACTERIZATION OF A PATHOTYPE OF CEREAL CYST NEMATODE, *HETERODERA AVENAE*, FROM CENTRAL SAUDI ARABIA. Cook, R.,¹ and A. S. Al-Hazmi.²

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Cereal cyst nematode has emerged as a damaging pathogen in irrigated wheat production systems in Saudi Arabia. Tests with an International Test Assortment of cereals (barley, oat, and wheat) were made to determine the pathotype classification of one population from the Al-Kharj region of central Saudi Arabia. Morphologically, cysts and juveniles of this population were identified as *Heterodera avenae*. Tests were made in controlled environments in Wales, UK, and Riyadh, Saudi Arabia, in 1995 and 1997. Three replicates of each cereal were inoculated with 525 hatched juveniles and the number of females per plant was determined after 60 days. On the control differentials barley cv. Emir, oat cv. Sun II, and wheat cv. Capa, there were 25, 0, and 42 females per plant, respectively. Based on its virulence on barleys, the Al-Kharj population was similar to pathotype Ha21. No oats were hosts, but all wheat differentials were wholly (Psathias, 22 females per plant) or partially susceptible (Loros, 10 females; AUS 10894, 12 females; Iskamish Katagan 2, 7 females). The virulence of this population on Loros wheat is different from that of the original Ha21 populations from northern Europe, where Loros is resistant. Characterization of additional Saudi populations is continuing.

STUBBY-ROOT SYMPTOMS ON COTTON INDUCED BY *BELONOLAIMUS LONGICAUDATUS*. Crow, W. T.,¹ D. W. Dickson,¹ and D. P. Weingartner.² ¹Department of Entomology and Nematology, and ²Department of Plant Pathology, University of Florida, Gainesville, FL 32611-0620.

Cotton production in Florida may be limited by plant-parasitic nematodes. Our objective was to observe and describe the effects of sting nematode, *Belonolaimus longicaudatus*, on cotton. Cotton was grown in field plots naturally infested with *B. longicaudatus* and in controlled environment chambers. Forty days after planting, plants were shorter in control plots than in plots treated with either aldicarb (1.68 kg a.i./ha) or 1,3-D (56.1 liters/ha). Plant height was highly negatively correlated with *B. longicaudatus*. Below-ground symptoms were striking in that the roots were severely abbreviated and "stubby" in appearance. Plants inoculated with *B. longicaudatus* and grown in chambers were much shorter and weighed less than uninoculated plants 30 days after planting. All inoculated plants showed severely abbreviated roots. *B. longicaudatus* appears to be highly virulent on cotton and induces stubby-root symptoms.

NEMATODE 18S rRNA SEQUENCE DATA AND THE CLASSIFICATION OF PLANT PARASITES. De Ley, P.,¹ I. T. De Ley,² A. Vierstraete,¹ J. Vanfleteren,¹ M. Blaxter,³ and A. Coomans,¹ ¹Instituut voor Dierkunde, Universiteit Gent, B-9000 Gent, Belgium; ²University of Southern Mindanao, 9407 Kabacan, Cotabato, The Philippines; ³Institute of Cell, Animal and Population Biology, University of Edinburgh, Edinburgh EH9 3JT, United Kingdom.

18s rRNA sequence analysis strongly suggests that many of the commonly accepted higher taxa in nematode systematics are paraphyletic. An emended classification based on monophyletic taxa will therefore require significant changes to nomenclature. As an example, we present some of the implications for the systematics of phytoparasitic taxa. Thus, 18S rRNA data do not place Trichodoridae close to Dorylaimida, and strongly suggest that both Tylenchina and Aphelenchina are most closely related to the free-living Cephalobina rather than to Diplogasterida. In both cases, this has important repercussions for the composition and number of order-level taxa in which these groups are to be classified. Also, 18S rRNA resolves evolutionary relationships between some families and superfamilies within Tylenchida.

ALTERNATIVES TO METHYL BROMIDE FOR CONTROL OF NEMATODE AND SOIL-BORNE DISEASES. Dickson, D. W. Department of Entomology and Nematology, University of Florida, Gainesville, FL 32611-0620.

Methyl bromide (MBr) was compared with other fumigants and nonfumigants for control of root-knot nematodes, soilborne diseases, and weeds in tomato grown in drip-irrigated polyethylene mulch culture in Florida. Plots were single row, 9.1 m long on 1.8 m centers with a 0.91 m bed width. MBr, chloropicrin (pic), 1,3D + pic (17% or 35%), and metham sodium were applied preplant. Oxamyl, tannic acid, and Abbott 9008 were applied postplant via drip irrigation. MBr, oxamyl, and pic treatments resulted in increased total marketable yields when compared to the control ($P \leq 0.05$). The remaining treatments provided higher numerical yields than the control but there were no statistical differences among them and the control. Oxamyl and MBr 98-2 increased the number of extra-large fruit compared to the control. Root-knot nematode galling indices were lower in soil treatments of MBr, pic, and 1,3-D + pic when compared to the control. Wilt induced by *Sclerotium rolfsii* was reduced to one or no plant hits per plot by MBr, pic, and all treatments containing 1,3-D + 35% pic except 1,3-D + 35% pic + oxamyl, when compared to the control.

CLONING SECRETION GENES FROM *MELOIDOGYNE INCOGNITA* USING RNA FINGER-PRINTING. Ding, X., R. I. Allen, and R. S. Hussey. Department of Plant Pathology, The University of Georgia, Athens, GA 30602-7274.

RNA fingerprinting was used to identify RNAs present in parasitic (up to 48 hours post-inoculation of soybean) second-stage juveniles (J2) of *Meloidogyne incognita*, but absent or reduced in preparasitic J2. A gene encoding a secretory protein was cloned from a *M. incognita* J2 cDNA library by probing with F29, a 0.5-kb fragment derived from fingerprinting. The full-length cDNA encodes 231-amino acids protein, with the first 21 amino acids being a putative secretory signal. In Southern blot analysis the gene was detected in *M. incognita*, but not in *Heterodera glycines* or *Caenorhabditis elegans*. In northern blot analysis a 1-kb transcript was detected in both preparasitic and parasitic J2, but not in adult females. The transcript was more abundant in parasitic J2 than in preparasitic J2. Comparing the predicted amino acid sequence with peptide sequence databases revealed significant similarity to ASP, a secretory protein from the infective J3 of *Ancylostoma caninum*, venom allergen antigen 5 family of proteins from Hymenoptera, and PR1, a pathogenesis-related protein from plants.

INTRASPECIFIC VARIATION IN POPULATIONS OF *ROTYLENCHULUS RENIFORMIS*. Dominguez, H. E., E. C. McGawley, and C. Overstreet. Department of Plant Pathology, Louisiana State University Agricultural Center, Baton Rouge, LA 70803.

Six populations of reniform nematode (*Rotylenchulus reniformis*) from Louisiana were subjected

to the host differential assay employed for identification of common root-knot species and races. Five populations were from cotton (1,3-6) and one was from cabbage (2). Significant variation in reproduction occurred among the populations on cotton ($R_f = 9.4-163.0$) and tobacco ($R_f = 8.2-144.9$). All the populations reproduced well on tomato ($R_f = 144.3-232.5$). Only population 1 reproduced on peanut ($R_f = 3.7$) and only population 6 reproduced on pepper ($R_f = 31.7$). Both peanut and pepper have been reported as resistant or immune. No reproduction occurred on watermelon. Seventeen additional populations from Louisiana were obtained from cantaloupe, sunflower, and seven vegetable crops (green bean, onion, pea, potato, pumpkin, sweet-potato, and turnip). Soil population densities ranged from 2 to 6,180 reniform nematodes per 100 cm³ of soil.

EFFECTS OF SELECTED BIOLOGICAL CONTROL PRODUCTS ON *CRICONEMELLA* SP. IN BENTGRASS. Donald, P. A., and B. S. Fresenburg. Plant Science Unit, University of Missouri, Columbia, MO 65211.

Three biological control products were tested for efficacy against *Criconemella* sp. on bentgrass golf green research plots. One product had a blue-green algal extract as the major component, one product was ground sesame stalks, and the third product was a heat-attenuated fungus. Efficacy was measured by comparing the number of *Criconemella* in the treated plots with the three control plot treatments (untreated, nematicide, and fertilizer [8% N]). Products were applied one to three times per growing season according to label directions. Data were collected at weekly intervals from June to September. The level of *Criconemella* found in the plots was below the damage threshold used in Missouri for golf greens (1,000/100 cm³ of soil). All treatments lowered *Criconemella* levels below the controls for at least part of the growing season. Numbers of *Criconemella* were similar to the controls in September for all treatments except the fungal product. *Meloidogyne* sp. was present in the research area but was below the detection level through the growing season in most plots.

CHARACTERIZATION OF PUTATIVE COFFEE LESION NEMATODES ASSOCIATED WITH FLORIDA CITRUS. Duncan, L. W.,¹ R. N. Inserra,² and D. T. Kaplan.³ ¹IFAS, Citrus Research and Education Center, University of Florida, Lake Alfred, FL 33850; ²DPI, Florida Department of Agriculture and Consumer Services, Gainesville, FL 32614-7100; ³USDA ARS, 2120 Camden Rd., Orlando, FL 32803-1419.

Several populations of putative coffee lesion nematodes collected from native and ornamental plants in Florida did not infect citrus. A principal component analysis based on 14 morphometric measurements suggested that the nematodes could be divided into three groups: citrus parasites; grass parasites; and those collected from a variety of plants. SEM comparison of the oral disks of the lesion nematode isolates indicated that lips were either divided into submedian and lateral segments or were undivided. The oral disk of citrus parasites was not divided. RAPD analysis indicated distinct genome organization for each isolate. Several lesion nematode species that appear morphologically similar to coffee lesion nematodes when examined by light microscopy may be present in Florida. We are attempting to clarify their taxonomy and to develop reliable and sensitive diagnostics to support Florida's citrus nursery-site certification program.

FINE STRUCTURE OF INFECTIVE JUVENILES OF *ONCHOCERCA VOLVULUS* DEVELOPED IN *SIMULIUM YAHENSE* IN LIBERIA. Endo, B. Y.,¹ and M. Trpis.² ¹Nematology Laboratory, Plant Sciences Institute, USDA, Beltsville, MD 20705, and ²Johns Hopkins University, School of Hygiene and Public Health, Department of Molecular Microbiology and Immunology, 615 N. Wolfe St., Baltimore, MD 21205.

Third-stage juveniles of *Onchocerca volvulus* were examined to elucidate the ultrastructure of the stoma, esophagus, intestine, and nervous system. The alimentary canal has a cuticularized stoma with triradiate lumen that extends through the muscular region of the esophagus. The lumen wall may be laterally appressed or may open into a stellate form in the glandular region. Posteriad

from the esophago-intestinal valve, the cylindroid lumen becomes partially occluded with microvilli formed by the intestinal epithelium. In cross-section the epithelial cells are delineated by junctional complexes. The alimentary canal terminates via a rectal valve and channel supported by somatic and neural cells. The nerve ring surrounds the muscular region of the esophagus. Related neurons support chemo- and tactoreceptors of sensilla and the extensive coelomyarian-meromyarian somatic muscles. Large accumulations of glycogen rosettes occur in muscle and hypodermal cells.

RELATING SOIL NEMATODE DIVERSITY TO ECOSYSTEM PROCESSES AND DISTURBANCES. Ettema, C. H. Institute of Ecology, University of Georgia, Athens, GA 30602-2202.

Reviewing the coarsely defined functions typically ascribed to the diverse soil nematode fauna, it appears that many species are redundant for soil ecosystem functioning. However, through analyses of field and microcosm data, it seems evident that the extent of nematode functional redundancy is greatly overestimated. First, while redundancy is common in single functions, distinct physiological and environmental requirements drive species of the same functional group to play widely different roles in soil ecosystem processes. Research on nematodes as indicators of disturbance illustrates this, though more natural history data are needed to refine our definition of the functions of individual species. Second, lack of correlation between nematode species and ecosystem functions is less likely a reflection of redundancy than of the logical error of linking organisms operating on smaller scales to processes measured at larger scales. This is a caveat for indicator research as well. Advances in techniques designed to measure process rates at scales more relevant to nematodes, as well as integration of population, community and ecosystem approaches, are needed to further develop the sustainable management of nematode diversity in human-influenced ecosystems.

NITROGEN MINERALIZATION BY BACTERIAL-FEEDING NEMATODES. Ferris, H., R. C. Venette, H. R. van der Meulen, and S. S. Lau. Department of Nematology, University of California, Davis, CA 95616.

Sand-column microcosms amended with an organic substrate, bacteria, and with or without bacterial-feeding nematodes, were leached at 3-day intervals. Cumulative N, as NH_4^+ or NO_3^- , leached from columns containing nematodes was consistently greater than from those without nematodes. For an organic substrate with C:N ratio of 11:1, rates of N-mineralization among species of different body size were similar, ranging between 0.0012 and 0.004 g N/individual/day, mainly as NH_4^+ . Smaller nematodes mineralized more N per unit of body weight than larger nematodes. We hypothesized that at low C:N ratios of the organic substrate bacterial growth is C-limited and N-immobilization will not occur; at high C:N ratios bacterial growth will be N-limited and there may be rapid immobilization of newly-mineralized N. Consequently, net N-mineralization in the presence of nematodes will be lower when the organic substrate has a high C:N ratio. In experiments with different nematode species, net mineralization and the apparent nematode contribution to mineralization generally decreased with increasing C:N ratio, consistent with the hypothesis; however, there were exceptions.

RIBOSOMAL DNA COMPARISONS OF *GLOBODERA TABACUM* WITH OTHER *GLOBODERA* SPECIES. Ferris, V. R., J. M. Ferris, and J. Faghihi. Department of Entomology, Purdue University, West Lafayette, IN 47907-1158.

Ribosomal DNA (rDNA) sequence data were obtained for *Globodera tabacum* from Connecticut, originally from L. I. Miller, and maintained on tobacco and/or *Solanum dulcamara*, and the Mexican species of *Globodera* known widely as "the Mexican cyst nematode", also from Dr. Miller and maintained on *S. dulcamara*. Sequence obtained included both internal transcribed spacers (ITS1 and ITS2) and the 5.8S rRNA gene between them. Phylogenetic analysis based solely on the rDNA data showed a close relationship between *G. tabacum* and a group of three

Globodera isolates from Mexico, including the Mexican cyst nematode and two Mexican *Globodera* isolates previously studied. *G. tabacum* rDNA sequence was 98% similar to that of the three Mexican isolates, 97.6% similar to that of *G. rostochiensis*, and 96.7% similar to the sequence of *G. pallida*. The *G. tabacum* sequence was only 88.7% similar to that of one of Miller's isolates of *G. virginiae* maintained on *S. dulcamara*.

DETERMINATION OF TILLAGE- AND CHEMICAL-SENSITIVITY RATINGS OF SOIL NEMATODE GENERA FOR USE IN SOIL HEALTH MONITORING. Fiscus, D. A.,¹ and D. A. Neher.² ¹Ecology Program, North Carolina State University, Raleigh, NC 27606, and ²Department of Biology, University of Toledo, Toledo, OH 43606.

Nematodes have been studied as potential indicators of agricultural soil health. Regional soil monitoring datasets include a range of sites for which multiple types of disturbance and natural variation are confounded. We used canonical correspondence analysis (CCA) and partial CCA to separate effects of tillage and chemical applications while accounting for non-anthropogenic environmental variation. One CCA ordination of nematodes comparing North Carolina conventional and no-till agricultural sites included 50 genera of nematodes plus soil sand content, pH, soil moisture, and tillage type. The ordination produced CCA axis 1 ($\lambda = 0.154$) and axis 2 ($\lambda = 0.116$), which together explained 50.4% of the species-environment relation. Then, relative tillage-sensitivity ratings were assigned to nematode genera on a scale of 1–3, least sensitive to most sensitive, based on projections of genus scores onto the tillage axis within this ordination. Chemical sensitivities were assigned similarly via other data.

ANALYSIS OF 1,3-DICHLOROPROPENE (1,3-D) AND NON-FUMIGANT NEMATOCIDES FOR CONTROL OF MELOIDOGYNE SPECIES IN PEST MANAGEMENT SYSTEMS. Fortnum, B. A.,¹ A. W. Johnson,² and S. A. Lewis.³ ¹Clemson University, 2200 Pocket Road, Florence, South Carolina 29506-9706; ²USDA ARS, P. O. Box 748, Tifton, GA 31793; ³Clemson University, Box 340377, Room 118, Long Hall, Clemson, SC 29634-0377.

Root-knot nematodes (*Meloidogyne* spp.) are major pathogens of tobacco. Pest management decisions typically are based on a systems approach where agricultural pesticides may be targeted at several pests. The economic benefits of a pesticide need to account for the complexity of the pest populations and multiple pest targets. Non-fumigant nematicides may reduce soil and foliar insects such as wireworms, cutworms, flea beetles, aphids, budworms, and hornworms. An economic analysis of the benefit of 1,3-D and selected non-fumigant nematicide/ insecticide combinations was performed using low to high nematode and insect populations in a cost matrix system. 1, 3-D provided superior yield enhancement and nematode control under low, moderate and high nematode pressure (302–1,280 kg/ha) when compared to the best nonfumigant (240–656 kg/ha). Although some nematicide/insecticides reduced the required number of foliar insecticide sprays, the cost of using a fumigant even in concert with an insecticidal rate of a soil insecticide/nematicide was comparable to the least expensive non-fumigant nematicide, when the cost of foliar insecticide applications was included in the cost estimates.

NEMATODE BIODIVERSITY AND SOIL HABITAT SUITABILITY IN HOT AND COLD DESERTS. Freckman, D. W.,¹ and R. A. Virginia.² ¹Natural Resource Ecology Laboratory, Colorado State University, Fort Collins, CO 80523, and ²Environmental Studies Program, Dartmouth College, Hanover, NH 03755.

We present a conceptual model that relates soil invertebrate biodiversity to the soil and environmental conditions in hot deserts and cold deserts (Antarctica). If soil invertebrates disperse to all locations, then communities of soil organisms should develop in all suitable habitats. In hot deserts, all soils examined contain nematodes from many trophic levels, including plant-feeding species. Their distribution and abundance is closely linked with plant distribution, spatial patterns of litter accumulation, the distribution of roots, and soil organic matter and nitrogen. In contrast,

only 65% of the Antarctic soils support invertebrates (tardigrades, rotifers, nematodes). There are no other soil systems known where nematodes represent the top of the food chain and where food webs appear so simple in structure. There appears to be no single soil property that defines a suitable or unsuitable habitat (invertebrates absent) in either desert system. The low diversity and low functional redundancy of the Antarctic soils, and to a lesser extent, hot deserts, suggests that these systems will be highly disrupted by the loss or decline of even a single species that is sensitive to environmental change.

PHORESY BETWEEN MYOLAIMUS HETERURUS AND A CRANE FLY, LIMONIA (RHIPIDIA) SCHWARZI. Giblin-Davis, R. M. University of Florida, 3205 College Avenue, Ft. Lauderdale, FL 33314-7799.

Myolaimus heterurus (Rhabditida: Myolaimidae) was found in cocoons of the weevil, *Metamasius hemipterus* (Coleoptera: Curculionidae) from the crown shaft of a live spindle palm, *Hyophorbe verschafeltii*, in southern Florida. *Myolaimus heterurus* adults were isolated from decaying palm tissue and xenically cultured on 1/10 tryptic soy broth agar (TSB). A variety of different invertebrates were dissected from the decomposing palm tissue, including all stages of *M. hemipterus*, rove beetles, earwigs, woodroaches, and millipedes, but only a crane fly, *Limonia (Rhipidia) schwarzi* (Diptera: Tipulidae), was associated with dauerlarvae of *M. heterurus*. Dauerlarvae were collected from the intersegmental abdominal folds of 42% of males (n = 48, range of dauerlarvae per host = 2–296, mean = 73) and 16% of females (n = 25, 1–190, 54) that were reared from decomposing palm tissue in screened cages. Dauerlarvae of *M. heterurus* were found on the cuticles of larvae and pupae of *L. schwarzi*, but were not found in the hemocoel of these stages. Dauerlarvae from crane flies were culturable to adults on TSB agar. The association appears to be phoretic.

TEMPORAL ASSOCIATION OF ENTOMOPATHOGENIC NEMATODES AND BACTERIA. Gouge, D. H., J. R. Van Berkum, L. L. Lee, and T. J. Henneberry. USDA ARS, Western Cotton Research Laboratory, Phoenix, AZ 85040.

Galleria mellonella larvae were infected with ten species or strains of *Steinernema* spp. and with *Heterorhabditis* spp. in petri dishes. Every six hours over a 48-hour period, larvae were dissected dorsally. Hemolymph was collected with a sterile loop and streaked on tryptic soy agar plates. Each of the ten nematode strains was incubated at 22, 27, and 32 °C. Subsequently isolated bacterial colonies were grown for 48 hours at 27 °C. Contaminants of insect origin were identified as *Salmonella* ssp. 1G and *Xanthomonas campestris* PV *viscatoria* B. After nematode application, bacteria identified from insect cadavers were *Enterobacter gergoviae*, *Vibrio* spp., *Pseudomonas fluorescens* type C, *Serratia marcescens*, *S. liquifaciens/grimesii*, and *Citrobacter freundii*. After 24 to 30-hour incubations, the primary symbiont occurred almost exclusively with the exception of *H. megidis* (M-145), in which the primary symbiont disappeared rapidly and was replaced after 24 hours by *S. liquifaciens/grimesii*. Although suppression occurred after 24 to 30 hours, these bacteria remained viable. Secondary associates generally occurred after the 24 to 30-hour period, often before the primary symbiont. Primary symbionts developed earlier at higher temperatures except for those of *S. feltiae* (St. 27) and *H. megidis* (M-145). Two nematode strains carrying only the primary symbiont (*S. riobravus* St. 355 and *H. bacteriophora* Cruiser) came from monoxenic, in vitro-produced commercial products. We conclude that not all *Galleria* mortality in entomopathogenic nematode bioassays is caused by the associated bacterial symbiont.

