EFFECT OF THE ENTOMOPATHOGENIC NEMATODE, *STEINERNEMA CARPOCAPSAE*, ON THE INVASION OF *RADOPHOLUS SIMILIS* INTO BANANA ROOTS. M. P. Aalten, Department of Agriculture, University of Reading, Earley Gate P.O. Box 236, Reading RG6 2AT, U.K.—Twenty-five small banana plants with an average height of 5. 5 cm (±1.1) and an average root fresh weight of 1.5 g (±0.7) were divided over 5 treatments. The 5 treatments involved an application of 500 *R. similis* for all treatments and 4 different concentrations of *S. carpocapsae*: i) 5 000, ii) 25 000, iii) 50 000 and iv) 100 000 (= 1×, 5×, 10× and 20× the recommended application rate for insect control). Seven days after inoculation, the root systems were harvested, washed free of soil, and treated with 0.1% acid fuchsin to stain the nematodes within the roots. The stained roots were then macerated, and the number of nematodes was estimated. The average number of *R. similis* invading the "control" roots was 285 (±51). There were no significant differences ($P > 0.05$) in the number of *R. similis* invading the roots among any of the 4 *S. carpocapsae* treatments. The average number of *R. similis* invading the roots of all 4 *S. carpocapsae* treatments combined was 147 (±55). The number of *R. similis* invading the *carpocapsae*-treated roots was significantly less ($P \leq 0.05$) than in the "control" roots. The concentration of *S. carpocapsae* used did not affect the reduction in invasion. Reasons why *S. carpocapsae* should affect the invasion ability of *R. similis* are speculative.

STEM NEMATODE, *DITYLENCHUS DIPSACI*, INFESTING FABA BEAN, *VICIA FABA*, IN MAGHREB REGION. F. Abbad Andaloussi, S. Sellami, and N. Kachouri, INRA, Département de Phytiatrie, BP 293, Kénitra, Morocco, INA Alger El Harach Algeria, and INRAT, Laboratoire de Zoologie, Tunis, Tunisia.—Stem nematode (*D. dipsaci*) is the most damaging nematode to faba bean in the Maghreb region (Morocco, Algeria, and Tunisia). Almost all faba bean areas are infested in these regions. The giant race of this nematode has been found in 64, 83, and 70% of the plant samples, respectively, from Morocco, Algeria, and Tunisia, which explains the importance of damage observed on *V. faba*. Research undertaken at INRA Morocco on the control of *D. dipsaci* in *V. faba* showed the limit of the use of resistance as a means of control due to the variability in the virulence of the stem nematode populations. On the other hand, the results indicate the efficiency of late sowing of *V. faba* to reduce the *D. dipsaci* populations. This cultural control method may have limited use in some regions where high temperatures prevail during the flowering stage. A research program has been initiated to develop control strategies for the stem nematode, *D. dipsaci*, in *V. faba* in the Maghreb region. The main objectives of this program are studying stem nematode virulence and screening for germplasm resistance.

CONTRIBUTION TO THE KNOWLEDGE OF THE FAMILY DORYLAIMIDAE DE MAN, 1876 (NEMATODA: DORYLAIMIDA) IN ANDALUCÍA ORIENTAL, SPAIN. J. Abolafia Cobaleda, and R. Peña Santiago, Departamento de Biología Animal, Vegetal y Ecología, Universidad de Jaén, Virgen de la Cabeza nº 4, 23008-Jaén, Spain.—Abundant material belonging to the family Dorylaimidae has been collected during the last 15 years mainly in natural areas from Andalucía Oriental (provinces of Almería, Granada, Jaén, and Córdoba), Spain. Nineteen species of the genera *Dorylaimus* (*D. asymphydorus, D. lineatus,* and *D.* sp.), *Prodorylaimus* (*P. acris, P. filiarum, P. mas,* and *P. uliginosus*), *Prodorylaimium* (*P. brigdammense*), *Mesodorylaimus* (*M.* sp. *cf. M. aegypticus, M. bastiani, M. litoralis, M.* sp. *cf. M. guarani, M. potus,* and 5 previously undescribed) and *Opisthodorylaimus* (*O. sylphoides*) have been identified. Measurements and illustrations of the most interesting species were given, and the distribution of the species in relation to 10 habitat types was analyzed and discussed.