DEVELOPMENT OF DITERA®—A BIOLOGICAL NEMATOCIDE. Grau, P., L. Rehberger, R. Hopkins, and P. Warrior. Abbott Laboratories, Agricultural Research Station, 17683 Avenue 6, Madera, CA 93637.

DiTera is a novel biological nematicide discovered and developed by Abbott Laboratories. It is produced by the submerged fermentation of the hyphomycete fungus *Myrothecium* spp.; the tech-

nical active ingredient has been formulated into stable liquid and granular formulations. The product exhibits very specific activity against various genera of plant-parasitic nematodes associated with several crops. Besides a direct kill of nematode juveniles and adults, DiTera inhibits hatching of nematode eggs. DiTera recently has been registered under the microbial guidelines by the U.S. EPA. An exemption from tolerance on all commodities also has been granted. Biological evaluations under field conditions have indicated that DiTera reduced damage due to nematodes on banana (*Musa* spp.), cole crops (*Brassica* spp.), and grape (*Vitis* spp.). Recent field trials have shown that at field rates of 28 to 112 kg/ha (25 to 100 lb/a), DiTera reduced nematode populations comparable to reduction by chemical nematicides. Current efforts are directed at formulation development and large-scale trials on banana, cole crops, grape, and turf.

EFFECT OF A NEMATODE-RESISTANT FODDER RADISH ON SOIL POPULATION DYNAMICS OF *HETERODERA SCHACHTII* IN SUGARBEET-BARLEY ROTATIONS. Gray, F. A., D. W. Koch, L. Yun, and J. M. Krall. Department of Plant, Soil and Insect Sciences, University of Wyoming, Laramie, WY 82071.

Cultivars of sugarbeet nematode-resistant fodder radish have been developed in Germany in response to the removal of nematicides due to groundwater contamination. When radish is planted in the fall following harvest of small grains, the soil population of *Heterodera schachtii* is reduced. Studies were conducted in Wyoming during 1992–1995 to evaluate the feasibility of integrating nematode-resistant radish into a malting barley-sugarbeet rotation. Effect of radish on soil population dynamics of *H. schachtii* during the rotation and the impact of radish on sugarbeet yield were determined at four sites. Initial soil populations of *H. schachtii* varied from 2.9 to 18.3 eggs/cm³ soil. Growing-degree-days (GDD) (base 4.4°C) following radish planting varied from 634 to 977, while reduction in nematode density following the radish crop varied from 19% to 75%. High number of GDD and control of volunteer barley, which provided maximum radish growth, resulted in the greatest reduction of *H. schachtii* and the greatest increase in sugarbeet yield. Increase in the soil population of *H. schachtii* during the sugarbeet crop was slower following the radish treatment, compared to the fallow treatment.

SENSING OF ATTRACTANTS IN A HETEROGENEOUS ENVIRONMENT BY NEMATODES: EXPERIMENT AND SIMULATION. Griffiths, B. S.,¹ I. M. Young,¹ A. R. A. Anderson,¹ B. D. Sleeman,² and W. M. Robertson.¹ ¹Scottish Crop Research Institute, Dundee DD2 5DA, and ²Department of Mathematics and Computer Science, University of Dundee, Dundee DD1 4HN, United Kingdom.

Nematodes rely on the sensing of chemical gradients to detect and locate food sources, and this is complicated by the heterogeneous structure of soil. Studies on chemotaxis have tended to ignore soil structure, because of the difficulties in observing soil processes at the micro-organism scale, and the lack of available systems to simulate such complex interactions. We have isolated the effect of soil structure in chemotaxis through a combined experimental and theoretical approach. At small scales structure slows gaseous diffusion and partitions gradients into discrete regions of high and low concentration. Structure also acts to impede nematode response to volatiles and also, under certain circumstances, guides the nematode through the gaseous gradients. These observations show how soil structure acts on processes, at two scales, in significantly different ways.

MODE OF REPRODUCTION AMONG ISOLATES OF *ROTYLENCHULUS RENIFORMIS* IN HAWAII. Gu, Y.-H., and B. S. Sipes. Department of Plant Pathology, University of Hawaii, Honolulu, HI 96822.

Virgin females from three populations of *Rotylenchulus reniformis* (from Oahu, Maui, and Molokai) were added to tomato plants. To half of the tomato plants, 25 males were also added. One month later, eggs were extracted from the tomato plants and counted. Eggs were collected on all

plants inoculated with males and females and on 11 of 12 plants inoculated with females only. A χ^2 test of the hypothesis that parthenogenetic reproduction occurred could not be rejected for any population. The number of eggs collected from plants with females only was low (241 eggs/plant) compared to the number of eggs from plants inoculated with both females and males (1909/plant). This suggests that only a small portion of females can reproduce amphimictically.

ANTAGONISTIC POTENTIAL OF RHIZOBACTERIA FOR CONTROL OF *PRATYLENCHUS PENETRANS* ON FRUIT CROPS. Hackenberg, C., A. Muehlchen, T. Forge, and T. Vrain. Agriculture and Agri-Food Canada, Summerland, BC V0H 1Z0, Canada.

Pratylenchus penetrans is a major pest of fruit crops of the Okanagan Valley (British Columbia, Canada). We evaluated bacteria isolated from roots of apple trees and local native plants, as well as selected plant growth-promoting rhizobacteria from various collections, for their potential to affect this nematode. Bacterial suspensions were drenched on roots of strawberry, raspberry and apple seedlings at planting time in *P. penetrans*-infested soil. Plants were grown for three months in a greenhouse, and numbers of *P. penetrans* in roots and soil were measured. A strain of *Pseudomonas chlororaphis* repeatedly and significantly reduced nematode numbers in roots of strawberry. An antibiotic-resistant mutant strain of *P. chlororaphis* was used to determine its durability in the rhizosphere. The density of this bacterium slowly declined after inoculation, but it was still present in the rhizosphere of strawberry plants 3 months after inoculation.

THE EFFECT OF COMPOSTING SUGAR BEET TARE DIRT ON VIABILITY OF SUGAR BEET CYST NEMATODE. Hafez, S.,¹ and R. Rynk.² ¹Plant, Soil and Entomological Sciences, and ²Biological and Agricultural Engineering, University of Idaho, Parma, ID 83660.

Tare dirt is the accumulated soil and plant residue collected during harvest and prior to processing. Because tare dirt contains nematodes and other plant pathogens, it cannot be returned to agricultural land. Composting is a potential way to eliminate nematodes and convert tare dirt to a useful agricultural soil amendment. Various methods of composting tare dirt, with and without dairy manure or onion culls, were investigated. The composted tare dirt was examined for the presence of the sugar beet cyst nematode, *Heterodera schachtii*. The results indicated that composting could effectively eliminate nematodes from tare dirt. In two of three years, no nematodes were found in any combination of raw materials and composting methods. Since temperatures in all compost piles in all years reached only moderate levels, factors other than high temperature probably are involved in elimination of nematodes. Composting converted the tare dirt to a material of consistent-texture, similar to rich topsoil and free of beet pieces and plant residue.

TYPE SPECIMENS ON DEPOSIT IN THE UNITED STATES DEPARTMENT OF AGRICULTURE NEMATODE COLLECTION. Handoo, Z., A. M. Golden, and D. Ellington. USDA ARS, Nematology Laboratory, Beltsville, MD 20705.

A list of the deposited type specimens, a detailed historical background, importance, maintenance procedures, and policies regarding the loan of type specimens are given for the type collection of the United States Department of Agriculture Nematode Collection. The type specimen section is one of the largest and most valuable in existence. It contains 1,430 species mounted and preserved on 5,177 metal and glass slides, and 404 vials. Including the type section, the other constituent divisions of the collection contains 34,000 permanent slides and vials, and 19,500 species entries. This list of deposited types is only a type specimen location reference and is not to be used for the status of type species. The complete title of the reference is not given for each species, only the author date, source, slide number(s), and authors(s) of designated types other than those of the original type. This collection preserves the type specimens of nematodes to serve as reference for research and identifications, and provides useful information on nematode hosts, occurrence and distribution.

ARABIDOPSIS THALIANA MUTANTS WITH ALTERED SUSCEPTIBILITY TO INFECTION BY *HETERODERA SCHACHTII*. **Hardy, K.,¹ H. Su,¹ S. R. Rodermel,² and T. J. Baum.¹**
¹Department of Plant Pathology and ²Department of Botany, Iowa State University, Ames, IA 50011.

Analysis of mutant plants has great potential for identification of genes involved in the cyst nematode-plant interaction. Ethyl methanesulfonate mutants of *Arabidopsis thaliana*, ecotype Columbia, with altered susceptibility to *Heterodera schachtii* infection were identified with a novel screening procedure. Individual M₂ plants were grown and inoculated under tissue culture conditions, which allowed in vivo observation of nematode infection and development. Mutant plants with altered infection frequencies or nematode development, relative to wild-type plants, were chosen and their progeny were rescreened to identify *bona fide* mutants. The screen revealed mutants with reduced nematode infection and development. However, plants that supported nematode frequencies higher than wild-type plants also were identified. Mutant plants currently are being back-crossed to wild-type plants, and mutant loci will be mapped in preparation for positional cloning.

GENETIC DIVERSITY IN INSECT-PARASITIC NEMATODES (RHABDITIDA: HETERORHABDITIDAE). **Hashmi, G., and R. Gaugler.** Department of Entomology, Rutgers University, New Brunswick, NJ 08903-0231.

Knowledge about the genetic structure of various species and populations of entomopathogenic nematodes is limited. We determined genetic variability within and between populations of seven *Heterorhabditis* species with random amplified polymorphic DNA (RAPD) markers. Mean percent similarity between different species, conspecific species, and different populations was 31.3%, 96.3%, and 83.8%, respectively. The banding patterns produced by RAPDs correlated well with described morphological classification. However, *H. hawaiiensis* could not be separated from *H. indicus*, nor *H. marelatus* from *H. hepialus*. RAPD markers were cloned and variations were analyzed by southern blot hybridization. Species-specific markers were identified for *Heterorhabditis bacteriophora*, *H. megidis*, *H. indicus*, *Steinernema feltiae*, *S. carpocapsae*, and *S. riobravus*. This report suggests that the molecular variability within and between populations will be useful for understanding the genetic structure of these important nematodes.

SEARCH FOR TRANSPOSON IN INSECT-PARASITIC NEMATODES. **Hashmi, G., and R. Gaugler.** Department of Entomology, Rutgers University, New Brunswick, NJ 08903-0231.

Insect-parasitic nematodes are close relatives of the free-living nematode *Caenorhabditis elegans*. *Heterorhabditis* and *Steinernema* species are the only nematodes that have evolved the ability to carry symbiotic bacteria. Efforts are ongoing to identify transposons in these nematodes. Extensive screening of five *Heterorhabditis* species containing several different isolates and nine *Steinernema* species were unsuccessful using Tc2-Tc5 as probes in Southern hybridization. Weak hybridization signals were observed with Tc3 in three isolates of *H. bacteriophora* and in four *Steinernema* species. PCR amplification was used to isolate putative transposons with similarity to the *C. elegans* transposon Tc1. PCR with a single primer resulted in the amplification of several products (1.0–1.8 kb) in different *Heterorhabditis* species. Several of these products were cloned and used to screen genomic DNA of *Heterorhabditis* species. Two clones, Tht-1 and Tht-2, were selected as being putative transposons. Homology of these elements with *C. elegans* was studied. These elements show a widespread distribution in different *Heterorhabditis* species and also are detectable in *C. elegans*.

GENETIC TRANSFORMATION OF ENTOMOPATHOGENIC NEMATODES. **Hashmi, S., and R. Gaugler.** Department of Entomology, Rutgers University, New Brunswick, NJ 08903-0231.

We have made significant progress in the genetic transformation of entomopathogenic nema-

todes. Our work has been facilitated by the enormous advances made in the free-living nematode, *Caenorhabditis elegans*, at both the genetic and molecular levels, especially in genetic transformation. We generated three different lines of transgenic *Heterorhabditis bacteriophora* carrying *C. elegans* *hsp16/lacZ*, *pGFP/mec4*, and *hsp70 A* genes. A transgenic *H. bacteriophora* strain for increased thermotolerance was developed by introducing a heat-shock protein gene (*hsp70 A*). The transformation is extrachromosomal yet heritable, and has retained stability for 15 generations. The transgenic nematode was field-released in 1996. We developed a simple, inexpensive, and efficient transformation system that makes use of arrays of microprobes fabricated using silicon machining technology. Genetic engineering shows early promise for the improvement of entomopathogenic nematodes. The development of transgenic strains will provide powerful new tools for the study of entomopathogenic nematode biochemistry, physiology, and ecology.

BIOLOGICAL CONTROL OF *MELOIDOGYNE ARENARIA* AT EPCOT, DISNEY WORLD. Hewlett, T. E.,¹ A. C. Schuerger,² and D. W. Dickson.¹ ¹Entomology and Nematology Department, University of Florida, Gainesville, FL 32611-0620, and ²The Land, EPCOT, P. O. Box 10000, Lake Buena Vista, FL 32830.

Pasteuria penetrans has been shown to control root-knot nematodes in heavily infested sites. Our objective was to determine if the bacterium would effectively control a heavy infestation of *Meloidogyne arenaria* race 2 on bean cv. Scarlet in The Land Pavilion at EPCOT. *P. penetrans* was added to steam-pasteurized soil as a root-powder formulation to form a concentration of 25,000 endospores/g of soil. This inoculum was added to infested sites in The Land and bean seedlings were transplanted. After 3 months, the plants were harvested and assayed. Data have been collected for three crop cycles. At the end of the third crop cycle the average gall index per plant had dropped from the initial index of 6.0 to 1.3 (10 = 100%, 1 = 10% of root system galled), and 62% of the second-stage juveniles (J2) sampled from *P. penetrans*-infested areas had endospores attached (mean = 28 endospores/J2). The percentage of females filled with endospores extracted from roots at specified sites ranged from 10% to 100%. *P. penetrans* increased rapidly in the site and is effectively controlling *M. arenaria* in more than 80% of the original infested area.

IN VITRO CULTURE AND FEEDING BEHAVIOR OF *BELONOLAIMUS LONGICAUDATUS* ON EXCISED *ZEA MAYS* ROOTS. Huang, X., and J. O. Becker. Department of Nematology, University of California, Riverside, CA 92521.

A greenhouse population of the sting nematode, *Belonolaimus longicaudatus*, obtained from an infested golf course in California's Coachella Valley, was surface-decontaminated and cultured on excised roots of *Zea mays* supported by Gamborg's B5 medium. At 26 to 27 °C females laid eggs and newly emerged juveniles of the second generation completed three molts within 29 days after egg deposition. Sixty days after inoculation with 60 females and 40 males, an average of 529 nematodes and 83 eggs were collected from the culture. The feeding process consisted of probing, stylet penetration, ingestion, and stylet retraction. Feeding seemed to be necessary before egg deposition or molting occurred. The sting nematode was observed feeding exclusively as an ectoparasite and preferably at the region of cell division and elongation. Vigorous feeding by many nematodes usually caused discoloration of root tips and termination of growth, with proliferation of lateral roots near the root tip. This population of sting nematodes has been continuously cultured in vitro for more than 10 months.

THE IN VITRO LIFE CYCLE OF *BELONOLAIMUS LONGICAUDATUS*. Huang, X., A. de Bever, and J. O. Becker. Department of Nematology, University of California, Riverside, CA 92521.

Effects of host tissue, nutrient medium, agar concentration, and temperature on in vitro reproduction of *Belonolaimus longicaudatus* were investigated. The optimum reproduction of *B. lon-*

gicaudatus occurred on *Zea mays* excised roots supported by Gamborg's B5 medium in 1.5% agar at 28 °C. Under these conditions the second stage juveniles (J2) fed and started molting to third-stage juveniles 2 days after inoculation (DAI), to fourth-stage juveniles 7 DAI, to males 13 DAI or to females 14 DAI. Nematode gender could be discerned by the end of the last molt. The first male appeared 15 DAI, and the first female 17 DAI, after which mating occurred. The fertilized females began to lay eggs 19 DAI. Feeding took place between each molt and before egg deposition occurred. Sexual reproduction continued with only the initial mating necessary during the female's life. The first-stage juveniles molted in the eggs 4 days after deposition, and J2 hatched from eggs 5 days after deposition. The life cycle from J2 to J2 was completed in 24 days.

FOOD QUALITY PROTECTION ACT OF 1996 AND ITS EFFECT ON NEMATOLOGY.

Huettel, R. N. United States Department of Agriculture-Cooperative State Research, Education, and Extension Service (USDA-CSREES), 901 D Street SW, Washington, D.C. 20250.

Implementing the "Food Quality Protection Act of 1996" will result in major changes in pesticide use on food crops. The nematology community will experience either complete losses in use or severely restricted uses of some of the few remaining chemical control agents now available. These agents include organophosphates, such as fenamiphos, and carbamates, such as carbofuran. Users may have to find alternatives more tailored to fit into integrated pest management programs. The focus of the law is to protect infants and children from pesticide risks, expand the public's right-to-know about pesticides, and use the best science in reaching regulatory decisions. To meet the challenges of the law, USDA-CSREES will focus on integrated pest management to develop and implement new pest management approaches to solve disease, insect, nematode, and weed problems through support of science and education-based solutions.

REACTIONS OF 20 PLANT CULTIVARS TO ROOT-KNOT NEMATODES IN EGYPT. **Ibrahim, I. K. A.**

Department of Plant Pathology, College of Agriculture, Alexandria University, El-Shatby, Alexandria, Egypt.

The reactions of 20 plant cultivars to *Meloidogyne incognita* (MI) and *M. javanica* (MJ) were determined in the greenhouse. Sunflower cultivars Haysun, Mayak, Giza 1, Molitchen, and H 101 G; kidney bean cultivars Giza 3, Giza 4, and Mareglobe; tomato cultivars Ace, Money Maker, Peto 86, and VF 145; and flax cultivars Giza 5, Giza 6, Giza 7, and Giza 8 were susceptible to both nematode species except flax cultivars Giza 7 and Giza 8, which were moderately susceptible to MJ. On the other hand, flax cv. Natasha and cotton (*Gossypium barbadense*) cultivars Giza 75, lupin cv. Giza 1, and sesame cv. Giza 2 were resistant to both MI and MJ.

A NEMATODE HAZARD INDEX. **Imbriani, J.,¹ and K. R. Barker.²** ¹Agronomic Division, North Carolina Department of Agriculture, Raleigh, NC 27607-6465, and ²Plant Pathology Department, North Carolina State University, Raleigh, NC 27695-7616.

Nematode management recommendations based on estimates of population density relative to damage threshold values are limited in their practical application. A flexible hazard index system was developed and implemented for North Carolina. Indexes and corresponding damage potentials are as follows: 0–19, very low; 20–39, low; 40–59, moderate; 60–79, high; and 80–100, very high. An index is assigned to a population density range based on damage potential of taxon, time of sampling, precision of damage function, and other factors. When the level of confidence is high, the hazard-index range is narrow; when low, the range is broad (e.g., 5–15 for a cotton *Rotylenchus reniformis* population of 10 to 499/500 cm³ soil vs. 15–50 for an unknown *Meloidogyne* population of 750 to 3,999/500 cm³). Highly destructive host-nematode combinations, such as *Belonolaimus longicaudatus* on peanut, generate a high hazard index even at low densities.

NEMAREC: A COMPUTERIZED PREDICTIVE NEMATODE-MANAGEMENT RECOMMENDATION SYSTEM. Imbriani, J.,¹ S. Saxena,² and D. Burns.² ¹Agronomic Division, North Carolina Department of Agriculture, Raleigh, NC 27607, and ²Keane, Inc., Durham, NC 27713.

An automated nematode-management system was designed and implemented for North Carolina. Nematode counts are entered on portable computers with keyboards mapped so each key represents a nematode taxon. Data are written to a database. Database packages are used to perform table look-ups according to a sample information model. The model considers alternative crops to be planted, crop and cultivar grown the last two seasons, location of farm (county), and sampling date. Crop-specific tables are constructed containing all of the possible combinations of nematode taxa and population densities. Sample recommendations are selected by reference to these tables. NemaRec assigns hazard indexes to each nematode species depending on population density and identifies explanatory documents to be sent with the report.

RECUTIL: A UTILITY FOR CUSTOMIZATION OF A COMPUTERIZED, NEMATODE-MANAGEMENT SYSTEM. Imbriani, J.,¹ S. Saxena,² and D. Burns.² ¹Agronomic Division, North Carolina Department of Agriculture, Raleigh, NC 27607-6465, and ²Keane, Inc., Durham, NC 27713.

A Windows™-based utility was designed for easy entry and modification of parameters used in computer-generated, nematode-management recommendations. System reference tables include crop, county, hazard indexes, and nematode taxa. Recommendation groups are created by sequential selection of one or more entries from the reference tables. The order of selection is next crop, county, previous crop, and crop before last. Relevant population density ranges are specified for each group. Recommendations are assigned to all possible combinations of taxa and population ranges. Recommendation groups are linked to a hazard index for each taxon population range. The utility also allows designation of conditions that will block report completion, such as samples requiring special extraction techniques or species identification.

MANAGING THE SOIL FOODWEB TO BENEFIT PLANT GROWTH: CARE AND FEEDING OF SOIL MICROHERDS. Ingham, E. R. Department of Botany and Plant Pathology, Oregon State University, Corvallis, OR 97331-2902.

The complexity of the soil foodweb can influence plant growth. Alteration of soil foodweb components (soil microherds), such as the loss of certain types of nematodes, fungi, protozoa, or bacteria, can lead to alteration of the plant community. Is there an appropriate soil foodweb structure which will increase or enhance growth of particular plants? Principles for assessing this possibility and examples of successful application have been developed.

CONTROL OF *MELOIDOGYNE CHITWOODI* AND CORKY RINGSPOT IN POTATO WITH METAM SODIUM AND OTHER NEMATICIDES. Ingham, R. E.,¹ and P. B. Hamm.² ^{1,2}Department of Botany and Plant Pathology, Oregon State University, Corvallis, OR 97331-2902, and ²Hermiston Agriculture Research and Extension Center, Hermiston, OR 97838.

In the Columbia Basin of Oregon, water-run metam sodium (WR-MS) at 468 liters/ha (50 gal/a) did not reduce root-knot nematode (RKN, *Meloidogyne chitwoodi*) or corky ringspot (CRS) infection in potato tubers, but the same rate shanked in at a depth of 100 cm controlled both infections. Shank-in MS at 281 liters/ha (30 gal/a) did not control CRS but did control RKN. WR-MS at 468 liters/ha (50 gal/a) plus 1,3 dichloropropene at 187 liters/ha (20 gal/a) controlled both RKN and CRS infection. WR-MS at 468 liters/ha (50 gal/a) plus ethoprop at 13.5 kg a.i./ha (12 lb a.i./a) broadcast and preplant-incorporated significantly reduced symptoms of CRS but had little effect on RKN infection. WR-MS at 468 liters/ha (50 gal/a) plus aldicarb at 3.4 kg a.i./ha (3 lb a.i./a) applied in-furrow reduced both CRS and RKN infection to acceptable levels. WR-MS at 468 liters/ha (50 gal/a) plus three foliar applications of oxamyl at 1.1 kg a.i./ha (1 lb a.i./a) via chemigation reduced RKN infection to acceptable levels and significantly decreased CRS.

INFECTION STRATEGY OF THE ENTOMOPATHOGENIC NEMATODE *STEINERNEMA CARPOCAPSAE*. Ishibashi, N., X.-D. Wang, and E. Kondo. Department of Applied Biological Science, Saga University, Saga 840, Japan.

Penetration and sex ratio of infective juveniles (IJ) of *Steinernema carpocapsae* were investigated using wax moth larvae. Nictation rates of IJ decreased from 22% on day-1 harvest to 18% and 9% on day-4 and day-8 harvests, respectively. Insect mortality declined as time to length of harvest increased. Penetration rates of female IJ harvested during the first 4 days were 18% after 6 hours in previously un-inoculated insects, and 8% in dead insects inoculated 48 hours earlier with a one-on-one exposure method. Male percentages were 69% and 49%, respectively. For insects injected with 50 IJ 0, 3, 6, or 9 hours before exposure to penetrating IJ, penetration rates were 45%, 43%, 21%, and 21% after a 48-hour exposure. The penetration rate into control (previously non-injected) insects was 45%. The results suggest that the nematode has two periods of active host-seeking and quiescence to avoid extinction, male IJ have a higher rate of penetration than female IJ, and IJ earlier established in the host inhibit invasion by later-arriving IJ.

FIELD EFFICACY AND ECOLOGY OF THREE ENTOMOPATHOGENIC NEMATODES WITH THE WESTERN CORN ROOTWORM. Jackson, J. J. USDA-ARS, Northern Grain Insects Research Laboratory, 2923 Medary Ave., Brookings, SD 57006.

Heterorhabditis bacteriophora (Lewiston), *Steinernema carpocapsae* (Mexican), a South Dakota isolate (*Steinernema* sp.), and the insecticide terbufos were evaluated as control agents for the western corn rootworm, *Diabrotica virgifera virgifera*. Nematodes were applied to the soil surface at 200,000/plant when 2nd-instar rootworm larvae were abundant. Nematode densities were highest in the top 5 cm of the soil profile on days 0 and 3 post-application and nearly equal in the 0–5, 5–10, and 10–15-cm depths from days 6 to 18. The South Dakota isolate and *S. carpocapsae* slightly reduced the population of 2nd-instar larvae. All nematodes significantly reduced the population of 3rd-instar rootworm larvae inhabiting the top 10 cm of the profile. The South Dakota isolate, *S. carpocapsae*, and terbufos equally protected roots and crop yield. *Heterorhabditis bacteriophora* effectively suppressed adult emergence and protected crop yield, but allowed more root damage than *S. carpocapsae* or the South Dakota isolate. Overall, the South Dakota isolate was as effective as the insecticide terbufos.

NEMATODES, FUNGI, AND ENCHYTRAEIDS. Jaffee, B. A. Department of Nematology, University of California, Davis, CA 95616-8668.

We observed enchytraeid worms moving on and through pelletized hyphae of several nematophagous fungi (*Hirsutella rhossiliensis* and *Monacrosporium geophyropagum*). A field exclosure experiment was conducted to test the hypothesis that enchytraeids (largely *Enchytraeus crypticus*) reduce the efficacy of these fungi when formulated as hyphae in alginate pellets. Pellets and soil were packed into 'cages' (PVC pipe, 3-cm-wide by 6-cm-long, 80 cm³ volume). The ends of each cage were covered with fine mesh (0.02 mm, too small for passage of enchytraeids) or coarse mesh (0.48 mm, sufficiently large for passage of enchytraeids). Cages were buried 15-cm-deep in field microplots and later recovered; fungus and enchytraeid population densities were determined. When cages had fine mesh, enchytraeids did not enter and fungus population density was large. When cages had coarse mesh, many enchytraeids entered (often > 5 enchytraeids/g of soil) and fungus population density was small. These data support our hypothesis that enchytraeids can interfere with biological control of plant-parasitic nematodes.

PEANUT-COTTON-RYE ROTATIONS AND CHEMICAL SOIL TREATMENT FOR MANAGING NEMATODES. Johnson, A. W., and N. A. Minton. USDA ARS, P. O. Box 748, Tifton, GA 31793.

Crop rotations including continuous peanut, continuous cotton, cotton-peanut, and peanut-cotton with and without rye and soil chemical treatments were conducted from 1988 to 1994. Population

densities of *Meloidogyne incognita* and *Belonolaimus longicaudatus* declined rapidly after the first crop of peanut in all rotations and remained low. Neither the winter rye cover crop nor applications of aldicarb + flutolanil on peanut and aldicarb on cotton had an effect on *M. incognita* or *B. longicaudatus* population densities. Cotton and peanut yields in the cotton-peanut rotations were 26% and 10% greater, respectively, than yields from monoculture over the 7-year study. Cotton and peanut yields were improved 9% and 4%, respectively, following rye vs. winter fallow. Soil chemical treatments increased yields of cotton 23% and peanut 32% over yields from untreated plots.

USING FUNCTIONAL GENOMICS WITH *CAENORHABDITIS ELEGANS* TO IDENTIFY TARGETS AND LEADS FOR NEW HUMAN THERAPEUTICS. Johnson, C. D. NemaPharm, Inc., Cambridge, MA 02139.

Most human genes have close counterparts in less complex animals, including nematodes. The soil nematode *Caenorhabditis elegans* is (compared to humans) a simple animal whose anatomy, development, behavior, and genome are the most thoroughly understood of any animal. NemaPharm uses *C. elegans*-based technologies that combine rapid isolation of gene knockouts and efficient phenotype analysis with high-throughput compound screening to identify new targets for the treatment of human disease.

EFFECTS OF ENDOPHYTE-INFECTED TALL FESCUE AND CHITINASE ON POPULATION STRUCTURE AND HATCHING OF *PRATYLENCHUS SCRIBNERI*. Jones, C. S., and E. C. Bernard. Entomology and Plant Pathology Department, P.O. Box 1071, University of Tennessee, Knoxville, TN 37901-1071.

Tall fescue and an endophytic fungus form a mutualistic association that leads to tall fescue resistance to many nematodes. Soil in which endophyte-infected (E+) and endophyte-free (E-) plants were growing was infested with *Pratylenchus scribneri*. Root systems were harvested 20, 40, and 60 days after infestation and stained to visualize nematodes and eggs. The experiment was performed three times. At 20 days, total nema numbers did not differ between E+ and E- tall fescue, but at 40 and 60 days numbers were much lower in E+ fescue. Egg and juvenile counts were similar at 20 days, but at 40 and 60 days eggs, J2, and J3 were nearly absent from E+ roots, suggesting that resistance in E+ tall fescue is due to interference with reproduction. In two other experiments, eggs extracted from alfalfa callus were exposed over a 15-day period to buffered aqueous solutions of chitinase in a 4-step dilution series of 0.017 units/ml to 17.7 units/ml, and a water control. Hatch in the control and 0.017–1.7 units/ml chitinase solutions was similar, but no hatching occurred at 17.7 units/ml. Presence of chitinase in E+ tall fescue may inhibit eggshell formation or interfere with normal embryonic development.

CHARACTERIZATION OF A SECRETED PROTEIN FROM THE POTATO CYST NEMATODE *GLOBODERA PALLIDA* WITH HOMOLOGS IN FREE-LIVING AND ANIMAL-PARASITIC NEMATODES. Jones, J. T.,¹ P. R. Burrows,² L. Robertson,¹ L. H. Duncan,¹ P. J. Wightman,¹ J. M. S. Forrest¹, and W. M. Robertson.¹ ¹Department of Nematology, Scottish Crop Research Institute, Invergowrie, Dundee, DD2 5DA, and ²Entomology and Nematology Department, IACR, Rothamsted, Harpenden, Herts, AL5 2JQ, United Kingdom.

Little is known about the molecular nature of the secretions of plant-parasitic nematodes despite their importance in the host-parasite relationship. We have used an antibody that recognizes surface secretions of the potato cyst nematode *Globodera pallida* (PCN) to isolate the gene coding for this molecule from an expression library. The gene, named *gp-sec-2*, is expressed throughout the life cycle of PCN and encodes a protein that has similarity to secreted proteins from animal-parasitic and free-living nematodes. The protein, GPSEC-2, has an extensively helical structure and may function as a binding protein for a range of hydrophobic molecules. Current work is aimed at expression of GPSEC-2 in order to allow functional studies on the expressed protein.

GENOME DIVERSITY AND CITRUS PARASITISM AMONG BURROWING NEMATODES (*RADOPHOLUS* SPP.) FROM HAWAII, CENTRAL AMERICA, AND FLORIDA. **Kaplan, D. T.,¹ and C. H. Opperman.²** ¹USDA ARS, 2120 Camden Rd., Orlando, FL 32803-1419, and ²Departments of Plant Pathology and Genetics, North Carolina State University, Raleigh, NC 27695-7616.

Burrowing nematodes from Central America, Dominican Republic, Florida, Guadeloupe, Hawaii, and Puerto Rico were characterized for their ability to parasitize citrus, but citrus parasites were found only in Florida. Sequence-tag sites originally amplified from a citrus-parasitic burrowing nematode were polymorphic among 38 burrowing nematode isolates and were not correlated with citrus parasitism, nematode isolate collection site, or with amplification of a 2.4kb sequence-tag site (DK#1). Results of a RAPD analysis and characterization of the isozymes phosphoglucose isomerase, lactate, and lactate dehydrogenase indicate that the burrowing nematode isolates were highly similar. Our findings did not confirm the presence of *Radopholus citrophilus* in Hawaii.

ARE HCH-1 GENES FOUND IN PLANT-PARASITIC NEMATODES? **Kay, E., R. I. Bolla, and P. Kozlowski.** Department of Biology, Saint Louis University, 3507 Laclede, St. Louis, MO 63103-2010.

The gene *hch-1* is found in *Caenorhabditis elegans*, where it encodes products involved in hatching and control of cell movement during embryogenesis. It is a member of the Tolloid-BMP gene family and encodes a zinc protease important in cell movement and pattern formation in several organisms, including *Drosophila melanogaster*. It is difficult and expensive to manage soybean cyst nematode (*Heterodera glycines*, SCN) by conventional procedures; thus, efforts are being made to develop biological controls. One such control might involve developing mechanisms to interfere with egg hatching. We have begun studies to understand the mechanisms and control of hatching of SCN eggs so as to design ways to interfere with embryogenesis and/or the normal hatching process. Hatching of SCN eggs is induced by zinc sulfate, suggesting involvement of a zinc-regulated enzyme or other enzyme requiring a zinc cofactor. Therefore, we investigated *hch-1* in SCN and its possible role in hatching. Using PCR primers designed from the *hch-1* gene sequence of *C. elegans* and genomic DNA from SCN eggs, we have identified a gene with homology to *hch-1* in SCN. This gene possibly may occur in other plant-parasitic nematodes.

SOYBEAN GALLING AND YIELD IN *MELOIDOGYNE ARENARIA*-INFESTED SOIL. **Kinloch, R. A.** University of Florida, WFREC, Jay, FL 32565.

Forty-two soybean cultivars and breeding lines were evaluated in similar tests at two field sites infested with *Meloidogyne arenaria* race 2 during 1996. Entries were replicated four times and planted on four-row plots, 7.6 m long on 0.9 m centers, during May. Gallling scores (0 = none; 0.2 = < 5%; 1 = 5–25%; 2 = 26–50%; 3 = 51–75%; 4 > 75% root surface galled) were taken from border row plants during September. Yields (kg/ha) across all entries (Y), taken from center rows during October, were related to gallling scores (X) by $Y = 2628 - 461X$ ($r = -0.6$; $P < 0.01$) at one site and by $Y = 2728 - 594X$ ($r = -0.7$; $P < 0.01$) at the other. Entries that were among the least galled and had among the highest yields at both sites were Northrup-King S65-50 (galling 2.2; yield 1,869), Hartz 798 (1.6; 1,711), and Perrin (2.4; 1,268), whereas Cobb (3.7; 370) and Sharkey (3.8; 333) were among the most galled and least yielding cultivars.

ASSESSMENT OF SELECTED PEANUT CULTIVARS FOR SUSCEPTIBILITY TO *MELOIDOGYNE ARENARIA*. **King, P. S., R. Rodríguez-Kábana, and D. G. Robertson.** Department of Plant Pathology, Auburn University, AL 36849-5409.

A nematode population dynamics study to compare the susceptibility of six peanut cultivars to the root-knot nematode *Meloidogyne arenaria* was conducted at the Wiregrass Substation near Headland, Alabama. The six peanut cultivars tested were Florunner, Andru 93, GK-7, AT-108, Sun

Oleic, and Southern Runner. Soil samples were collected to assess nematode populations at six intervals during the season, ranging from 62–140 days after planting. Nematode development in Sun Oleic and Southern Runner reached 50% of their maximum populations in less than 80 days after planting. Florunner, Andru 93, GK-7, and AT-108 reached 50% of their maximum populations at approximately 100 days after planting. Although there is no resistance to *M. arenaria* in these cultivars, slower rates of nematode development may indicate some tolerance.

A SIMPLIFIED VIDEO TELECONFERENCING SYSTEM FOR DISTANCE EDUCATION IN NEMATOLOGY. Kirby, H. W. Department of Crop Sciences, University of Illinois, Urbana-Champaign, IL. 61801.

A graduate level course in plant nematology was taught at three remote sites in Illinois in the fall of 1996 using a video teleconferencing system (VTS) and on-campus laboratory sessions. The system is a commercial program marketed as Vis-a-Vis and consists of a data bridge and teleconferencing program to permit multipoint conferencing with interactive audio. This VTS system is compatible with any Windows-based computer system and uses ordinary telephone lines for communication with the data bridge located at the University of Illinois. Students were provided with monitors, keyboards, and an audio link, as well as course lecture outlines. Two laboratory sessions were held on campus on Saturdays to provide experience in extraction, identification, and handling of plant-parasitic nematodes.

SEASONAL POPULATION VARIATION OF *XIPHINEMA AMERICANUM* WITH DIFFERENT CROPPING SYSTEMS OF CORN AND SOYBEAN. Kirby, H. W.,¹ and D. C. Feltes.²

¹Department of Crop Sciences and ²Illinois Cooperative Extension Service, University of Illinois, Urbana, IL. 61801.

An experiment was conducted in 1995 and 1996 in northwestern Illinois to monitor population dynamics of *Xiphinema* sp. in a sandy soil (89% sand). Plots were sampled monthly beginning in May (preplant) and continuing until October (harvest). Cropping systems were: i) continuous corn, ii) continuous corn treated with terbufos (Counter CR formulation at 1.5 kg a.i./ha), iii) corn with terbufos followed by soybeans, and iv) corn-soybean rotation with no terbufos. Populations peaked in July in 1995 and in June in 1996, with the nematicide-treated plots having the highest populations and greatest monthly variation. Continuous corn had the least monthly variation throughout the sampling period.

INFLUENCE OF A *MELOIDOGYNE INCOGNITA*-RESISTANT TOMATO CULTIVAR ON NEMATODE DENSITY AND PERFORMANCE OF DOUBLE-CROPPED CUCUMBERS.

Kirkpatrick, T. L.,¹ and P. D. Colyer.² ¹University of Arkansas Southwest Research and Extension Center, Hope, AR 71801, and ²Louisiana State University Red River Research Station, Bossier City, LA 71113.

Fresh market vegetable producers in the Red River Valley of Arkansas and Louisiana routinely double-crop cucurbits after a spring tomato crop. *Meloidogyne incognita* (Mi) is a limiting factor in this system. Mi population increases on the tomato crop have been particularly damaging to subsequent cucurbit crops. Studies were initiated at the LSU Red River Research Station near Bossier City, LA, to determine the potential of growing Mi-resistant tomato cultivars for lowering Mi population densities prior to the cucurbit crop. The experiment, conducted in 1995 and 1996, was designed as a 2 × 2 factorial with four replications, where treatments were either Mi-resistant 'Celebrity' or susceptible 'Heatwave' tomatoes followed by cucumber ('Dasher II') with the nematicide ethoprop or with no nematicide. In both years, cucumber root gall indices were lower and premium and marketable cucumber yields were higher following Celebrity. Mi population densities also were lower at the termination of the cucumber crop following the resistant cultivar. Root gall indices were lower but Mi population density at cucumber harvest was not affected by ethoprop. Application of ethoprop did not improve cucumber yield or marketability.

ISOLATION AND INFECTIVITY OF ENTOMOPATHOGENIC NEMATODES FROM OHIO. **Klein, M. G., and J. J. Moyseenko.** USDA ARS, Application Technology Research Unit, 1680 Madison Ave., Wooster, OH 44691.

Although golf courses are treated with many pesticides, epizootics of *Heterorhabditis megidis*, *H. bacteriophora*, and *Steinernema carpocapsae* have been noted in larvae of the Japanese beetle, *Popillia japonica*, infesting golf course turf. We surveyed turf sites in northern Ohio over a five-year period and isolated nematodes from about 20% of the samples using the *Galleria* bait technique. Approximately 70% of all isolates were *Steinernema* spp. All isolates of heterorhabditids were *H. bacteriophora*. Despite numerous attempts, we were unable to reisolate *H. megidis* from its original location on a northern Ohio golf course. Most epizootics and the *H. megidis* type site were associated with insecticide stress on the Japanese beetle larvae. No isolates of the scarab pathogen *S. glaseri* were found in northern Ohio. Only five of the 32 isolates of *Steinernema* spp. tested in the laboratory gave more than 75% control of scarab larvae. Nineteen of the 20 *H. bacteriophora* isolates caused greater than 75% mortality. Selected isolates of our Ohio *H. bacteriophora* performed as well in field tests as did commercially available nematode formulations.

CHANGES IN POPULATION DENSITIES OF PLANT-PARASITIC NEMATODES IN COTTON FIELDS AMENDED WITH POULTRY LITTER. **Koenning, S. R., and K. R. Barker.** Plant Pathology Department, Box 7616, North Carolina State University, Raleigh, NC 27695-7616.

The impact of fall or spring application of poultry litter at rates of 4 to 26 t/ha on population densities of plant-parasitic nematodes was evaluated in several North Carolina cotton fields. Mid-season (August) population densities of *Hoplolaimus columbus* were negatively related to the amount of poultry litter applied, but end-of-season numbers of this nematode generally were not. Similarly, numbers of *Meloidogyne incognita* at midseason were inversely related to the amount of poultry litter applied, whereas J2 numbers at cotton harvest were positively related to the rate of litter application. Numbers of *Paratrichodorus minor*, *Helicotylenchus dihystra*, and *Tylenchorhynchus claytoni* varied widely within these experiments, but were only suppressed at relatively high rates of litter application. The suppression of *H. columbus*, *P. minor*, and *M. incognita* at midseason was accompanied by a significant increase in cotton lint yield. Fall applications of litter were more efficacious than spring applications in affecting suppression of numbers of *H. columbus* one year, but not in the subsequent year.