TAXONOMIC AND BIOGEOGRAPHIC DIVERSITY WITHIN HETERORHABDITIS: A MOLECULAR PHYLOGENETIC PERSPECTIVE. B. Adams, A. M. Burnell, and T. O. Powers, Department of Plant Pathology, University of Nebraska, Lincoln, Nebraska 68583, U.S.A., and Department of Biology, St. Patrick's College, Maynooth, Co. Kildare, Ireland.—Phylogenetic relationships within the genus *Heterorhabditis* were examined for 7 described species (*H. hawaiiensis, H. indicus, H. bacteriophora,*

H. argentinensis, H. zealandica, H. hepialus, H. megidis) and 10 laboratory strains. DNA sequences from the ITS 1 and portions of the 18s and 5.8s flanking regions of the nuclear ribosomal RNA gene provided 127 phylogenetically informative characters of 742 aligned base pairs. Parsimony, distance, maximum likelihood, and combinable components analyses were performed under a variety of alignments and character codings. All methods produced a single tree of identical topology. Tree robustness was measured by bootstrap and decay index analysis. Different tree topologies were evaluated in terms of maximum likelihood ratios, tree lengths, and topology dependent PTP tests. Results suggest that although the genus comprises a monophyletic group, taxonomic boundaries based on morphological species concepts within the "bacteriophora group" are paraphyletic. Alternatively, taxonomic boundaries based on biological or phylogenetic species concepts are not compromised, but require that at least 2 taxa be elevated to species status. The "bacteriophora" clade is shown to be plesiomorphic, and of the continental isolates, congruence between geologic and phylogenetic history requires only 2 dispersal events. Low sequence divergence between dispersing taxa and the other members of their clade suggest that these events were relatively recent.

AN SEM STUDY ON *TERATORHABDITIS ANDRASSYI* AND *CRUZNEMA TRIPARTITUM* (MESORHABDITINAE:RHABDITIDA). I. Ahmad, T. A. Husseni, and Q. Tahseen, Department of Zoology, Aligarh Muslim University, Aligarh 202002, India.—Two species of Mesorhabditinae inhabiting similar habitats were studied with the scanning electron microscope. In *T. andrassyi*, the lip region is low, lips are separate with strongly sclerotized and flattened inner margins. Lateral lips are slightly smaller than the subdorsal and subventral. Amphidial apertures are small, elliptical, and located at the base of lateral lips. Labial papillae are in 2 circlets with the inner ones being setose and directed towards the star-shaped stomal opening. Lateral fields are represented by 2 ridges, and the longitudinal lines are most prominent adjacent to the lateral fields. Males have a strongly developed bursa with crenate margins and 10 pairs of caudal papillae. In addition, there are a median pre-cloacal and paired post-cloacal subventral papillae. Phasmids are open on the dorsal aspect of the bursa between the seventh and eighth papilla. The spicules are fused at the tips. In *C. tripartitum*, the lips are large and rounded with single papilla each. Amphidial apertures are minute, slightly off the lateral axis. Lateral fields have 3 ridges. Longitudinal ridges/lines are prominent, crossed by transverse striations forming a corn-cob pattern. Longitudinal ridges on the tail are irregular. In males, the bursa is well-developed but small with 9 pairs of caudal papillae. Margins of bursa are not crenate. Median pre-cloacal and paired post-cloacal papillae also are present. The cuticle breaks up into mamillations in the cloacal region.