CONTROL OF *MELOIDOGYNE HAPLA* IN HERBACEOUS PERENNIAL ORNAMENTALS BY SANITATION AND RESISTANCE. **LaMondia, J. A.** Department of Plant Pathology and Ecology, Connecticut Agricultural Experiment Station Valley Laboratory, 153 Cook Hill Road, Windsor, CT 06095.

Meloidogyne hapla can be spread in bare-root herbaceous perennial propagation material and may be difficult to control in new fields or in the landscape. Root pruning of bare-root plants was investigated as a means of reducing spread and establishment of *M. hapla*. Plants inoculated two months previously with 10,000 eggs per plant were root-pruned to remove either a portion or most of the fibrous root system without removing underground stems, buds, tubers or tuberous roots. Root-pruning *Aconitum*, *Ajuga*, *Anemone*, *Geranium*, and *Trollius* significantly reduced or eliminated *M. hapla* galls and egg production in plants 1-4 months after propagation. In other trials, planting *M. hapla*-resistant plants such as *Rudbeckia* and *Aster* into pots infested with 10,000 eggs/pot eliminated *M. hapla* populations after 2-6 months of growth. Tomato plants grown after *Rudbeckia* and *Aster* were free of galls and eggs, while bioassay tomato grown after susceptible plants such as *Coreopsis* and *Lobelia* were heavily galled with a large number of egg masses.

PLANT-PARASITIC NEMATODES ASSOCIATED WITH NON-DELTA COTTON PRODUCTION IN MISSISSIPPI. **Lawrence, G. W.,¹ F. Killebrew,² and K. S. McLean.³** ¹Department of Entomology and Plant Pathology, ²Mississippi Cooperative Extension Service, Mississippi State

University, Mississippi State, MS 39762-9775, and ³Department of Agriculture, Northeast Louisiana University, Monroe, LA 71209.

Sixty cotton fields, representing 58,528 ha in fifteen counties in the non-delta region of Mississippi, were surveyed in 1996 for plant-parasitic nematodes associated with cotton production. Four randomly selected cotton fields in each county were sampled, each sample representing approximately 4 ha. *Rotylenchulus reniformis* and *Aphelenchus*, *Criconebella*, *Helicotylenchus*, *Hoplolaimus*, *Meloidogyne*, *Paratrichodorus*, *Pratylenchus*, *Scutellonema*, and *Tylenchorhynchus* spp. were collected in this survey. The most pathogenic taxa identified included *Meloidogyne* spp., *Rotylenchulus reniformis*, and *Hoplolaimus* spp., which were detected in 10%, 15%, and 40%, respectively, of the fields sampled. Sixty percent of the cotton fields sampled contained at least one species of plant-parasitic nematode pathogenic to cotton. Based on 1995 cotton hectareage of our survey area, 35,117 ha contain one or more species of nematodes with the potential to reduce cotton yields.

REACTION OF COTTON TO POPULATION DENSITIES OF *ROTYLENCHULUS RENIFORMIS*. Lawrence, G. W.,¹ and K. S. McLean.² ¹Department of Entomology and Plant Pathology, Mississippi State University, Mississippi State, MS 39762, and ²Department of Agriculture, Northeast Louisiana University, Monroe, LA 71209.

Field microplots were infested with initial population levels (Pi) of 0, 1,000, 2,500, 5,000, 7,500, and 10,000 *Rotylenchulus reniformis* juveniles and vermiform adults per 500 cm³ of soil and planted with 10 seeds each of Delta and Pineland cotton to give 20 plants per microplot. Final nematode population densities generally increased with increasing Pi levels. A Pi of 7,500 produced the highest nematode population of 20,343 nematodes/500 cm³ soil at harvest. Nematode reproduction ($R = \text{final population}/\text{initial population}$) was inversely related to increasing inoculum densities. At harvest, initial inoculum levels were negatively correlated with seed cotton yield. Cotton yields were lower in all plots infested with *R. reniformis*. Yields were significantly reduced by Pi levels of 2,500 and higher. Yield losses of 30 percent were produced in plots that received the at-plant population density of 5,000 *R. reniformis* per 500 cm³ of soil.

END-PRODUCTS OF *MELOIDOGYNE* MITOCHONDRIAL DNA RECOMBINATION. Lunt, D. H., and B. C. Hyman. Department of Biology, University of California, Riverside, CA 92521-0427.

Polymerase chain reaction (PCR) amplification and DNA sequence analysis were used to identify deletions in the non-coding repeat region of the *Meloidogyne javanica* mitochondrial genome. Although genetic recombination is not thought to occur within animal mitochondrial DNA (mtDNA), unusual nucleotide sequence organization at the deletion junctions suggested the involvement of just such a mechanism in the generation of these variant mtDNA molecules. We developed an inverse PCR assay to document the presence of sub-genomic, mini-circular excision products produced during mtDNA deletion that, together with deleted mitochondrial genomes, represented the anticipated end-products of mtDNA recombination. Sequence analysis of cloned mini-circles revealed a sequence organization reciprocal to that of the parent mtDNA molecules that had incurred a deletion. Reciprocity of sequence order among end-products of mtDNA rearrangement events provides the first evidence entirely consistent with genetic recombination in animal mtDNA. This novel finding will impact many areas of biology, require careful interpretation of mtDNA variability, and underscores the utility of phytonematodes as useful animal models.

BURROWING NEMATODE RESISTANCE IN BLACK SIGATOKA-RESISTANT BANANA HYBRIDS. D. H. Marin,^{1,2} K. R. Barker,¹ T. B. Sutton,¹ D. T. Kaplan,³ and C. H. Opperman.¹ ¹Department of Plant Pathology, North Carolina State University, Raleigh, NC 27695-7616;

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Resistance of black Sigatoka-resistant banana hybrids FHIA-01, FHIA-02, FHIA-03, and FHIA-21 to the burrowing nematode, *Radopholus similis*, was assessed under greenhouse conditions. Potential interactions of FHIA hybrids and *R. similis* populations from Honduras and Costa Rica also were evaluated. Each hybrid plant was inoculated with 200 monoxenically-reared nematodes. Banana plants produced by tissue culture were grown in 0.4-liter styrofoam cups containing a 1:1 mix of coarse and fine sand at 25 °C and 80% RH. Plants were held in acclimation for 3 to 4 weeks prior to inoculation. Plant height, fresh shoot and root weights, nematode population levels, and root necrosis indices (0–100) were determined 8 weeks after inoculation. *Pisang jaribuaya* (accession III-106) and Grande Naine (*Musa* AAA, Cavendish subgroup) were used as resistant and susceptible controls, respectively. Nematode population numbers and root necrosis indices did not differ among FHIA-01, FHIA-03, and the susceptible control (Grande Naine). Although *R. similis* reproduced poorly in *P. jaribuaya*, extensive root necrosis (index value ~50%) was associated with this pathogen. The Costa Rican *R. similis* reproduced more rapidly on banana than the Honduran nematodes, but both caused similar root damage.

A COMPETITIVE ELISA FOR DETECTION AND QUANTIFICATION OF FMRFAMIDE-LIKE PEPTIDES IN FREE-LIVING AND PARASITIC NEMATODES. **Masler, E. P.,¹ E. S. Kovaleva,² and T. G. Kingan.¹** ¹Nematology Laboratory, USDA ARS, Beltsville, MD 20705-2350, and ²Department of Zoology, University of Maryland, College Park, MD 20742.

A peptide family with a characteristic RFamide C-terminus ("FMRFamides") and possessing myoactive properties has been reported in both free-living and animal-parasitic nematodes. Primary structures reported thus far probably represent only a fraction of those present in these animals. Immuno-screening typically has been done with radioimmunoassays. We have developed an enzyme-linked immunosorbent assay (ELISA), which detects 1 fmol FMRFamide, as an additional biochemical tool. The assay has been used to monitor FMRFamide-like activity in extracts of *Panagrellus redivivus* (mixed developmental stages) and in subsequent chromatographic fractions. To facilitate characterization of new peptide sequences, we are developing an isolation protocol for FMRFamide-like peptides from *P. redivivus* and other free-living and plant- and animal-parasitic nematodes using the ELISA screen.

ENTOMOPATHOGENIC NEMATODES AGAINST FOLIAR PESTS: DEVELOPMENT OF APPLICATION TECHNOLOGY. **Mason, J. M., M. N. Patel, and D. J. Wright.** Department of Biology, Imperial College of Science, Technology and Medicine, Silwood Park, Ascot, Berkshire, SL5 7PY, United Kingdom.

Successful application of entomopathogenic nematodes against foliar pests has been limited. Three major abiotic factors (temperature, desiccation, and UV radiation) are generally considered limiting factors in successful use of these nematodes. However, further emphasis needs to be placed on spray application technology and correct timing of applications. Low-volume application technology has great potential for application of entomopathogenic nematodes against pests in crucifer-lepidopteran pest complexes in the tropics. One aspect of this technology is optimization of nematode application with spinning disc spraying systems. With these systems, both the size of the droplets produced and spray coverage can be manipulated. Spinning disc systems can be calibrated over a wide range of nematode concentrations and sizes, with infective juveniles ranging in length from 500 to 1000 µm. The effects of adjuvants on the efficiency of low-volume application of entomopathogenic nematodes appears to be variable.

EVALUATION OF THE INVOLVEMENT OF NEMATODES IN CORK OAK DECLINE. **McGawley, E. C.,¹ M. M. Mota,² and M. J. Sousa.²** ¹Department of Plant Pathology and Crop Physiology, Louisiana State University Agricultural Center, Baton Rouge, LA 70803, and ²Departamento de Biologia, Universidade de Évora, 7000 Évora, Portugal.

Cork Oak Decline (COD) is a malady of unknown etiology present throughout the Mediterranean area that is especially widespread and severe in southern Portugal. A total of 81 trees (*Quercus suber* L.) from 10 widely separated geographical sites were sampled during the period of September 1996 to January 1997. Each tree was classified as symptomatic or asymptomatic for COD. Four soil and root samples (15-cm diam. \times 1-m deep) were collected at the drip-line, bulked, and a 500-g subsample processed by the centrifugal-flotation method for extracting nematodes. Additionally, infestation by endoparasitic and dorylaimoid species was determined after 48 hours of incubation on Baermann funnels. There were no differences in either nematode density (21 plant parasites/500 cm³ of soil) or diversity (3 to 5 genera) between symptomatic and asymptomatic trees.

IMPACT OF SYSTEMIC HERBICIDES ON NEMATODES WITHIN WOODY ROOTS. McKenry, M. V., T. Buzo, S. Kaku, and R. Ashcroft. UC Kearney Agricultural Center, 9240 S. Riverbend Ave., Parlier, CA 93648.

In July 1996 the roots in a 20-year-old Lovell Peach orchard, *Prunus persica*, were observed to be heavily galled by root-knot nematode and supporting 10 to 100 juveniles of *Meloidogyne incognita*/g root. For the purpose of killing the old root system before replanting, various systemic herbicides were painted onto cut tree trunks on August 1. Sixty days later portions of the root systems were excavated and assayed for root death and changes in nematode population level. None of our treatments provided visible root death, but populations of *M. incognita* second-stage juveniles (J2) were significantly reduced by each of three treatments across four replicates in the heavily infected portion of the orchard. The untreated trees provided 19 J2/g root whereas roots from trees painted with 50 ml Garlon 3A® (DowElanco) + 25 ml diesel fuel, 50 ml Roundup® (Monsanto) + 25 ml diesel fuel, or 25 ml Roundup + 8 ml fosthiazate + 25 ml diesel fuel produced only 0.05, 0.05, and 1.0 J2/g root, respectively. Population levels of *Meloidogyne* spp. in woody roots may provide a more sensitive bioassay for root death than vital stains or visual assessments.

IMPLICATIONS OF SOIL NUTRIENT MANIPULATION ON NEMATODE MANAGEMENT. Melakeberhan, H., M. E. Kelly, and M. Omer. Department of Entomology, Michigan State University, East Lansing, MI 48824.

The relationship between nematode and soil nutrient dynamics was investigated under greenhouse conditions (25 ± 2 °C) by growing single plants in 800 cm³ sandy loam soil for 5–7 weeks. Daily Hoagland solution (HS) treatment resulted in lower population densities of *Heterodera glycines* and *Meloidogyne incognita* on *H. glycines*-resistant (Bryan) and susceptible (Tracy M) soybean cultivars, as well as higher soil macronutrients, but lower nodulation compared with twice-weekly HS treatment. This suggests that alternative nutrient sources that can increase nutrients to the levels that affect nematodes without decreasing nodulation need to be developed. When water, HS, HS-N, or HS-K were compared, soil NO₃, P, K, Mg, and Ca were the lowest in water and the highest in HS treatment; N and K in roots increased proportionally to source with little effect on other elements; nodulation in water and HS-N treatments was up to eight times more than in HS and HS-K while the amount of NO₃ in soil from water and HS-N was less than 20% of HS or HS-K treatments. The study shows that longer duration and/or alternative sources are needed to accumulate sufficient N levels.

EVALUATION OF 15 TRIFOLIUM SPP. AND OF ALFALFA AS HOSTS OF FOUR ROOT-KNOT NEMATODE SPECIES FOUND IN NEW ZEALAND. Mercer, C. F., and K. J. Miller. AgResearch Grasslands, PB 11-008, Palmerston North, New Zealand.

The dominant root-knot nematode in New Zealand pasture was originally identified as *Meloidogyne hapla* but is now recognized as *M. trifoliophila*. Clarification was needed on the host range of these two species on legumes found in New Zealand pasture. In a glasshouse test, 15 clovers (*Trifolium* spp.) and alfalfa (*Medicago sativa*) were inoculated with eggs of *Meloidogyne trifoliophila*, *M. hapla*, *M. incognita*, or *M. javanica*. All legumes tested were hosts to some degree to each

of the root-knot nematodes used except for *T. striatum* and *M. sativa*, which did not support *M. trifoliophila*. Low galling rates occurred on *T. glomeratum* infected by *M. hapla* (mean of 2% of the root system galled) and *T. semipilosum* infected by *M. trifoliophila* (2%). The most heavily parasitized were *T. repens* infected by *M. trifoliophila* (92%), *T. pratense* infected by *M. incognita* (91%), and *T. argutum* infected by *M. trifoliophila* (80%) and by *M. incognita* (88%).

RHIZOSPHERE ACTIVITY OF THE NEMATODE-ANTAGONISTIC FUNGUS *VERTICILLIUM LECANII*. Meyer, S. L. F.,¹ D. P. Roberts,² and W. P. Wergin.¹ ¹Nematology Laboratory and ²Biocontrol of Plant Diseases Laboratory, USDA ARS, BARC-West, Beltsville, MD 20705-2350.

Strains of the fungus *Verticillium lecanii* have been shown to reduce hatching rates of soybean cyst nematode (SCN, *Heterodera glycines*) eggs and to decrease SCN population densities in greenhouse pots. To determine affinity of the fungus for soybean roots, plugs of mycelium or aqueous spore suspensions of *V. lecanii* were placed on excised soybean roots in Petri dish cultures. The fungus grew in close association with roots in many cultures, with mycelium extending up to 67% of the root length. Scanning electron microscopy indicated that *V. lecanii* also penetrated root cells. In greenhouse and growth chamber studies, soybean seeds were planted in soil contained in plastic cylinders (20 cm high); the cylinders were constructed of rings (each 2 cm in height). The fungus was applied in alginate prills near the seeds. At harvest, soil and root sections from the rings were sampled for *V. lecanii*. Movement of *V. lecanii* in the rhizosphere varied with growth conditions of the plants, ranging from little movement to a 16-cm depth.

COMPARATIVE INFECTIVITY AND FERTILITY IN RFLP PHENOTYPES OF *PRATYLENCHUS COFFEA* ON SWEET POTATO. Mizukubo, T.,¹ and K. Hanada.² ¹Plant Nematology and ²Molecular Plant Pathology Laboratories, Kyushu National Agricultural Experiment Station, Kumamoto 861-11, Japan.

Eleven isolates of *Pratylenchus coffea* differed in sizes of PCR amplification products of rDNA using F194 and F195 primers: ca. 1,080 bp in one Indonesian (type locality) and nine Japanese isolates, and about 1,020 bp in a Guatemalan isolate. Eight Japanese isolates were tested at 26 ± 2 °C for their infectivity on a susceptible sweet potato cultivar (SSWP). Two isolates with higher reproduction than the other six were more virulent as expressed by lesion indices on storage roots of SSWP 90 days after inoculation. The 11 isolates were grouped into four clusters based on DNA fragment patterns obtained after Hinf 1, Alu 1, Dde 1, and Hha 1 endonuclease digestions: A) one nonvirulent and two virulent isolates, and the Indonesian isolate; B) five nonvirulent isolates; C) a tobacco isolate; D) the Guatemalan isolate. Although there was cross-compatibility among the RFLP phenotypes A, B, and C, reciprocal breedings of A and D failed to produce progeny. Inbreeding among the F1 from A × C, A × B, and B × C generated some F2 in 20%, 33%, and 67% of the replications, respectively.

ECOLOGY OF *PARATRICHODORUS ALLIUS*, A TOBACCO RATTLE VIRUS VECTOR, FROM THE PACIFIC NORTHWEST. Mojtahedi, H., G. S. Santo, and C. R. Brown. Washington State University, USDA ARS, Prosser, WA 99350.

Paratrichodorus allius was identified from around roots of various crops, including potato, in Washington and Oregon. Greenhouse studies showed that *P. allius* was sensitive to low soil moisture, and increased 1–30 fold on 20 potato cultivars and breeding clones at high moisture levels. The low moisture regime corresponded to conditions commonly found in commercial potato fields, and may explain why *P. allius* does not increase on field-grown potato. In population dynamics studies, *P. allius* on potato initially declined and remained low throughout the growing season. The population was evenly distributed through the 0–90 cm soil profile, and no evidence of downward migration was observed. In soil columns, *P. allius* migrated only 30 cm upwards

when transmitting tobacco rattle virus to indicator plants. The more deeply placed nematodes transmitted the virus when plant roots were allowed to grow toward the nematodes.

SOIL TILLAGE AND PLANT EFFECTS ON NEMATODE COMMUNITIES IN SOUTHERN PORTUGAL. Mota, M. M.,¹ M. J. Carvalho,² G. Basch,² E. C. McGawley,³ and D. F. Murchio.¹ ¹Departamento de Biologia and ²Departamento de Fitotecnia, Universidade de Évora, 7000 Évora, Portugal, and ³Department of Plant Pathology and Crop Physiology, Louisiana State University Agricultural Center, Baton Rouge, LA 70803.

Roots and soil associated with stunted seedlings of 'Centauro' wheat and 'Joanilho' triticale employed in soil mobilization trials at two locations (Abóboda [A] and Revilheira [R]) in southern Portugal were naturally infested with lesion nematodes (*Pratylenchus thornei* and *Pratylenchus* sp.) and trace numbers of several other plant-parasitic nematode species. Trials compared agronomic benefits of direct seeding (DS) vs. plowing (P). For both crop species at both locations, significantly greater numbers of nematodes per 500 cm³ of soil were associated with DS (A: wheat = 481, triticale = 535; R: wheat = 202, triticale = 428) than with P (A: wheat = 115, triticale = 251; R: wheat = 98, triticale = 223). Soil from A was treated in a subsequent greenhouse test with five replications of five treatments: aldicarb nematicide (N) at 1 g/4 kg-capacity clay pot; metalaxyl fungicide (F) at 0.3 ml/pot; both chemicals (B); untreated control (CK1); soil heated at 90 °C for 8 hours (CK2). Within six weeks after establishment and relative to CK1, there were visible increases in plant height and tillering associated with N and F, and an even greater increase associated with B.

MORPHOLOGICAL AND GENETIC VARIATION AMONG MELOIDOGYNE ARENARIA POPULATIONS IN JAPAN. Narabu, T.,¹ and M. Harada.² ¹National Agriculture Research Center, Tsukuba, Ibaraki 305, and ²Faculty of Horticulture, Chiba University, Matsudo, Chiba 271, Japan.

Three isolates of *Meloidogyne arenaria* race 2 (A2-O, A2-J, and MA1) were identified from 31 populations in Japan. These isolates were differentiated from each other based on perineal patterns, host ranges, esterase phenotypes, and fragment sizes of mtDNA sequences amplified by polymerase chain reaction (PCR). A2-O had perineal patterns typical of *M. arenaria*, the two-isozyme (A2) phenotype, and a 1.1 kb PCR product. A2-J had *M. javanica*-like perineal patterns, the A2 phenotype, and a 1.7 kb product. MA1 had *M. incognita*-like perineal patterns, the one-isozyme (A1) phenotype, and a 1.7 kb product. Only A2-J reproduced on sweet potato cv. K-14. Morphologically, all populations of males were typical of *M. arenaria* based on head shape and stylet structure. Attachment specificity of *Pasteuria penetrans* isolates was also similar in all populations. A2-O and MA1 were detected in limited regions of southern Japan, whereas A2-J was widespread throughout the country except in regions where A2-O occurs.

EFFECTS OF RHIZOBACTERIA ON HATCHING AND ROOT INVASION OF THE SUGAR-BEET CYST NEMATODE, HETERODERA SCHACHTII. Neipp, P., and J. O. Becker. Department of Nematology, University of California, Riverside, CA 92521.

One hundred fifty rhizobacteria strains, isolated from a soil suppressive to the sugarbeet cyst nematode (*Heterodera schachtii*), were evaluated for biocontrol activity against hatching and root infection by second-stage juveniles (J2). The effects of the bacteria on J2 root penetration were initially examined in growth pouches. Microtiter plates or small soil columns were used to determine bacterial effects on hatching. Several isolates were selected for further testing in the greenhouse. Five of eleven isolates tested reduced J2 infection of sugarbeet up to 48% when J2 were used as inoculum. Two isolates that reduced hatching of the nematode in soil columns also reduced root infection of sugarbeet up to 73% when eggs were added as inoculum.

SOIL MOISTURE GRADIENTS AND THE DISTRIBUTION OF NEMATODES WITHIN ALFALFA PLANTS AND FIELDS IN COLORADO. Niles, R. K., and D. W. Freckman. Natural Resource Ecology Laboratory, Colorado State University, Fort Collins, CO 80523.

Alfalfa plants expressing the symptoms of stem nematode disease usually are infected with *Ditylenchus dipsaci* (the disease causal agent) and other nematode species. Small-scale patterns of nematode distribution within plants may be influenced by a large-scale soil moisture gradient within fields. In 1996, we sampled alfalfa fields on linear transects that paralleled water flow. Gravimetric soil moisture related positively to the distance from the head of the field, where flood-irrigation water enters the field. Plant-parasitic nematode species, selected fungal-feeding species, selected bacterial-feeding species, and trophic groups were enumerated in eight microhabitats: alfalfa leaves, stems, crowns, crown necromass, crown soil, rhizosphere soil, bulk soil, and bulk litter. Nematode richness differed among microhabitats, and plant-parasites showed less sensitivity to soil moisture than other nematodes.

DEVELOPMENT OF TECHNIQUES TO ASSAY FUNGUS CULTURE BROTH FOR METABOLITES TOXIC TO ROOT-KNOT AND SOYBEAN CYST NEMATODES. Nitao, J. K., S. L. F. Meyer, and D. J. Chitwood. Nematology Laboratory, USDA ARS, BARC-West, Beltsville, MD 20705-2350.

Fungi of potential use for biological control of plant-parasitic nematodes may reduce nematode populations by secreting toxic metabolites as well as by directly parasitizing hosts. To identify such metabolites, in vitro bioassay techniques were developed to screen fungal cultures for inhibitors of soybean cyst nematode (*Heterodera glycines*) and root-knot nematode (*Meloidogyne incognita*) egg hatch. Methods were developed for use of eggs obtained from greenhouse-grown plants and for fungal cultures grown in two standard media, potato-dextrose broth and Czapek-Dox broth. Media alone strongly inhibited soybean cyst nematode egg hatch when compared to water. Neither adjustment of pH nor addition of zinc sulfate sufficiently countered the inhibition. This necessitated a dilution of the media prior to being tested or the transfer of the eggs to water after exposure to media. In contrast, root-knot nematode eggs were found to hatch at consistent levels in both media, allowing direct screening of fungal broths after sterile filtration and addition of antibiotics.

TILLAGE AND ROTATION EFFECTS ON POPULATION DYNAMICS OF *HETERODERA GLYCINES*. Noel, G. R., and L. M. Wax. USDA ARS and Department of Crop Sciences, University of Illinois, Urbana, IL 61801.

In a 3-year experiment, the population development of *Heterodera glycines* was determined under conventional tillage and no-till production systems planted with *H. glycines*-resistant 'Fayette' and susceptible 'Williams 82' soybean grown in rotation with maize. Ratios of final populations of eggs to initial populations ($R_f = P_f/P_i$) were determined. Under conventional tillage, R_f values for Fayette and Williams 82 were 2.5 and 14.9, respectively. Under no-till, R_f 's were 4.9 for Fayette and 28.6 for Williams 82. In 1995 maize was planted, and R_f values were 0.94 and 0.96, respectively, for conventional and no-till plots planted to Fayette in 1994, and 0.31 for both conventional and no-till plots planted to 'Williams 82' in 1994. In 1996 soybean was planted, and R_f 's under conventional tillage were 9.7 and 5.2 for Fayette and Williams 82, respectively. Under no-till, R_f values were 7.8 for Fayette and 23.1 for Williams 82. R_f values in 1996 indicate that the second cropping of Fayette increased the ability of *H. glycines* to reproduce on 'Fayette'. The effect of tillage on population development was inconclusive.

RELATIVE LETHAL DOSE, A TIME-TEMPERATURE MODEL OF THERMALLY INDUCED MORTALITY OF NEMATODES. Noling, J. W. University of Florida, IFAS, CREC, Lake Alfred, FL 33850.

Suspensions of eggs and juveniles of *Meloidogyne incognita* were immersed in a thermostatically controlled water bath at 43, 46, 49, 52, 54, 57, or 60 °C for exposures of up to 240 minutes.

Nematodes also were subjected to sequential treatments of combinations of higher or lower temperatures of varying times to examine cumulative heat stress effects. Nematode survivorship was estimated by the root-gall index of cucumber plants grown for 45 days in soil infested with tsetse eggs and juveniles. For single-temperature exposures, the root-gall index was well described by negative linear functions between temperature and \log_{10} time. Exposure times necessary to prevent galling of assay plants were less than 4 minutes at temperatures above 50 °C. Combination treatments of sublethal doses were not cumulatively additive in producing a lethal effect. For ambient environments, the relative lethal dose model may prove useful for predicting thermally induced mortality of nematodes.

SUSCEPTIBILITY OF GUARDIAN™ PEACH ROOTSTOCK TO *MELOIDOGYNE* SPP. Nyczepir, A. P.,¹ T. G. Beckman,¹ and G. L. Reighard.² ¹USDA-ARS, Southeastern Fruit and Tree Nut Research Laboratory, 111 Dunbar Rd., Byron, GA 31008, and ²Clemson University, Box 340375, Clemson, SC 29634.

Grower demand resulted in commercial availability of Guardian peach rootstock before all root-knot nematode testing was completed. Preliminary reports suggested that Guardian rootstock was resistant to *Meloidogyne* spp. Guardian-USDA and Guardian-Clemson seed sources were evaluated for their susceptibility to *M. incognita* and *M. javanica*. Host suitability of the two Guardian seed sources and Lovell (susceptible) and Nemaguard (resistant) peach was determined 110 days after inoculation. Both Guardian commercial seed sources were poor hosts for the populations of *M. incognita* and *M. javanica* tested. Reproduction by *M. incognita* and *M. javanica*, as indicated by number of eggs and number of eggs per egg mass, was less on Guardian plants than on Lovell. *Meloidogyne incognita* infective juveniles penetrated Guardian roots and formed galls, but most did not complete their life cycle.

SELF-PROTECTION AND CROP ROTATION VALUE OF RESISTANT COTTON IN THE MANAGEMENT OF *MELOIDOGYNE INCOGNITA*. Ogallo, J. L.,¹ P. B. Goodell,² J. Eckert,² and P. A. Roberts.¹ ¹Department of Nematology, University of California, Riverside, CA 92521, and ²University of California, Kearney Agricultural Center, Parlier, CA 93648.

NemX, a new cultivar of Acala-type upland cotton with high resistance to *Meloidogyne incognita* (Mi), was grown in rotation with Mi-susceptible cotton cultivars Maxxa and Pima S7, lima bean cv. Henderson, resistant alfalfa cv. WL525HQ, and resistant cowpea cv. CB88 during 1994–1996. In three consecutive plantings of NemX, the average preplant nematode population density changed from 143 J2/500 g soil in the first year, to 29 and 17 in the second and third year, respectively. Lint yield of NemX averaged 1,111 kg/ha, and did not vary significantly from year to year. One or two consecutive plantings of NemX had the same effect in protecting the susceptible cotton cv. Maxxa or Lima bean cv. Henderson from root-knot nematodes. A crop of Maxxa planted after one season of NemX, alfalfa, cowpea, Pima, or Maxxa yielded more lint following cowpea (1,409 kg/ha) than following alfalfa (1,208), NemX (1,046), Pima (713), and Maxxa (665). The results demonstrated both the self-protection value and the rotation value of NemX against *M. incognita*. Since no other common species of *Meloidogyne* is known to reproduce well in upland cotton, use of NemX could be very important in cultural management of the nematode.

COMPARATIVE EVALUATION OF NEMX, A NEW CULTIVAR OF UPLAND COTTON WITH HIGH RESISTANCE TO *MELOIDOGYNE INCOGNITA*. Ogallo, J. L.,¹ P. B. Goodell,² J. Eckert,² and P. A. Roberts.¹ ¹Department of Nematology, University of California, Riverside, CA 92521, and ²Kearney Agricultural Center, University of California, Parlier, CA 93648.

Resistance to *Meloidogyne incognita* (Mi) in a new cotton cultivar, NemX, was compared to that in four resistant lines (N6072, N8577, N901, and N903), and four susceptible cultivars (Maxxa, SJ2, Royale, and NemX) in growth pouch, pot, and field tests. Plants in growth pouches each received a Pi of 500 second-stage juveniles/pouch. Resulting egg mass numbers on NemX roots

were 14% of those on the susceptible cultivars. Plants in pot tests were each inoculated with a P_i of 30,000 eggs/plant. The nematode reproduction factor ($R_f = P_f/P_i$) in NemX and the resistant lines was one-seventh that of the susceptible cultivars. Root galling in pot and field tests averaged 15% in NemX and the resistant lines, and 74% in the susceptible cultivars. In fields where NemX and Maxxa were planted in adjacent plots with a high P_i (> 300 J2/g soil), NemX yielded 1,050 kg/ha of lint, exceeding Maxxa's yield by two-fold. In lightly infested plots ($P_i < 100$ J2/g soil) Maxxa yielded 1,450 kg/ha, exceeding yield of NemX by 6%. Wilting due to the *M. incognita*-Fusarium wilt disease complex occurred on 22% of NemX and 65% of Maxxa plants in pots with a $P_i > 300$ J2/g soil.

DEVELOPMENT OF STRIPED FLEA BEETLE LARVAE (*PHYLLOTRETA STRIOLATA*) INFECTED WITH *HOWARDULA PHYLLOTRETAE*. Okada, H. Department of Upland Farming, Tohoku National Agricultural Experiment Station, Arai, Fukushima City 960-21, Japan.

Newly hatched larvae of *Phyllotreta striolata* were infected with female juveniles of *Howardula phyllotretae*. The developmental process of infected and uninfected larvae was compared. Each larva was kept in a petri dish and reared on radish root at 25 °C in darkness. The larvae were observed daily to check molting and survivorship until they died or molted into the third (final) instar. Ninety percent of the uninfected larvae survived to the third instar. Durations of both the first and second instars of uninfected larvae were three days. Ninety-five percent of the infected larvae survived to the second instar. All second-instar larvae, however, died before the third instar. Duration of infected first-instar larvae was four days, while that of the second varied from 2 to 29 days. The infected larvae disseminated an average of ca. 600 nematode juveniles. Females of various body sizes and juveniles were found in larval cadavers.

LIPIDS AND SUGARS: INGREDIENTS FOR PROLONGED INFECTIVITY OF *STEINERNEMA CARPOCAPSAE*? Patel, M. N., and D. J. Wright. Department of Biology, Imperial College of Science, Technology and Medicine, Silwood Park, Ascot, Berkshire SL5 7PY, United Kingdom.

Neutral lipids form the major energy reserve in infective juveniles (IJ) of entomopathogenic nematodes (up to 30% dry weight.) but secondary sources such as glycogen may also be important in some species, comprising up to ca. 20% nematode dry weight. In three species, *Steinernema riobris*, *S. feltiae*, and *S. glaseri*, infectivity declined with neutral lipid content. In contrast, *S. carpocapsae* remained highly infective even when neutral lipid reserves were almost exhausted. Using a glycolytic inhibitor, iodoacetamide, we found that glycogen was important for maintaining infectivity in aged IJ of *S. carpocapsae* but not in newly emerged individuals. Given the commercial value of entomopathogenic nematodes, the data generated in this study have important implications for production.

THE FATTY ACID COMPOSITION OF INFECTIVE JUVENILES OF ENTOMOPATHOGENIC STEINERNEMATID NEMATODES. Patel, M. N., and D. J. Wright. Department of Biology, Imperial College of Science, Technology and Medicine, Silwood Park, Ascot, Berkshire SL5 7PY, United Kingdom.

The fatty acid composition of different lipid fractions (neutral lipids, free fatty acids, and phospholipids) from infective juveniles (IJ) of four entomopathogenic nematodes, *Steinernema carpocapsae*, *S. riobris*, *S. feltiae* and *S. glaseri*, was determined. Newly emerged IJ of all four species had similar fatty acid profiles for each of the different lipid fractions. In the neutral lipids, the fatty acids C18:1n-9 (43–49 mol %), C16:0 (18–23%), C18:2n-6 (8–14%) and C18:0 (4–8%) were the most abundant and the unsaturation index ranged from 90–120. The composition of the free fatty acid fraction mirrored that of the neutral lipids, while in the phospholipid fraction the major fatty acids were C18:1n-9, C16:1n-7, C16:0, and C16:4, and the unsaturation index was

about 40% greater than that of the neutral lipids. Storing IJ at 25 °C resulted in significant changes in the fatty acid composition of the neutral lipids and phospholipids.

ELECTROPHYSIOLOGICAL ANALYSIS OF RESPONSES OF MALES OF *GLOBODERA* SPP. TO SOME CHEMICALS. Perry, R. N.,¹ and E. Riga.² ¹Entomology and Nematology Department, IACR-Rothamsted, Harpenden, Herts., AL5 2JQ, United Kingdom, and ²Pest Management Research Centre, Vineland Station, Ontario L0R 2C0, Canada.

Electrophysiology has been used to determine the responses of live, intact adult males of *Globodera rostochiensis* and *G. pallida* to various chemicals. Exposure of all males of both species to acetylcholine elicited strong electrophysiological responses. By contrast, neither glycine nor citric acid induced any marked electrophysiological responses and only males of *G. pallida* showed significant behavioral responses, moving toward glycine and away from citric acid. The electrophysiological and behavioral responses to γ and α -aminobutyric acids were complimentary, with *G. rostochiensis* showing significant response only to the latter and *G. pallida* responding significantly only to the former. Males of both species responded significantly in both electrophysiological and behavioral assays to L-glutamic acid but not to D-glutamic acid.

COMPARISON OF NUCLEOTIDE SEQUENCES FROM THE INTERGENIC SPACER REGION OF THE THREE DESCRIBED RACES OF *MELOIDOGYNE CHITWOODI*. Petersen, D., and T. Vrain. Agriculture and Agri-Food Canada, Summerland, BC V0H 1Z0, Canada.

The size of the ribosomal intergenic spacer region (IGS) is known to vary between closely related root-knot nematode species. We examined whether this same region can also be used to discriminate between races. Genomic DNA from all three described races of *Meloidogyne chitwoodi* was obtained and the IGS region was sequenced. Within the resulting 1678-bp sequence, several repeated regions were found, including fourteen consecutive repetitions of a 25-bp sequence. While each repetition varied slightly, the identical variation was present in each race. This region immediately follows the 5 S gene, which exhibits over 90% homology with the 5 S gene sequence in *M. arenaria*. These results support the hypothesis that *M. chitwoodi* races are evolving either faster than, or independently of, the ribosomal IGS.

SPECIES-SPECIFIC PROBES FOR THE DETECTION OF *MELOIDOGYNE CHITWOODI* AND *M. FALLAX*. Petersen, D.,¹ C. Zijlstra,² J. Wishart,³ V. Blok,³ and T. Vrain.¹ ¹Agriculture and Agri-Food Canada, Summerland, BC V0H 1Z0, Canada; ²IPO-DLO, Postbus 9060, 6700 GW Wageningen, The Netherlands; ³Scottish Crop Research Institute, Invergowrie, Dundee, United Kingdom.

Amplified length polymorphisms within the ribosomal intergenic spacer region (IGS) of several root-knot nematode species make this DNA region an excellent choice for developing hybridization probes to detect and differentiate DNA from *Meloidogyne* species. IGS sequences were obtained by sequencing the corresponding PCR-amplified DNA. Alignment of the nucleotide sequences from three root-knot nematode species (*M. chitwoodi*, *M. fallax*, *M. hapla*) revealed localized areas of dissimilarity. Oligonucleotide probes were then synthesized and used as PCR primers to specifically detect *M. chitwoodi* and *M. fallax*. Multiplex-PCR amplification of DNA from a single juvenile successfully differentiated over 20 populations of these species from *M. arenaria*, *M. hapla*, *M. incognita*, *M. javanica*, and *M. mayaguensis*.

VARIABILITY OF VIRULENCE AMONG ISOFEMALE LINES DEVELOPED FROM TWO *MELOIDOGYNE INCOGNITA* ISOLATES FROM THE SAME FIELD SITE. Petrillo, M. D., and P. A. Roberts. Department of Nematology, University of California, Riverside, CA 92521.

The *Rk* gene in cowpea, *Vigna unguiculata*, confers resistance to the root-knot nematode, *Meloidogyne incognita*. Isofemale lines were developed from cultures of two *M. incognita* isolates originally from the same field site, with one expressing high virulence to *Rk* and another selected

for avirulence to *Rk*, by culturing on susceptible tomato after field isolation. The isolates were confirmed as having enhanced virulence (average egg mass production of 120% of the susceptible check) and reduced virulence (5%), respectively, before isofemale lines were developed. Isofemale lines were tested by measuring egg mass production on susceptible cowpea genotype CB3 and resistant genotype CB46, grown in seed-growth pouches maintained at 27 °C in a controlled-environment chamber. The isofemale lines from the selected avirulent isolate separated into three categories: high virulence ($\geq 100\%$), low virulence ($< 7\%$), and avirulence (0%). The isofemale lines from the virulent isolate expressed moderate (48 to 85%) to high ($\geq 100\%$) virulence. These data indicated that isofemale lines developed from the same culture of a field isolate vary significantly in virulence to *Rk*.

PATHOGENICITY, FITNESS, AND MOLECULAR CHARACTERIZATION OF *PRATYLENCHUS GOODEYI* POPULATIONS ON BANANA. Pinochet, J.,¹ M. C. Jaizme,² C. Fernández,¹ and M. Jaumot.¹ ¹Departamento de Patología Vegetal, IRTA, Crta Cabrils s/n, 08348 Cabrils, Barcelona, Spain, and ²Departamento de Protección Vegetal, ICIA, Valleguerra, Tenerife, Islas Canarias, Spain.

Comparative pathogenicity among four isolates of *Pratylenchus goodeyi* from the Canary Islands was evaluated on Grand Naine banana (*Musa* AAA). The reproductive fitness of the four Canary Island isolates and two African isolates was assessed in monoxenic carrot cultures. The isolates also were compared with a RAPD-PCR technique. A Kenya isolate was more pathogenic than one from Las Arucas, Gran Canarias, on banana. The Telde isolate (Gran Canaria) showed a higher reproductive fitness than Tanzania and Kenya isolates under in vitro conditions. RAPD primers OPB-15 and OPB-17 produced bands that allowed differentiation among isolates. The Las Arucas isolate was the most dissimilar. When an isolate from Tenerife was tested against several known sources of resistance, plant material showed different levels of susceptibility. Resistance was not detected, although the accession Yangambi Km 5 proved to be a poor host for *P. goodeyi*.

ARGININE KINASE IN *STEINERNEMA* SP. Platzer, E. G.,¹ W. Wang,¹ S. N. Thompson,² and D. Borchardt.³ Departments of ¹Nematology, ²Entomology, and ³Chemistry, University of California, Riverside, CA 92521.

In the infective third-stage juveniles (J3) of *Steinernema carpocapsae* we have found moderate activity of arginine kinase (AK), 4.6 micromol/minute/mg protein, in the forward reaction (ATP formation) at pH 7.5. At the same pH, the reverse reaction (arginine phosphate formation) was 3.2 micromol/minute/mg protein. AK (reverse reaction) in adults of *S. carpocapsae* and J3 of *S. feltiae* and *S. riobravus* were 2.3, 1.9, and 4.9 micromol/minute/mg protein, respectively. In previous NMR studies, the presence of arginine phosphate in the J3 of *S. carpocapsae* was directly dependent on normoxic conditions. Hence, the moderate AK activity of these entomopathogenic nematodes appears to be correlated with an aerobic habitat.

DIFFERENCES IN RESISTANCE TO *MELOIDOGYNE INCOGNITA* BETWEEN MELON (*CUCUMIS MELO*) CULTIVARS AND HORNED CUCUMBER (*C. METULIFERUS*). Ploeg, A. T. Department of Nematology, University of California, Riverside, CA 92521.

The resistance of seven melon cultivars, three melon lines, and one line of horned cucumber to a race 3 population of root-knot nematode, *Meloidogyne incognita*, was studied in greenhouse pot tests and in growth chamber tests in paper growth pouches. The nematodes produced severe galling on the roots of all melon cultivars, intermediate galling on the melon lines, and very little galling on horned cucumber. Correspondingly, numbers of egg masses and eggs were greatest on the melon cultivars and lowest on horned cucumber. Results from the greenhouse and growth-pouch tests were similar. The latter system required less time and space and could be used successfully for screening melons for root-knot nematode resistance. Root staining revealed that numbers of *M.*

incognita second-stage juveniles invading susceptible melon and resistant horned cucumber were similar, but that in horned cucumber more juveniles developed into males than into females.