BIOCONTROL OF RENIFORM AND ROOT-KNOT NEMATODES BY NEW BACTERIAL ISOLATES. A. H. Ali, Cairo University, Faculty of Agriculture, Nematology Division, Cairo, Egypt.—Five bacterial antagonists were tested for the control of *Meloidogyne javanica* and *Rotylenchulus reniformis* on sunflower. Results indicated that reproduction of both nematode species was affected by application of the bacterial isolates. Inoculum levels and timing of these bacterial applications relative to nematode inoculation also had significant effects on both nematode species. In this regard, application of bacteria one week prior to nematode inoculation surpassed that of with, or one week post nematode inoculation treatments. In all cases, suppressive effect of the bacteria relative to check ranged between 46% to 96% for *M. javanica* and 39% to 100% for *R. reniformis*. The carry over effect of the 5 bacterial isolates continued up to 15 months, at which time reduction in nematode reproduction was between 41% and 91% for *R. reniformis* and 5% to 88% for *M. javanica*. Bacteria could be re-isolated from the soil after a 15-month period from the initial inoculation.

USE OF SOME VEGETABLE AND ORNAMENTAL PLANT WASTES AND NEMATICIDES FOR IMPROVING GROWTH, YIELD AND NEMATODE CONTROL OF POTATO PLANTS. F. A. Ali, and M. E. Sweelam, Horticulture and Economic Entomology and Agricultural Zoology Departments, Faculty of Agriculture Menoufia University, Shebin El-Kom, Egypt.—Two field experiments were conducted at Shebin El-Kom, Egypt during 1994 and 1995 seasons to study the effects of wastes of some vegetable and ornamental plants on growth and yield of potato cv. Agria and its role in nematode control. These wastes were added either as ground dry matter to soil or by steeping the tuber seeds in water extracts 2 hrs before planting. Plants used were *Melia azedirach, Lantana camara, Shinus terebenthifolius, Cymbopogon citratus, Artemisia cinia, Allium cepa,* and *Allium sativum.* Nemacur and *Bacillus thuringensis* were added as nematicides. Results showed that the improved germination was increased by all treatments as compared with untreated control. Adding the wastes of *C. citratus* as a ground powder and *A. cinia* as a water extract gave the largest yield of tubers. Growth character such as plant height, number of leaves, and dry weight of whole plant parts (leaves, stems and tubers) were significantly increased by adding the wastes. Harvest index was significantly affected by the using treatments. However, no significant differences were found in specific gravity, vitamin C, and total soluble solids of tubers. A total of 12 nematode genera belong to 9 families and 2 orders were recovered and identified from the experimental area. The most dominant genera belonged to *Meloidogyne* spp. and *Rotylenchulus reniformis.* All wastes showed nematicidal properties against nematode populations, resulting in significant differences in their control, as well as Nemacur and *B. thuringensis.* The best results in nematode control as well as potato yields were obtained by Nemacur, *C. citratus, S. terebenthifolius, A. cinai,* and the bacterium when used as a ground powder.

USE OF CELL CYCLE MARKERS TO STUDY EARLY EVENTS OF PLANT-NEMATODE INTERACTIONS. J. de Almeida Engler, M. Van Montagu, G. Engler, and G. Gheysen, Laboratorium voor Genetica, via the Department of Genetics, affiliated to the Flanders Interuniversity Institute for Biotechnology, Universiteit Gent, K.L. Ledeganckstraat 35, B-9000 Gent, Belgium.—Sedentary plant-endoparasitic nematodes are pathogens that infect a wide range of economically important crop plants causing severe losses to agriculture. In a compatible interaction, they induce specialized feeding sites: giant cells embedded in galls (root-knot nematodes) or syncytia (cyst nematodes). To get more insight into which developmental programs are switched on during early events of plant-nematode interactions, we used 3 cell cycle markers. Tritiated-thymidine incorporation was used to monitor which nuclei DNA synthesis occurred and was used as a marker for the S phase of the cell cycle. Expression analysis of the cell cycle marker genes cdc2a and cyc1 was performed by mRNA *in situ* hybridization and promoter-β-glucuronidase analysis. The cyc1 gene was used as a marker for the G_2 phase, whereas the cdc2a gene was used as a marker for all cell cycle phases as well as for the competence to divide. Infected seedlings were treated with the cell cycle inhibitors, hydroxyurea (blocks in S phase) and oryzalin (blocks in M phase) to investigate the relevance of DNA synthesis and mitosis on the development of the feeding sites. All experiments were carried out at different time points after inoculation on *Arabidopsis thaliana* roots infected with the root-knot nematode, *Meloidogyne incognita,* and the cyst nematode, *Heterodera schachtii.* A strong correlation was observed between initiation of feeding cells by both root-knot and cyst nematodes with DNA synthesis and the induced expression of cdc2a and cyc1 genes.