RESPONSE OF SOIL NEMATODES AND NEMATODE COMMUNITY INDICES TO IRRIGATION IN A CITRUS ECOSYSTEM. Porazinska, D. L.,¹ R. McSorley,¹ L. W. Duncan,² and J. Graham.² ¹Entomology and Nematology Department, University of Florida, Gainesville, FL 32611-0620, and ²Citrus Research and Education Center, University of Florida, Lake Alfred, FL 33850-2299.

Availability of a measure indicating status of the soil ecosystem is crucial, particularly for monitoring programs assessing impacts of agricultural activities on environmental quality. This study investigated the effects of various irrigation intensities (an example of an agricultural management practice) on the structure of the nematode community. Numbers of individual nematode genera were assessed and related to irrigation intensity, as were ecological measures such as community structure indices, diversity indices, and maturity indices. Maturity index was an effective measure in distinguishing differences between irrigation regimes, but other indices of community structure were not particularly effective. Of various nematode genera and trophic groups, only omnivores and the omnivorous genera *Aporcelaimellus* and *Eudorylaimus* responded to the irrigation treatments. This trend was observed across seasons.

CHARACTERIZATION OF HOST GENES IN THE EARLY STAGES OF ALFALFA-MELOIDOGYNE INCOGNITA INTERACTION. Potenza, C.,¹ E. A. Higgins,² S. H. Thomas,² and C. Sengupta-Gopalan.¹ ¹Department of Agronomy and Horticulture, and ²Department of Entomology, Plant Pathology, and Weed Science, New Mexico State University, Las Cruces, NM 88003.

Root-knot nematodes invade roots and induce development of specialized feeding sites that act as metabolic sinks, diverting plant nutrients for usage by this major agricultural pathogen. Our goal is to isolate and characterize genes that are induced or repressed in the host following nematode invasion, but whose expression is altered prior to establishment of functional feeding sites. Towards this goal, we have identified a critical time period during which these events occur in our alfalfa/*Meloidogyne incognita* model system and produced a cDNA library from infected roots. We have developed a differential method of screening this library using magnetic beads, isolated a number of plant host genes whose expression is altered with nematode infection, and begun initial characterization of selected plant host genes.

LINKING MOLECULAR AND MORPHOLOGICAL CHARACTERS IN TAXONOMIC STUDIES OF NEMATODES. Powers, T. O., B. J. Adams, and A. L. Szalanski. Department of Plant Pathology, University of Nebraska-Lincoln, Lincoln, NE 68583-0722.

Molecular profiles derived by PCR-RFLP and nucleotide sequence data are rich sources of taxonomic characters. However, without corresponding morphological characterization of nematode samples, molecular data are of limited reliability. We have taken two approaches to providing a morphological-molecular linkage. Samples for molecular characterization are first measured, photographed, and then processed for PCR. A second approach, used with adenophorean nematodes, involves generating a PCR product from a portion of the nematode body and then processing the remainder as a glycerin-infiltrated voucher specimen mounted on a glass slide.

ENHANCED DEGRADATION OF 1,3-DICHLOROPROPENE. Riegel, C.,¹ D. W. Dickson,¹ and L. G. Peterson.² ¹Entomology and Nematology Department, University of Florida, Gainesville, FL 32611-0620, and ²DowElanco, 1861 Capital Circle, NE Suite 104, Tallahassee, FL 32308.

Enhanced degradation of 1,3-D has been reported. Our objective was to determine if enhanced degradation affects the efficacy of 1,3-D. Microplots infested with *Meloidogyne arenaria* were broadcast-fumigated with 1,3-D (112 liters/ha) applied 30 cm deep. Each plot was sampled 15, 30,

and 45 cm deep 24 hours before and 24, 48, 72, and 96 hours after fumigation. Soil samples (200 cm³) taken from each depth were divided equally and one portion was bioassayed (assay 1). Second-stage juveniles (J2) from the second portion were extracted, counted, and bioassayed within 24 hours (assay 2). Galls and eggs were counted 6 weeks later. In assay 1, the number of galls and eggs was lower in all samples after fumigation. Eggs were not detected on roots from soil exposed to 1,3-D for 24 hours, and a maximum of 5 of 162 root samples per sampling time had galls. In assay 2, J2 extracted within 24 hours galled roots, whereas J2 extracted after 24 hours caused few or no galls.

ANOTHER SOIL STORY. Riegel, C., T. E. Hewlett, and D. W. Dickson. Entomology and Nematology Department, University of Florida, Gainesville, FL 32611-0620.

Nematodes are found in all types of habitats in abundant numbers and impact the lives of almost everyone, yet most people are not aware that they exist. One way to improve public awareness of nematodes is to educate children at an early age. This can be done in schools and by parents using teaching aids. Elementary school level literature about the flora and fauna in soil is lacking. "Another Soil Story" is a book with an emphasis on nematodes designed to educate while entertaining children about soil organisms. It tells the story of Rhonda the root-knot's journey through soil. The reader follows Rhonda's life from hatching to the establishment of a feeding site inside a bean plant. This project will enhance public awareness of nematology, which is needed to preserve and promote our science in the future.

ROTATION OF RESISTANCE TO CONTROL *HETERODERA GLYCINES*. Riggs, R. D., L. Rakes, and T. Hart. Department of Plant Pathology, University of Arkansas, Fayetteville, AR 72701.

Rotation studies, which included a susceptible soybean cultivar (Hutcheson), two resistant cultivars (Walters, Asgrow A5979), and a nonhost of soybean cyst nematode (SCN, *Heterodera glycines*), were conducted to determine the effect of rotating soybean cultivars with different sources of resistance. The nonhost varied among tests. Hutcheson and A5979 were each planted continuously. The tests were run on soil infested with SCN race 9. Among the rotation crops, Hutcheson usually had the highest population increase in a season, but at times Walters did. A5979 had SCN levels nearly as low as the nonhost. At the end of the test the SCN level was always lower in plots planted continuously to a susceptible cultivar, than on the susceptible cultivar in rotation plots. When A5979 was planted continuously for five years, the ability of *H. glycines* to reproduce on it increased.

AMENDED POLYTOMOUS CODES FOR SIX *LONGIDORUS* SPECIES RECENTLY DESCRIBED OR REDESCRIBED. Robbins, R. T.,¹ and D. J. F. Brown.² ¹Nematology Laboratory, University of Arkansas, Fayetteville, AR 72701, and ²Scottish Crop Research Institute, Invergowrie, Dundee DD2 5DA, Scotland.

A recent polytomous key for *Longidorus* by Chen et al. did not include the polytomous code for the following 3 species from Alaska described in 1996 by Robbins and Brown: *L. alaskaensis*, A4-B3-C3-D12-E2-F23-G12-H12-I2; *L. paralaskaensis*, A45-B3-C3-D12-E2-F3-G12-H12-I1; *L. bernardi*, A2-B1-C2-D4-E12?-F2-G2-H3-I1. The polytomous codes for 3 *Longidorus* species originally described by Thorne and redescribed by Robbins and Brown in 1995 are amended as follows: *L. crassus*, A4-B3(24)-C23-D1-E12?-F3(24)-G2-H1(2)-I1; *L. fragilis*, A3-B2-C2-D1-E1?-F3-G2-H56-I1; *L. sylphus*, A2-B12-C2-D2(3)-E12?-F2-G12-H23-I1. The codes for Thorne's species differ somewhat from those given by Chen et al. mainly because the redescriptions represent a range of observations, not an average. We found the code for amphidial pouches (E) to be the least reliable, primarily because they often are obscured and often are seen in an oblique instead of a lateral view.

ISOLATION AND CHARACTERIZATION OF A SECRETED MOLECULE FROM THE POTATO CYST NEMATODES *GLOBODERA ROSTOCHIENSIS* AND *G. PALLIDA*. **Robertson, L.,¹ L. H. Duncan,¹ J. T. Jones,¹ M. A. J. Ferguson,² and W. M. Robertson.¹** ¹Department of Nematology, SCRI, Invergowrie, Dundee DD2 5DA, and ²Department of Biochemistry, University of Dundee, Dundee, United Kingdom.

Secretions of plant-parasitic nematodes are thought to have important roles in various aspects of the host-parasite relationship, including induction and maintenance of feeding sites. The serotonin agonist 5-dimethoxytryptamine oxalate was used to induce the production of secretions from *Globodera rostochiensis*. These secretions were used as immunogen to produce an antibody designated GR-1. Immunofluorescence and immunogold labelling showed that GR-1 reacts with subventral gland cell components of *G. rostochiensis* and *G. pallida* second-stage juveniles. GR-1 has been used to immunoscreen a *G. pallida* cDNA library and a full length sequence has been obtained. The cloned gene, named *gp-sec-3*, is expressed in a range of nematode life-cycle stages.

CONTROL OF *GLOBODERA* AND *MELOIDOGYNE* SPP. WITH CANAVANINE AND ITS EFFECT ON BACTERIAL FEEDERS. **Robertson, W. M., A. N. E. Birch, I. E. Geoghegan, and B. S. Griffiths.** Scottish Crop Research Institute, Dundee DD2 5DA, United Kingdom.

Canavanine, derived from legume seeds, is a naturally occurring plant product with nematocidal properties, and was tested in vitro and in vivo for its ability to control *Globodera rostochiensis* and *Meloidogyne incognita* on potato. When purified canavanine was added to root diffusate, hatching efficiency and survival of hatched juveniles were significantly reduced at 100 and 200 ppm. When canavanine was applied as a soil drench, the development of females on roots grown in soil was increasingly inhibited by concentrations of 12.5, 25, and 100 ppm. Both the total number of cysts and the number of eggs per cyst were affected. Practical application of canavanine, especially in a sustainable agricultural scenario, probably would be as a diluted plant extract containing a mixture of nematocidal compounds. Applying an extract of jack bean had no effect on the development of beneficial bacterial-feeding nematodes, except at unrealistically high doses.

NEW SOURCES OF NEMATODE RESISTANCE IN WILD ACCESSIONS OF UPLAND COTTON FROM MEXICO. **Robinson, A. F., A. E. Percival, and A. C. Bridges.** USDA ARS, SCRL, 2765 F & B Road, College Station, TX 77845.

Forty-six USDA race stock accessions of upland cotton (*Gossypium hirsutum*) and two of *G. barbadense* were examined for resistance to *Meloidogyne incognita* race 3 and *Rotylenchulus reniformis* in environmental growth chamber experiments. Only the *G. barbadense* accessions (TX-1347, TX-1348) supported significantly less reproduction by *R. reniformis* than the susceptible control, Deltapine 16. Both accessions were highly susceptible to *M. incognita* race 3. The *G. hirsutum* accessions TX-1174, TX-1440, TX-2076, TX-2079, and TX-2107 had levels of resistance to *M. incognita* race 3 comparable to that of Wild Mexican Jack Jones, a primary source of resistance in the most resistant breeding lines of *G. hirsutum* available. No accession was as resistant as the highly resistant line Auburn 623 RNR. Resistant accessions were from the contiguous coastal states of Quintana Roo, Yucatan, Campeche, Tabasco, and Veracruz. Reproduction by *R. reniformis* populations from Alabama, Mississippi, Louisiana, and Texas, and by *M. incognita* race 3 populations from Mississippi, Texas, and California, did not differ appreciably on resistant genotypes.

LONG-TERM EFFECTS OF CROPPING SYSTEMS WITH SOYBEAN, CORN, AND PEANUT ON SOYBEAN YIELDS AND NEMATODE POPULATIONS. **Rodríguez-Kábana, R., P. S. King, and L. W. Wells.** Department of Plant Pathology, Auburn University, Alabama Agricultural Experiment Station, Auburn, AL 36849.

A long-term rotation study was conducted to assess the effects of corn and peanut on yield and nematode populations of soybean. Yield declined continuously with soybean monoculture. Yield of

soybean following two years of corn (S-C-C) did not decline and was superior to those obtained with monoculture or with soybean following one year of either corn or peanut. *Meloidogyne arenaria* did not reproduce in soybean or corn but significant populations of *Pratylenchus* spp. were associated with these crops. *Heterodera glycines* was detected in very low numbers in 1987 in plots with soybean monoculture; numbers of juveniles in soil increased rapidly from 1987 to 1994 in plots with soybean. *Heterodera glycines* juvenile populations in soil were highest in monoculture soybean and lowest in S-C-C; juvenile populations declined sharply in 1995 and the nematode was not detected in any plot in 1996.

CROP ROTATION WITH CORN AND SOYBEAN AND MANAGEMENT OF ROOT-KNOT NEMATODE AND SOUTHERN BLIGHT ON PEANUT. Rodríguez-Kábana, R., D. G. Robertson, C. F. Weaver, and L. W. Wells. Department of Plant Pathology, Auburn University, Alabama Agricultural Experiment Station, Auburn, AL 36849.

A field experiment was initiated in 1983 to assess the value of rotations with corn and soybean for the management of *Meloidogyne arenaria* and other soilborne pathogens of peanut in Alabama. The field was severely infested with *M. arenaria* and *Sclerotium rolfsii* (southern blight). Monocultured peanut from 1983 to 1996 resulted in declining yields and increased southern blight. Incidence of southern blight increased in a linear manner in response to the number of years in monoculture. Peanut following two years of corn (C-C-P) suppressed southern blight and sustained peanut yields above the yields obtained with peanut monoculture or with peanut following one year of either corn (C-P) or soybean (S-P). Neither C-P nor S-P were effective in suppressing *S. rolfsii*. Populations of *M. arenaria* in monocultured peanut were as high as those in peanut with C-C-P, C-P, or S-P.

HOST RANGE OF PARATRICHODORUS ALLIUS AND RESISTANCE OF POTATO TO THE CORKY RINGSPOT DISEASE. Santo, G. S., H. Mojtahedi, C. R. Brown, P. E. Thomas, and J. M. Crosslin. Washington State University, Prosser, WA 99350, and USDA-ARS, Prosser, WA 99350.

Paratrichodorus allius, a vector of the tobacco rattle virus that causes corky ringspot disease (CRS) on potato, was isolated from potato fields in Pasco, WA and Umatilla, OR. Both isolates reproduced successfully on crops commonly rotated with potato as well as on most weeds associated with this crop in the Pacific Northwest. Our field data showed that there were varying degrees of resistance to CRS in seven potato cultivars and breeding clones. However, resistance to CRS was not correlated with reproduction of *P. allius* in greenhouse experiments. The average reproductive factor (RF = final number of nematodes/initial inoculum, in 55 days) of *P. allius* from Umatilla was 10-fold greater than the Pasco isolate on the selected potato cultivars.

ENVIRONMENTAL ADAPTATIONS FOR THE IN VIVO CULTURE AND BIOASSAY OF HETERODERA GLYCINES. Sardanelli, S.,¹ and W. J. Kenworthy.² ¹Department of Plant Biology, and ²Department of Natural Resource Sciences and Landscape Architecture, University of Maryland, College Park, MD 20742.

Limited availability of spatial facilities with adequate environmental control can be a substantial limiting factor for in vivo culture and bioassay. The culture and bioassay of *Heterodera glycines* (SCN) race 3 within a transportable, moisture replacement system was evaluated in controlled environment chambers, a greenhouse, and in a laboratory environment. Manipulation of one or more edaphic factors in the three environments, in conjunction with the system, has circumvented seasonal testing restrictions. Adjustments require minimal input and materials are readily available. Trials involving resistance screening and race determination have resulted in consistent development of SCN females. Initial testing of *Meloidogyne incognita* in culture and bioassay on 'Rutgers' tomato also indicates potential application of the system to studies of root-knot nematodes.

MAXIMIZING RESOURCES FOR THE IN VIVO CULTURE AND BIOASSAY OF PLANT-PARASITIC NEMATODES. **Sardanelli, S.,¹ and W. J. Kenworthy.²** Departments of ¹Plant Biology and ²Natural Resource Sciences and Landscape Architecture, University of Maryland, College Park, MD 20742.

Reliable and repeatable procedures are essential for successful evaluation of practices for managing plant-parasitic nematodes. A new method for culture and bioassay of plant-parasitic nematodes has been developed and tested extensively with *Heterodera glycines* race 3. A special reservoir delivers a constant and measurable supply of soil moisture. Individual units for culture and bioassay require a minimal amount of growing medium. Direct seeding, using breeder-selected seed for a number of soybean cultivars, provided 100% germination. Techniques for evaluation of nematode and host are adaptable and have been successfully incorporated during experimentation. Preliminary trials with *Meloidogyne incognita* on 'Rutgers' tomato produced favorable results as to plant growth and nematode response.

EXTRACTION OF ROOT-KNOT NEMATODES FROM POTATO TUBERS. **Schneider, S. M., J. E. Cochran, and S. M. Santoy.** Vegetable and Forage Crops Research Unit, USDA-ARS, Prosser, WA 99350.

A reliable, efficient method for extracting all root-knot nematode stages from potato tubers was developed. Tuber tissue was excised to a depth of 12 mm in 3-mm intervals from 10 tubers with varying levels of nematode infestation. Tissue was incubated in a pectinase + cellulase solution on a shaker overnight at room temperature. Sugar flotation-centrifugation was used to separate nematodes from plant tissue. Nematodes in the supernatant were stained with acid fuchsin and counted by stage and sex. Nematodes remaining in the pellet were also counted. The supernatant contained 97% of the total nematodes, with 3% remaining in the pellet. The percentage remaining in the supernatant varied significantly with stage: 99.7% for J2, 97% for J3 + J4/4, 86% for females, and 93% for males. These percentages did not vary significantly with tuber or depth of tissue. Population estimates based on this technique were significantly higher than those based on counts of nematodes in stained tuber tissue.

VARIABLE AND SINGLE-RATE APPLICATION OF TEMIK 15G IN COTTON FIELDS INFESTED WITH ROOT-KNOT NEMATODE. **Schuster, G. L.,¹ T. A. Wheeler,² and H. W. Kaufman.²** ¹100 E. Bedford, Dimmitt, TX 79027, and ²Texas A&M University, Rt. 3, Box 219, Lubbock, TX 79401.

Cotton fields infested with low (46 second-stage juveniles [J2]/500 cm³ soil) and high (604 J2/500 cm³ soil) *Meloidogyne incognita* densities were sampled intensively. Field rows were divided into thirds, and soil collected from each one-third block (either 8 or 16 rows wide) was assayed for nematodes before planting. Block thirds were assigned a Temik® 15G rate based on J2 soil density in the spring. One half of the block was treated with the variable assigned rate, while the other half of the block was treated with the standard producer rate. Yields were taken for the variable vs. standard rate application treatments. In the field with a high density of *M. incognita*, the variable rate application used 2.04 kg/ha more of Temik 15G than the single rate treatment, but yielded 235.2 kg/ha more in lint. In the field with a low density of root-knot nematode, the variable rate treatment used 2.52 kg/ha less Temik 15G and yielded 100 kg/ha more in lint than the standard rate treatment. Yields were significantly different.

EFFECTS OF DIFFERENT CROPS ON THE DIVERSITY AND POTENTIAL OF FUNGAL EGG PATHOGENS OF CYST NEMATODES. **Schuster, R.-P., A. Pyrowolakis, and R. A. Sikora.** Soil-Ecosystem Phytopathology, Institut für Pflanzenkrankheiten, University of Bonn, Nussallee 9, 53115 Bonn, Germany.

The rhizospheres of different crops were investigated for the presence of fungal egg pathogens in the soil at 4 months. The effect of intercrops on the occurrence and degree of egg infection in

two different sugar beet fields infested with *Heterodera schachtii* was studied. Degree of fungal egg infection was measured using *Globodera pallida* eggs incubated in slide frames as a test nematode after staining with rose-bengal. The fungal egg pathogens studied were isolated directly from nematode eggs plated on water agar. Fungi isolated were identified microscopically as well as by fatty acid-methyl ester analysis (FAME). This was supported by analysis with different cluster analysis systems. Microbial diversity of soils was compared by calculation of indices for species richness, diversity and evenness.

ENDOPHYTIC FUNGI ISOLATED FROM BANANA ROOT SYSTEMS AND THEIR POTENTIAL FOR THE BIOLOGICAL CONTROL OF *RADOPHOLUS SIMILIS*. Schuster, R.-P., and R. A. Sikora. Soil-Ecosystem Phytopathology, Institut für Pflanzenkrankheiten, University of Bonn, Nussallee 9, 53115 Bonn, Germany.

Endophytic fungi isolated from banana roots were tested for their ability to recolonize root tissue and to control *Radopholus similis*. Culture filtrate tests demonstrated nematostatic and nematocidal activity toward the burrowing nematode *R. similis*, as well as toward other species. Up to 26% of the screened fungi caused more than 90% mortality. Bioassays with excised roots were used to test for endophytic colonization, with some of the isolates having active metabolites demonstrated strong endophytic growth. In pot experiments colonization percentages varied according to the application system, from 40% to 80 % for the main roots and from 9% to 39 % for the root tip region. Reductions of greater than 60% in *R. similis* populations and enhanced growth of the banana plantlets were observed in greenhouse tests. Endophytic fungi provide an excellent opportunity for biocontrol for tissue culture plantlets because of the small amount of inoculum needed.

REPRODUCTIVE POTENTIAL AND DAMAGE TO COFFEE BY THE KONA ROOT-KNOT NEMATODE IN FOUR DIFFERENT SOILS. Serracin, M., D. P. Schmitt, and B. S. Sipes. Department of Plant Pathology, University of Hawaii, Honolulu, HI 96822.