GREEN MANURE CROPS WITH POTENTIAL FOR MANAGEMENT OF COLUMBIA ROOT-KNOT NEMATODE, *MELOIDOGYNE CHITWOODI* RACE 2. S. Al-rehiayani, and S. L. Hafez, University of Idaho, Parma R&E Center, 29603 University of Idaho Lane, Parma, Idaho 83660, U.S.A.—The suitability of oil radish, buckwheat, sudangrass, sorghum-sudangrass, horsebean, velvetbean, castorbean, rapeseed, mustard, and corn as hosts of *Meloidogyne chitwoodi* race 2 was evaluated in greenhouse pots. Barley and tomato were included as susceptible hosts. Host status was based on reproductive factor, Rf (Pf/Pi). Cultivars of tomato, barley, and mustard were excellent hosts (Rf > 7). Oil radish and

sudangrass 'Hidan 36' were also hosts but supported fewer nematodes (Rf < 3) than other hosts. Buckwheat, rapeseed, sudangrass 'Trudan 8', sorghum-sudangrass 'Sordan 79', horsebean, velvetbean, castorbean, and corn were either poor or nonhosts (Rf < 1). Five g (dry wt) stems and leaves of the crops were incorporated as green manure into 500 cm^3 of sterile soil. Four g of nonfumigated field soil was added to each pot to facilitate decomposition of plant tissue. The soil in each pot was inoculated with *M. chitwoodi* (5 000 juveniles/pot). A three-week-old tomato seedling was planted in each pot and left to grow for 7 weeks. Green manure significantly reduced the total number of *M. chitwoodi* race 2. Horsebean, velvetbean, castorbean, oil radish 'PHP', sudangrass 'Hidan36', rapeseed 'Humus', and corn were most effective as green manure (percent reduction ranged from 79 to 94%) when compared to barley (77%) or tomato (68%). Buckwheat was least effective in reducing nematode number (< 65%). Poor host status combined with green manure effects may reduce nematode population to a manageable level. Further work is being conducted to evaluate these crops under field conditions.

PATHOGENIC VARIABILITY OF *DITYLENCHUS DIPSACI* POPULATIONS AND ITS INFLUENCE ON THE SUSCEPTIBILITY OF GARLIC CULTIVARS. M. F. Andres, and S. Lopez-Fando, Departamento de Protección Vegetal, CCMA, CSIC, Serrano 115, 28006-Madrid, Spain.—The stem and bulb nematode, *Ditylenchus dipsaci*, is a serious pest of garlic crops in Spain. The pathogenic variability of 4 *D. dipsaci* Spanish populations and their interaction with garlic ecotypes and cultivars were investigated under controlled conditions. Five Spanish ecotypes were infected by 4 populations of *D. dipsaci* as selection criterium. Populations SO and JA showed the greatest pathogenic variability based on nematode reproduction rate and plant damage. The interaction of these populations (SO and JA) with 3 Spanish, 1 French, 1 Chinese, and 1 Californian garlic cultivar was also evaluated. One Spanish cultivar (RC) and the Chinese cultivar showed the highest tolerance level for both populations. Spanish cultivar BV seemed to be the most susceptible among the other cultivars with different degrees of susceptibility. Similar results were obtained under greenhouse conditions. However, a smaller nematode reproduction index were observed, which attenuated the susceptible response of the BV cultivar.