The influence of four soils with different physical and chemical properties on *Meloidogyne konaensis* reproduction and growth of *Coffea arabica* cv. Guatemala was determined under greenhouse conditions. Selected soils were an inceptisol, a mollisol, and two oxisols representing coffee-growing zones of the Hawaiian islands. Coffee seedlings with eight pairs of leaves (ca. 9 months-old) were transplanted into 24.5-cm-diam. pots containing the different soils and inoculated with 2,500 *M. konaensis* second-stage juveniles. Nematode galling, reproduction, and root and soil populations as well as plant fresh shoot and root weight were determined at harvest (120 days after inoculation); results were compared among soils and against non-inoculated controls. Factors related to soil moisture retention properties and soil pore size distribution in addition to different nematode reproductive rates may affect pathogenicity of *M. konaensis* to coffee.

DIFFERENCES IN REPRODUCTION AMONG POPULATIONS OF *RADOPHOLUS SIMILIS* FROM HAWAII ON THREE HOSTS. Sipes, B. S. Department of Plant Pathology, University of Hawaii, Honolulu, HI 96822.

Six populations of burrowing nematode, putatively identified as *Radopholus similis similis* (Ba1 and Ca1) and *R. similis citrophilus* (An1, An2, An3, and An5), were evaluated for reproduction on *Anthurium andreanum* 'Midori', *Chamaedorea elegans*, and *C. seifritzii*. The plants were inoculated with 2,000 mixed life stages of the populations and maintained in the greenhouse for 5 months. At harvest nematodes were extracted from plant tissue in a mist chamber and plants subsequently were dried to a constant weight. *Anthurium* was generally a better host than the *Chamaedorea* spp. for all nematode populations. *Chamaedorea seifritzii* was a better host than *C. elegans* for all populations except An1. *Anthurium* was the best host for Ba1, Ca1, An2, An3, and An5, and *C. elegans* was the best host for An1. Differences in reproduction among the populations on the three host plants may reflect differences in pathogenicity or virulence for these populations.

BIOLOGICALLY-BASED PRODUCTS FOR ROOT-KNOT NEMATODE CONTROL IN FRESH BASIL. Sipes, B. S.,¹ R. T. Hamasaki,² and D. P. Schmitt.¹ ¹Department of Plant Pathology, University of Hawaii, Honolulu, HI 96822, and ²Cooperative Extension Service, Kaneohe, HI 96744.

Nematrol, Clandosan, and Telone II were evaluated for control of root-knot nematode, *Meloidogyne javanica*, in basil. A field experiment with 4 replications was established, sampled for nematodes, and treated with Telone II (224 liters/ha), Nematrol (258 kg/ha), Clandosan (4.5 t/ha), or left untreated. Basil, *Ocimum basilicum* cv. HCES 1A, was planted 2 weeks later. Leaf harvest was recorded as the plants matured. Final harvest was collected after an epidemic of *Fusarium*. Basil plants were cut at ground level, weighed, and a soil and root sample collected. No statistical differences were detected but trends were evident. Soil population densities of root-knot nematode increased to similar levels in all treatments. Galling was lowest and plant biomass was greatest in the Telone II-treated plots followed by the Nematrol, Clandosan, and untreated treatments. Nematodes per gram of root were lowest in the Nematrol-treated plots. Nematrol had a greater effect on nematode population densities than expected and may offer potential for nematode control. The higher yield associated with the Clandosan treatment may have been a fertilizer effect.

EXTRACTION OF ROOT-ASSOCIATED MELOIDOGYNE INCOGNITA AND ROTYLENCHULUS RENIFORMIS. Stetina, S. R., E. C. McGawley, and J. S. Russin. Department of Plant Pathology and Crop Physiology, Louisiana Agricultural Experiment Station, Louisiana State University Agricultural Center, Baton Rouge, LA 70803-1720.

A technique based on physical maceration of root tissue was developed to extract vermiform and swollen stages of *Meloidogyne incognita* and *Rotylenchulus reniformis*. Experiments conducted on soybean and tomato evaluated the efficiency of method (stir, grind), NaOCl concentration (0%, 0.5%), and duration (1x, 2x) on extraction of nematodes and eggs from 60-day-old populations. Root-associated populations of *R. reniformis* were considerably lower than those of *M. incognita*, so development of the method focused on the latter. Grinding liberated more nematodes than stirring, but the reverse was true for egg extraction. Among grinding treatments, a duration of 10 seconds in 0.5% NaOCl provided the most efficient extraction of nematodes and eggs. Among stirring treatments, a duration of 10 minutes in 0.5% NaOCl provided the most efficient extraction of eggs. These techniques were compared on soybean roots 30 days older than those on which the procedures were first evaluated, with consistent results.

THE POTENTIAL OF GEOGRAPHIC INFORMATION SYSTEMS IN AGRICULTURE. Strickland, R. M., D. R. Ess, and S. D. Parsons. Agricultural and Biological Engineering Department, 1146 ABE Building, Purdue University, West Lafayette, IN 47907-1146.

The use of new technologies including Geographical Information Systems (GIS), Global Positioning Systems (GPS), Variable Rate Technology (VRT), and Remote Sensing is becoming the trend for today's high-technology, precision agricultural industry. GIS provides the ability to link multiple data values for the same geo-referenced location, and provides the user with a graphical visualization of what the data look like. When GIS is coupled with GPS, management decisions can be applied in a precise "micro-managed" manner utilizing VRT techniques, which holds the potential to save significant sums of money and reduce the potential for crop and environmental damage.

MOLECULAR SYSTEMATICS OF TYLENCHIDA USING 18S RIBOSOMAL DNA. Szalanski, A. L., B. J. Adams, and T. O. Powers. Department of Plant Pathology, University of Nebraska-Lincoln, Lincoln, NE 68583-0722

Thirty-three tylenchid taxa representing 28 genera were compared by nucleotide sequence analysis of approximately 635 bp of 18S ribosomal DNA. Trees were constructed with parsimony, maximum likelihood, neighbor-joining, and UPGMA algorithms. Diverse methods to evaluate

phylogenetic signal and tree robustness supported several traditionally recognized nematode groupings. Four cyst-producing genera consistently formed a clade that joined a grouping that included *Hoplolaimus*, *Scutellonema*, *Rotylenchus* and *Helicotylenchus*, but excluded *Meloidogyne*. Criconeematidae and Anguinidae were strongly supported. Little support was observed for Belonolaimidae, Telotylenchinae, Merliniinae, or subfamily groupings in Tylenchidae. Species of *Meloidogyne* were remarkably divergent from each other in 18S sequence, and genetically distant from other genera in Tylenchida.

MANAGEMENT OF *MELOIDOGYNE INCOGNITA*: USE OF A RESISTANT PEPPER AS A ROTATION CROP. Thies, J. A.,¹ J. D. Mueller,² and R. L. Fery.¹ ¹U.S. Vegetable Laboratory, ARS, USDA, Charleston, SC 29414, and ²Edisto Research and Education Center, Clemson University, Blackville, SC 29817.

A two-year study was conducted to evaluate the potential of using resistant pepper cultivars as a rotation crop for managing *Meloidogyne incognita*. The study was conducted in a field infested with *M. incognita*; the experimental design was a split-plot. In 1994, the main plots were planted to either the highly resistant cultivar Carolina Cayenne or its susceptible sibling line PA-136. In 1995, Carolina Cayenne and the susceptible bell cultivars California Wonder and Keystone Resistant Giant were grown as subplots in each of the original main plots. The response of Carolina Cayenne to *M. incognita* was unaffected by the previous crop. Previous cropping history, however, had a significant impact on the response of the bell cultivars, e.g., the mean galling response was less and the yield was 2.3 times greater in the main plots previously cropped with Carolina Cayenne than in those previously cropped with PA-136. These results suggest that resistant pepper cultivars have considerable merit as a rotation crop for managing *M. incognita* infestations in soils used for growing high value vegetables.

INTERACTIONS AMONG *MELOIDOGYNE INCOGNITA*, ANNUAL OR PERENNIAL WEEDS, AND CHILE PEPPER. Thomas, S. H.,¹ J. Schroeder,¹ and L. W. Murray.² ¹Department of Entomology, Plant Pathology and Weed Science, and ²Department of Economics and International Business, New Mexico State University, Las Cruces, NM 88003-0003.

A microplot experiment was conducted during 1996 to determine the effects of presence or absence of *Meloidogyne incognita* (RKN) on competition between *Capsicum annuum* (chile) and the annual weed *Anoda cristata* (spurred anoda = SA) or the perennial weeds *Cyperus esculentus* (yellow nutsedge = YNS) or *C. rotundus* (purple nutsedge = PNS). Chile growth was reduced by all three weeds and early season RKN numbers were greater in chile when weeds were present. Chile reduced root growth and increased RKN numbers on all weeds. RKN reduced chile leaf area and dry weight throughout the season, but had no effect on leaf area, leaf weight, or root dry weight of any of the weeds, and did not reduce nutsedge tuber count. These data support the hypotheses that SA, YNS, and PNS enhance RKN infection in chile, and that all three weeds are unaffected by RKN levels that are pathogenic to the crop.

A DNA SEQUENCE-BASED AID TO NEMATODE SPECIES IDENTIFICATION. Thomas, W. K.,¹ J. G. Baldwin,² M. Mundo,² and L. M. Frisse.¹ ¹Division of Molecular Biology and Biochemistry, University of Missouri, Kansas City, 5007 Rockhill Road, Kansas City, MO 64110, and ²Department of Nematology, University of California, Riverside, CA 92521.

Within nematode taxonomy, there is an urgent need for new tools that can aid in the rapid identification of species. We have developed a molecular approach based on the amplification and automated sequencing of a section of the nuclear large ribosomal RNA subunit. Preliminary tests suggest that this molecular approach is especially promising because: (i) it can be applied to a wide range of nematode taxa; (ii) this method produces unambiguous, comparable character sets that can be rapidly identified by computer aided analysis; (iii) results can be obtained from all developmental stages and both sexes, and from short-term formalin-fixed nematodes; (iv) empirical data

suggest that the sequence varies at an appropriate level for species discrimination. This approach allows the use of DNA-sequence characters to strengthen and supplement the extant morphology-based taxonomy.

PARASITISM OF *HETERODERA GLYCINES* EGGS IN THE RHIZOSPHERE BY ISOLATES OF ARF. Timper, P., and R. D. Riggs. Plant Pathology Department, University of Arkansas, Fayetteville, AR 72701.

Isolates of ARF, a promising fungus for biological control, are separated into two groups based on the appearance of their sclerotia-like structures: ARF-C (compact) and ARF-L (loose). A previous study showed that, on agar plates, ARF-C isolates were aggressive egg parasites, whereas ARF-L isolates were weak egg parasites. Our objective was to determine whether ARF-C and ARF-L isolates differed in their ability to parasitize eggs of *Heterodera glycines* in the rhizosphere of soybean cv. Lee 74. To determine parasitism of eggs, plants with gravid nematodes were transplanted into pots containing fungus-infested soil. After 20 days, eggs from 90 cysts/ARF isolate were examined for parasitism. The percentage of eggs parasitized was 55% to 92% for ARF-L isolates and 0% to 23% for ARF-C isolates. The discrepancy between egg parasitism on agar and in the rhizosphere suggests that ARF-L isolates are better rhizosphere colonizers than are ARF-C isolates.

GLUCOSINOLATE BREAKDOWN PRODUCTS FOR MANAGEMENT OF *HETERODERA GLYCINES*. Tylka, G. L.,¹ D. H. Soh,¹ and J. R. Coats.² ¹Department of Plant Pathology and ²Department of Entomology, Iowa State University, Ames, IA 50011.

Glucosinolates are secondary plant metabolites, a few of which are known to be insecticidal or nematocidal. Research was conducted to determine whether glucosinolate breakdown products, or aglycones, could be developed as nematicides for the soybean cyst nematode, *Heterodera glycines*. In vitro hatching of free *H. glycines* eggs in 10% and 100% $\mu\text{g/ml}$ cyanohydroxypropene and cyanohydroxy-propene propionate was 0.4% to 4.6% of that in deionized water after 24 days. Hatching did not increase when eggs were transferred from glucosinolate breakdown products to deionized water or 3 mM zinc sulfate after 24 days; *H. glycines* egg hatching was irreversibly inhibited or the eggs were no longer viable. In another experiment, hatching of *H. glycines* eggs in deionized water was inhibited by 79.4% after 12 days by volatiles from 100 $\mu\text{g/ml}$ cyanohydroxy-propene solution. These glucosinolate breakdown products may be useful as soil fumigants for management of *H. glycines* if hatching, particularly that of encysted eggs, is irreversibly inhibited by volatile fractions from the compounds.

POPULATION DENSITIES OF *HETERODERA GLYCINES* DETERMINED WITH GLOBAL POSITIONING TECHNOLOGY. Tylka, G. L., and S. K. Souhrada. Department of Plant Pathology, Iowa State University, Ames, IA 50011.

Growers are adopting global positioning system (GPS) technology for site-specific crop management. To assess the utility of GPS technology for corn and soybean production, two 20.2-ha study areas were established in 1996 in large (>100 ha) fields, one planted with corn and the other with soybean. The study areas were divided into 0.2-ha cells, and pre-plant and post-harvest soil samples were collected from randomly selected sites located within each cell with a hand-held GPS unit. The soybean cyst nematode, *Heterodera glycines*, was found in all but two cells in both study areas. Initial *H. glycines* egg densities in infested cells ranged from 50 to 29,000 eggs/100 cm³ soil, and cells with high initial *H. glycines* egg densities were aggregated in both study areas. During the growing season, detected *H. glycines* egg densities increased as much as 300-fold in cells in the soybean study area, but densities generally decreased in the cells of the corn study area. Egg population densities then could be correlated with other soil and plant variables measured in these cells.

YIELD LOSSES OF *HETERODERA GLYCINES*-RESISTANT SOYBEAN CULTIVARS IN IOWA IN 1996. Tyłka, G. L., and S. K. Souhrada. Department of Plant Pathology, Iowa State University, Ames, IA 50011.

Resistant soybean cultivars are used by Iowa soybean growers to manage the soybean cyst nematode, *Heterodera glycines*. Experiments are conducted each year to evaluate the agronomic performance of maturity group (MG) I, II, and III, *H. glycines*-resistant soybean cultivars in *H. glycines*-infested and nearby uninfested fields in north-central, central, and southeast Iowa. Several susceptible cultivars of each MG also are included in the experiments. In 1996, average yields of the resistant soybean cultivars, combined by MG, were 16.6% to 44.2% greater than those of susceptible cultivars of the same MG in *H. glycines*-infested fields. However, yields of the resistant cultivars averaged 14.1% to 33.5% greater in the noninfested fields than in the infested fields. Individual resistant cultivar yields were 2.5% to 47.5% greater in noninfested fields than in infested fields, indicating variation in tolerance of the resistant cultivars to the nematode. Resistant soybean cultivars consistently yield less in *H. glycines*-infested fields than in nearby noninfested fields in Iowa, which emphasizes the need for integrated management of the nematode.

ROOT-KNOT NEMATODES IN VEGETABLE CROPS IN SPAIN. Verdejo-Lucas, S.,¹ C. Ornat,¹ and F. J. Sorribas.² ¹Departamento Patología Vegetal, IRTA, Crta de Cabrils s/n., 08348 Barcelona, Spain, and ²Departamento Agronomía, ESAB, Comte d'Urgell 187, 08036 Barcelona, Spain.

A survey was conducted to detect *Meloidogyne* spp. in vegetable crops grown intensively in coastal areas of northeast Spain. Sixty-six greenhouses and 59 fields were sampled before planting the spring tomato crop, and at harvest. *Meloidogyne* spp. occurred in 39% of the sites sampled. Species detected included *M. arenaria*, *M. incognita*, and *M. javanica*. These nematodes were more frequent and in higher numbers in greenhouses than in fields. Changes in nematode population densities were assessed in nine greenhouses through the crop sequences cultivated during a growing season. In general, population densities increased after the spring tomato crop, reached the highest densities after the summer cucumber crop, and decreased after the autumn-winter crop. In most greenhouses, plant damage was observed after the summer cucumber crop but not after the spring tomato or autumn-winter crop.

CHANGES IN POPULATION DENSITIES OF *TYLENCHULUS SEMIPENETRANS* IN SPANISH CITRUS ORCHARDS. Verdejo-Lucas, S.,¹ and F. J. Sorribas.² ¹Departamento Patología Vegetal, IRTA, Crta. de Cabrils s/n., 08348 Cabrils (Barcelona), Spain, and ²Departamento Agronomía, ESAB, Comte d'Urgell 187, 08036 Barcelona, Spain.

Changes in population densities of *Tylenchulus semipenetrans* were monitored in two citrus orchards located in Amposta and Moncada. Both sites were planted to Troyer citrange and sampled for three consecutive years starting in April 1994. Changes in nematode population densities were more pronounced in the Amposta site than in the Moncada site. A peak in number of juveniles and males per 250 cm³ soil occurred earlier in the season in the Moncada site (April) than in the Amposta site (July) in 1994 and 1995, but little fluctuation was observed in 1996. Females per g root peaked in July or October in both sites. Numbers of eggs per g root peaked in the Amposta site in October 1994 and 1996, and in January 1995, whereas egg production in the Moncada site showed little change throughout the study.

MAPPING A NOVEL HEAT-STABLE RESISTANCE TO *MELOIDOGYNE* IN *LYCOPERSICON PERUVIANUM* LA 2157. Veremis, J. C.,¹ S. van Heusden,² and P. A. Roberts.¹ ¹Department of Nematology, University of California, Riverside, CA 92521, and ²CPRO-DLO, P.O. Box 16, NL-6700AA Wageningen, The Netherlands.

All tested entries of the wild tomato, *Lycopersicon peruvianum* LA 2157, were resistant to *Mi*-avirulent *Meloidogyne* spp. biotypes at 32 °C. The novel resistance locus was mapped with an

RFLP linkage map of F₂-lines from *L. esculentum* cv. Solentos × *L. peruvianum* LA 2157. The inheritance of this heat-stable resistance was evaluated in 100 F₃ lines derived from two F₁ interspecific hybrids. Phenotypic classification of individual F₃ lines as homozygous resistant, homozygous susceptible, and segregating (susceptible and heterozygous resistant) was used to determine the linkage with markers on chromosome 6. The position of the novel heat-stable resistance of LA 2157 was localized in the resistance genes cluster close to the location of gene *Mi*. Cuttings of the above F₃-lines expressed resistance to *Mi*-avirulent *M. incognita* and *M. javanica* biotypes at 32 °C (a temperature at which *Mi* resistance is not expressed), but not to a *Mi*-virulent *M. incognita* biotype at 30 °C.

INBRED NEMATODES ELUCIDATE RELATIONSHIPS BETWEEN DIFFERENT SOURCES OF RESISTANCE. R. A. Vierling,¹ J. Faghihi,² V. R. Ferris,² and J. M. Ferris.² ¹Indiana Crop Improvement Association and Department of Agronomy, Purdue University, West Lafayette, IN 47907-1150, and ²Department of Entomology, Purdue University, West Lafayette, IN 47907-1158.

Mapping of soybean cyst nematode (SCN, *Heterodera glycines*) resistance loci using field populations has led to contradictory results. The same SCN inbred (race 3 phenotype) used to map resistance from 'Hartwig' soybean was also used to map resistance from 'Peking' soybean. Four unlinked RFLP markers were associated with SCN resistance from Peking. Two of the four Peking loci are in common with two SCN resistance loci we previously mapped in Hartwig, and two loci are not shared with Hartwig. To determine if SCN inbreds could be used to identify race-phenotype specific resistance loci, we challenged progeny from a Williams 82 × Hartwig cross with a race 4-phenotype inbred nematode. Comparison of results obtained with the two inbred nematode lines showed shared and unique resistance loci. Using inbreds for molecular mapping of SCN resistance can distinguish relationships among different sources of SCN resistance and allow for the identification of both unique and shared resistance genes.

MIGRATION BEHAVIOR OF ROOT-KNOT NEMATODES IN ROOTS. Von Mende, N. Entomology and Nematology Department, IACR Rothamsted, Harpenden, Herts. AL5 2JQ, United Kingdom.

The migration of *Meloidogyne incognita* in *Arabidopsis thaliana* roots recently has been described in detail. In the present study, the migratory pathway and the behavior of three root-knot nematode species, *M. incognita*, *M. arenaria*, and *M. javanica*, in *A. thaliana*, tomato, and balsam were compared. The three nematode species behaved in tomato and balsam in a way similar to that observed in *Arabidopsis*. Plants were grown in growth pouches at 24 °C and inoculated with 150 infective juveniles per root tip. The rate of invasion was evaluated every 6 hours. Within 6 hours after inoculation juveniles of *M. arenaria* were already detected in the cortex heading towards the root tips, whereas juveniles of *M. incognita* were detected in the root only after a further 6 hours. This difference in speed of invasion and migration was confirmed by video recorded microscopy of migrating juveniles in *A. thaliana* roots, demonstrating that inside these roots, *M. incognita* is the slowest of the three species tested.

DESMODIUM, LEUCAENA, SENNA, AND SESBANIA TISSUES AS AMENDMENTS TO ROOT-KNOT NEMATODE-INFESTED SOIL. Walker, J. T.,¹ J. B. Morris,² and J. Melin.¹ ¹Department of Plant Pathology, University of Georgia, and ²USDA, PGRCU, Griffin, GA 30223-1731.

Few semi-tropical forage or medicinal legumes have been evaluated as organic soil amendments. Dried tissues from *Desmodium*, *Leucaena*, *Senna* and *Sesbania* species were mixed individually at 0%, 1%, 2%, and 5% (w/w) with pasteurized soil containing 6,000 eggs/kg of *Meloidogyne incognita*, then placed in polyethylene bags for one week at 21 to 28 °C. Tissue of all species significantly reduced the number of galls on Rutgers tomato by more than 50% after 8 weeks; however, there was an interaction between tissue type and root-gall rating, tomato heights, and dry

weights. *Desmodium gangeticum* was extremely phytotoxic, whereas *D. sandwicense* was not, yet the latter reduced gall numbers by 52%. *Leucaena leucocephala* decreased gall numbers at all rates and was phytotoxic only at 5%. *Senna corymbosa*, *S. occidentalis*, and both samples of *Sesbania* reduced the numbers of root galls, but varied in their toxicity to tomato. These and related legumes may have potential herbicidal and or nematicidal properties as soil amendments.

EFFECTS OF RESISTANCE TO *MELOIDOGYNE INCOGNITA* ON THE INTERACTION OF *M. INCOGNITA* AND *THIELAVIOPSIS BASICOLA* IN COTTON. Walker, N. R.,¹ T. L. Kirkpatrick,² and C. S. Rothrock.¹ ¹Department of Plant Pathology, University of Arkansas, Fayetteville, AR 72701, and ²University of Arkansas Southwest Research and Extension Center, Hope, AR 71801-9729.

Studies were established to determine if resistance to *Meloidogyne incognita* (Mi) affects the severity of the interaction between Mi and *Thielaviopsis basicola* (Tb) in cotton. Mi-resistant Auburn 634 and M-315 and susceptible Deltapine 5415 cotton seed were germinated, then transplanted into pots containing 1,500 g of methyl bromide-fumigated, sandy loam soil. Pots were infested with one of the following treatments: 20 chlamydospores of Tb/g of soil, 2,000 Mi eggs/pot, or both. Un-infested pots served as controls. The studies were conducted for 50 days in a growth chamber maintained at 21 °C. Nematode reproduction on Deltapine 5415 was lower in the Mi + Tb treatment than in Mi alone. Reproduction on the resistant lines was low and did not differ between Mi alone and Mi + Tb treatments. Root necrosis was similar for all cultivars in the Tb and Mi + Tb treatments, but necrosis was more severe than when Mi alone was used. Cultivars did not differ in seedling height-to-node ratio (HNR) within treatments, but HNR was lowest for seedlings in the Tb treatment.

EFFECTS OF COVER CROPS ON NEMATODE POPULATIONS AND SOIL MICROORGANISMS IN A PINEAPPLE FIELD. Wang, K.-H., and B. S. Sipes. Department of Plant Pathology, University of Hawaii, Honolulu, HI 96822-2279.

Three cover crops, marigold (*Tagetes patula*), yellow mustard (*Sinapis alba*), and Sunn hemp (*Crotalaria juncea*), were examined for their effects on nematode and soil microorganism populations in a pineapple field and in soil in the greenhouse. Incorporation of *C. juncea* reduced reniform nematode mobility under greenhouse conditions by 80% compared to 46% in the weed control, and supported higher fungivorous nematode populations in the field test (100/250 cm³ soil) compared to the weedy fallow control (10/250 cm³ soil) before cover crop incorporation. Fungal propagules were more common in *C. juncea*-treated field plots, but no nematode-predacious or parasitic fungi were observed on fungus-selective media. Compared to pineapple, the three cover crops were poor hosts for reniform nematode (*Rotylenchulus reniformis*). Reduction of *R. reniformis* numbers by *C. juncea* may be due to indirect effects of soil microorganisms enhanced by the plant and its nonhost status to *R. reniformis*.

IDENTIFICATION AND CHARACTERIZATION OF CUTICLE COLLAGEN GENES OF PARASITIC STAGES OF *MELOIDOGYNE INCOGNITA*. Wang, T. Y., and R. S. Hussey. Department of Plant Pathology, The University of Georgia, Athens, GA 30602-7274.

The change in cuticle structure of parasitic stages of *Meloidogyne incognita* is accompanied by significant biochemical changes. To clone genes encoding cuticle collagens of *M. incognita*, a degenerate probe based on seven conserved amino acids (GPPGPPG), was used to screen an adult female cDNA library. Several complete or partial collagen genes were cloned, including two very similar genes, *col-1* and *col-2*, which share 89% nucleotide sequence identity. The predicted amino acid sequences encoded by these genes contained several domains and sequence motifs common to other nematode collagens. Analogs of the collagen genes were present in *Meloidogyne* spp. but not in *Caenorhabditis elegans* or *Heterodera glycines*. These genes were developmentally expressed following the onset of parasitism. Identification of cuticle collagen genes of parasitic stages

will provide a better understanding of collagen synthesis and changes in cuticle structure during *M. incognita* maturation.

EXPRESSION OF FUSION PROTEINS OF SECRETORY GENES FROM THE ESOPHAGEAL GLANDS OF THE SOYBEAN CYST NEMATODE. Wang, X., Y. Yan, and E. L. Davis. Department of Plant Pathology, North Carolina State University, Raleigh, NC 27695-7616.

A cDNA encoding a putative soybean cyst nematode (SCN, *Heterodera glycines*) dorsal gland secretory protein recognized by monoclonal antibody (MAb) 5B9, and two cDNAs encoding subventral gland proteins (putative cellulases 1 and 2) recognized by MAB MGR48, were cloned into expression vector pET-28 and overexpressed in *E. coli*. The expressed proteins were N-terminal fusions to His-Tag peptides with molecular weights of about 52,000 (5B9 protein), 38,000 (cellulase 1), and 54,000 (cellulase 2). Their identities were confirmed on Western blots with MABs 5B9 and MGR48. The 5B9 fusion protein was affinity-purified, and both thrombin-digested and undigested 5B9 fusion proteins were used to immunize rabbits to produce polyclonal antibodies. Both cellulase fusion proteins were purified under denaturing conditions and used to generate polyclonal antibodies. The polyclonal antibodies are useful for localizing these secretory proteins in SCN-infected soybean roots.

OVERWINTERING OF *HETERODERA GLYCINES* AND *H. CAROTAE* IN MICHIGAN. Warner, F. W., M. F. Berney, and G. W. Bird. Department of Entomology, Michigan State University, East Lansing, MI 48824-1115.

Overwintering of soybean cyst nematode, *Heterodera glycines*, and carrot cyst nematode, *H. carotae*, was evaluated under Michigan conditions. Populations of both species appeared to increase during the winter. *H. glycines* research was done under field conditions, and in microplots of three soil textures (sand, sandy loam, and sandy clay loam) and four soybean cultivars. Soil samples were collected in the fall and spring, and processed with a modified centrifugation-flotation procedure. In 1995 and 1996, egg and second-stage juvenile populations of *H. glycines* were higher in spring-collected than fall-collected samples. Survival generally was better in sandy clay loam than in sand, with sandy loam intermediate. Differences in overwintering survival associated with soybean cultivars were not consistent in 1995 and 1996. Population dynamics of *H. carotae* in organic soil was monitored monthly from 1988–1990, and cyst densities usually reached a maximum in the February following carrot production.

COMPARISON OF VELVETBEAN AND BAHIAGRASS EFFECTS ON YIELD AND NEMATODE POPULATIONS OF SOYBEAN. Weaver, C. F.,¹ R. Rodríguez-Kábana,¹ D. B. Weaver,² and D. G. Robertson.¹ ¹Department of Plant Pathology, and ²Department of Agronomy and Soils, Auburn University, AL 36849.

The effects of velvetbean (*Mucuna deeringiana*) and bahiagrass (*Paspalum notatum*) on yield and nematode populations of soybean were compared in a 3-year field experiment in southern Alabama. The field was severely infested with root-knot nematodes (*Meloidogyne arenaria*, *M. incognita*), soybean cyst nematode (*Heterodera glycines*), and a spiral nematode (*Helicotylenchus dihystera*). Yield and nematode populations were assessed in seven soybean cultivars following two years of velvetbean and two years of bahiagrass. Overall yield increases (relative to soybean monoculture) in response to velvetbean and bahiagrass were 138% and 124%, respectively. The velvetbean system suppressed root-knot and cyst nematodes effectively while the bahiagrass system did not. Both systems supported significant levels of spiral nematodes.

ADVANTAGES OF LOW-TEMPERATURE SCANNING ELECTRON MICROSCOPY (SEM) FOR RECORDING OBSERVATIONS OF UNIQUE SPECIMENS. Wergin, W. P.,¹ E. Hoberg,² and E. F. Erbe.¹ ¹Nematology Laboratory, and ²Biosystematics and National Parasite Collection Unit, Agricultural Research Service, U.S. Department of Agriculture, Beltsville, Maryland 20705.

Systematic nematologists who wish to use scanning electron microscopy to help describe nematode features are frequently confronted by three problems: (i) the number of specimens may be extremely limited; (ii) the specimens are commonly shipped in alcohol and are partially dehydrated; (iii) conventional preparation procedures, such as chemical fixation, dehydration, and critical point drying, often cause considerable shrinkage and distortion. To eliminate these problems a protocol was developed that enabled multiple orientations and observations of a single specimen. In brief, a single specimen could be rehydrated, frozen to a specimen holder and observed with low-temperature SEM. Following these observations, the specimen could be removed from the instrument, thawed, reoriented, and refrozen for further observation.

TRANSFER OF SUPPRESSIVENESS AGAINST *HETERODERA SCHACHTII* INTO A FUMIGATED CONDUCTIVE FIELD SITE. Westphal, A., and J. O. Becker. Department of Nematology, University of California, Riverside, CA 92521.

A soil transfer study was conducted in a field at the agricultural research station, UC Riverside, CA, which had been demonstrated to be suppressive against *Heterodera schachtii*. In a randomized complete block, methyl bromide was applied with a hot-gas fumigation method at a rate of 336 kg/ha to all plots, except the controls. Suppressive soil from other parts of the field was rototilled into the upper 10 cm of the treated plots at a rate of 1% and 10%. After one month the experimental plots were infested with greenhouse-reared *H. schachtii*. One month after inoculation all plots were planted with seedlings of Swiss chard (*Beta vulgaris*). Decreased nematode activity was observed in the suppressive soil compared with the conducive soil starting at planting. Ten weeks later, at harvest, nematode populations had reached 120 cysts/g root dry mass in the suppressive soil and 273 cysts/g root dry mass in the conducive soil. Amendments of both 1% and 10% of suppressive soil suppressed cyst nematode populations to a level where they were not significantly different from the suppressive control.

DEVELOPMENT OF *HETERODERA SCHACHTII* POPULATIONS IN A SUPPRESSIVE VS. A CONDUCTIVE FIELD SOIL. Westphal, A., and J. O. Becker. Department of Nematology, University of California, Riverside, CA 92521.

During two consecutive years the population development of *Heterodera schachtii* was monitored in plots containing suppressive and conducive soils in a field at the Agricultural Research Station, UC-Riverside, CA. The soil type was a Hanford sandy loam. Soil fumigation with metam sodium rendered the suppressive plots into conducive ones. The entire trial site was then reinfested with greenhouse-reared sugar beet cyst nematodes. In consecutive order the plots were cropped to Swiss chard (*Beta vulgaris*), canola (*Brassica napus*), Swiss chard, and cabbage (*Brassica oleracea*). At each harvest, yield and nematode population densities were determined. The plots were then rototilled and rebudded for the following crop. During the entire length of the experiment the number of cysts per gram of soil remained significantly higher in the fumigated conducive soil than in the untreated suppressive plots. The egg number per gram of soil was consistently low in the suppressive soil (mean = 33 eggs/g soil in the root zone). In the conducive soil the egg numbers peaked in the second growing season (3× that of the suppressive soil) and then declined to approximately 1.5× the numbers in the suppressive soil.

DISTRIBUTION OF ROOT-KNOT NEMATODE DENSITIES IN COTTON FIELDS AND PROPOSED NEMATOCIDE RATES. Wheeler, T. A.,¹ H. W. Kaufman,¹ and G. L. Schuster.²

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The distribution of root-knot nematode in cotton may determine the success of variable nematocide rate application technology. Seven fields were intensively sampled for root-knot nematode. The following threshold levels for second-stage juveniles (J2) per 500 cm³ soil in the fall were: <250; 250-999; 1,000-2,499; and ≥2,500 for Temik 15G rates of 3.0, 4.2, 6.0, or 8.4 kg/ha,

respectively. Critical spring J2 levels per 500 cm³ soil were: <50; 50–199; 200–499; ≥500 for Temik 15G rates of 3.0, 4.2, 6.0, or 8.4 kg/ha, respectively. These threshold levels were used to develop frequency histograms for Temik use in each field. In 5 of 9 fields, one rate was recommended for >50% of the area sampled. Nematicide-treated fields were 80, 0, 92, 76, 17, 0, 4, 13, and 4% overtreated by growers, and 0, 100, 0, 0, 21, 59, 75, 37, and 83% undertreated, according to the decision rules. Variable rate application of nematicides or nematode sampling programs should increase net profits and(or) reduce amounts of applied but unnecessary pesticide.

RESPONSE DIFFERENCES OF A FREE-LIVING NEMATODE, *PANAGROLAIMUS SUPERBUS*, TO LEAD AND CADMIUM. Williams, M. S. R.,¹ and S. Seraphin.² ¹Ecology and Evolutionary Biology Department, and ²Materials Science and Engineering Department, University of Arizona, Tucson, AZ 85721.

Two isolates of *Panagrolaimus superbus* were separately exposed to 500 ppm lead and 500 ppm cadmium for an exposure time of 20 minutes. Isolates were collected from Oxford, UK, and Tucson, Arizona, and cultured on *Enterobacter cloacae* on agar. Control nematodes were exposed to deionized water. TEM sections from the oesophageal and intestinal regions revealed differing responses to the two heavy metals. Lead particulates were identified in the esophageal gland region and in the lumen of the semicircular canals and triradiate oesophagus. Control nematodes had no evidence of particles. Particle elemental analysis was carried out with electron diffraction and energy dispersive X-ray spectroscopy (EDS). This is the first report of lead biomineralization in free-living nematodes. There were no detectable heavy metal particulates in cadmium-exposed nematodes. Both lead and cadmium produced a decrease in intestinal villi length compared WITH controls. This is consistent with earlier findings for cadmium. Villi length reduction appeared greater by a factor of two in cadmium-exposed worms.

TOTAL NEMATODES AS INDICATORS OF ECOSYSTEM CONDITION. Yeates, G. W. Landcare Research, Private Bag 11052, Palmerston North, New Zealand.

In agroecosystems the total soil nematode fauna is commonly positively correlated with productivity. In such ecosystems the contribution of nematode grazing to microbial turnover and nutrient cycling outweighs losses due to plant-feeding nematodes, and the abundance of plant feeders reflects the size and quality of the plant resource. In some soil types or climates, microfauna other than nematodes may effectively control nutrient cycling. Use of predators or bacterial:fungal feeding ratios as indicators is limited by differences in soil structure or organic matter between soil types and land uses. Aquatic sediments, fed by organic detritus, do not represent ecosystems and their nematode faunas should be compared to those of terrestrial organic accumulations; indices based on them may not be applicable to agroecosystems. There is need for further work to (i) relate 'instantaneous' soil nematode populations to the system sampled; (ii) assess whether dominant taxa (e.g., Rhabditidae, Heteroderidae, Steinernematidae) should be discounted; and (iii) determine how abundance values can be compared among crops, soil types, districts, regions, nations, and biomes.

SPECIES RICHNESS IN SOIL NEMATODE ASSEMBLAGES. Yeates, G. W.,¹ and B. Boag.²

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In terrestrial ecosystems, soil nematodes are typically numerically abundant, taxonomically diverse, and play critical roles in soil processes. The total number of species (= species richness) present at a site is central to studies of biodiversity but is not given by indices such as H', maturity index (MI), and ΣMI. Published species counts average 49 (1–228), but such estimates are based on a wide variety of sampling regimes and number of specimens identified. After categorizing 134 published counts as single, repeated, or intensive sampling regimes, we 'normalized' species richness in vegetational and latitudinal groups. Average species richness was found to be greatest in temperate broadleaf forest (62), followed by 53 species in cultivated soil, 42–49 species in

coniferous forest, tropical rainforest, and grassland, and 23 species in polar regions. Sites at 0–10° latitude averaged 81 species, while maximum species richness was 94 at 30–40°. Such results can be viewed not only in terms of mechanisms underlying biological diversity but also to highlight vegetation types and latitudes for which few data are available.

PLANTING EARLY-MATURING SOYBEANS IN APRIL IN TENNESSEE REDUCES NUMBERS OF *HETERODERA GLYCINES* EGGS. Young, L. D. USDA ARS, 605 Airways Blvd., Jackson, TN 38301-3201.

Maturity group IV soybean cultivars Asgrow A4138 (resistant) and Asgrow A4341 (susceptible), and maturity group V cultivars Asgrow A5403 (resistant) and Hutcheson (susceptible) were planted 11 April, 16 May, and 17 June 1996 in a field infested with *Heterodera glycines* race 3. Yields of all cultivars planted in April were lower than when cultivars were planted in May or June. Yield of Asgrow A5403 was significantly greater than the yields of other cultivars at each planting date, except for yield of Hutcheson planted in April. However, at the 4 October sampling, plots planted to Asgrow A4341 in April had significantly fewer eggs compared to those planted in May or June. Numbers of eggs for Hutcheson plots did not differ across planting dates. Nematode suppression gained from early planting of early maturing cultivars was not sufficient to offset lower yields experienced with this treatment.

COMPARISON OF PCR-AMPLIFIED IGS, ETS, AND ITS OF RIBOSOMAL DNA OF FIVE *PRATYLENCHUS* SPP. OF ONTARIO. Yu, Q., and J. Potter. AAFC, PMRC, Vineland Station, 4902 Victoria Ave., Vineland, L0R 2E0, Ontario, Canada.

Intergeneric spacer (IGS), external transcribed spacer (ETS), internal transcribed spacer 1 (ITS1), internal transcribed spacer 2 (ITS2), and the combined region of ITS1 and ITS2 of ribosomal DNA of *Pratylenchus crenatus*, *P. neglectus*, *P. penetrans* isolates 1 and 2, *P. thornei*, *P. zaei*, and *P. sp.* of Ontario origin were PCR-amplified with four sets of primers. The sizes of amplified ITS1 of these species were different, ranging between 300 and 600 bp. The size of ITS2 of *P. neglectus* was close to that of *P. penetrans* isolate 1, while ITS2 of *P. zaei* was close to that of *P. sp.* All ITS2 were smaller than ITS1 of same species, but also ranged between 600 and 300. The ITS1 and ITS2 combined regions of these species were different, ranging from 600 to 1,000 bp. IGS and ETS regions of *P. neglectus*, *P. penetrans* isolate 2, *P. thornei*, and *P. zaei* were the same size, about 1,770 bp, the size of IGS and ETS of *Caenorhabditis elegans*.

POTENTIAL FUNGAL BIOCONTROL AGENTS ISOLATED FROM CHICKEN MANURES AND LITTER. Zhang, F., and J. P. Noe. Department of Plant Pathology, University of Georgia, Athens, GA 30602.

Thirty fungal species isolated from chicken litter were tested for growth rates under varying conditions and evaluated as possible biological control agents for root-knot nematode, *Meloidogyne arenaria*. Most fungi grew well on V8, PDA, and CMA media but only 30% grew on chicken litter agar. Although culture filtrates from more than 10 fungal species showed limited toxic effects on second-stage juveniles (J2) of *M. arenaria*, only the filtrate from *Myrothecium verrucaria* significantly reduced penetration of soybean roots by J2 in a greenhouse soil mix. The greatest toxic effects were observed from culture filtrates of *M. verrucaria* grown on PDB liquid medium on a shaker. After fermentation for two weeks, culture filtrates from PDB liquid medium applied as aqueous dilutions of 12% to 100% filtrate inhibited penetration of *M. arenaria* on soybean. By comparison, only the pure culture filtrate of *M. verrucaria* from V8 liquid medium reduced nematode penetration. Several fungi show potential for production of nematocidal metabolites, but more information is needed on the conditions required for fermentation, and on the chemical structures having nematocidal activity.

FINE STRUCTURE OF *PRATYLENCHUS PENETRANS*. Zunke, U.,¹ B. Y. Endo,² and W. P. Wergin.² ¹University of Hamburg, Institute of Applied Botany, 20355 Hamburg, Germany, and ²Nematology Laboratory, Plant Sciences Institute, USDA, Beltsville, MD 20705.

Observations were made on the fine structure of various developmental stages of *Pratylenchus penetrans* by means of transmission (TEM) and low-temperature scanning electron microscopy (LTSEM). Comparisons also were made between TEM observations of isolated nematodes and those previously studied with video-enhanced light microscopy (VECM). Secretory granules in ampullae of the subventral glands are small and have moderate electron opacity. In comparison, secretory granules in the dorsal gland extension in the metacarpus may appear uniformly small but show a wide range in size and electron opacity within the procorpus. Spermatocytes in the growth zone of the testis develop into amoeboid spermatids with electron-opaque spherical nuclei that appear to lack a membrane. The vaginal cuticle forms a flat, contoured channel, which is convoluted near the vulva.