CORRELATION OF FIELD POPULATIONS OF *MELOIDOGYNE INCOGNITA* WITH GROWTH RESPONSES OF POINTED GOURD (*TRICHOSANTHES DIOICA* ROXB) FOR DETERMINING RELATIVE INVOLVEMENT OF OTHER NEMATODES. A. Anwar, and A. C. Verma, Department of Nematology, N.D. University of Agriculture and Technology, Kumarganj, Faizabad, India.—A survey of field plots in eastern U.P. India was conducted to assay the population density of plant-parasitic nematodes in relation to growth parameters of pointed gourd. The strongest correlations between population densities of root-knot nematodes and growth responses occurred when soil assayed for nematodes were made on first, second, and third-year-old crop. *Meloidogyne incognita* was the most damaging parasite as evidenced by high negative correlations between population densities and plant growth responses. *Rotylenchulus reniformis, Tylenchorhynchus vulgaris, Hoplolaimus indicus, Tylenchus* sp., and *Helicotylenchus* sp. were involved to varying degrees, depending on age of crop and initial densities of these nematodes. The negative correlation of *R. reniformis* to crop damage was higher than other nematodes, although reniform nematodes in such polyspecific communities contributed a severe degree of damage in pointed gourd. Symptoms were small rootlets in the first year, while second and third-year-old crops showed marked decline in growth, and sometimes crop failure. Similar correlation analyses showed that apparent antagonistic or synergistic population density relationships among nematodes under field conditions depend on the composition of the nematode community under study.

SPREAD OF SOYBEAN CYST NEMATODE (*HETERODERA GLYCINES*) BY WIND IN MINAS GERAIS STATE, BRAZIL. N. E. Arantes, and E. C. H. Pereira, EMBRAPA-CNPSo, C. P. 351, 38001-970 Uberaba, MG, Brazil, COPAMIL, and Rod. Dim 070, Km 01, 38510-000 Iraí deMinas, MG, Brazil.—Since its initial discovery in Brazil, *Heterodera glycines* has spread very quickly and now it is a seri-

ous pest of soybean (*Glycine max* [L.] Merrill). The spread of soybean cyst nematode by wind was investigated in the counties of Iraí de Minas and Nova Ponte in Minas Gerais State, Brazil. In August and September 1995, when the wind was very strong and the land was being tilled for summer planting, 20 aluminum plates were placed on woody stakes, 1.2 m high. A water lamina of 6 cm was kept in each plate. The suspensions obtained in the plates were poured through nested 840µm-pore and 250-µm-pore sieves. The residues from the 250-µm-pore sieve were poured through filter paper and observed in a stereoscopic microscope. A few cysts were recovered from some samples showing that they may be spread by wind.

COMPARISON OF THREE METHODS OF NEMATODE RECOVERY FROM BANANA (*MUSA* AAA) ROOTS. M. Araya, W. Carrillo, and A. Ramírez, Costa Rican National Banana Corporation Research Department, Apdo 390-7210 Guápiles, Costa Rica.—Individual comparisons between the traditional method (maceration of plant roots) with jar incubation and the mist extraction system were made regarding recovery of *Radopholus similis, Helicotylenchus* spp., *Meloidogyne* spp., and *Pratylenchus* spp. from *Musa* AAA roots. A much better recovery of the 4 genera was obtained with the traditional method in comparison with the jar incubation. The mist extraction system allowed an equal recovery of *Meloidogyne* spp. and *Pratylenchus* spp., however, both genera were found in lower densities in the root samples. The traditional method yielded higher densities of *R. similis* and *Helicotylenchus* spp. than the mist extraction system. No simple linear, quadratic, or cubic relationship was found between population densities the methods compared to allow a conversion factor to be developed. Additional research is needed to elucidate the effect of water addition to bring volume up to 200 cm^3 on nematode distribution and its influence on counting dish and, therefore, on nematode density estimation.

ORGANIZATION OF THE WORK WITH ENTOMOPATHOGENIC NEMATODES IN CUBA. E. Arteaga,[1] M. Montes,[1] E. Fernández,[2] B. Chang,[1] V. Calzadilla,[2] O. Vásquez,[1] O. Vásquez,[2] G. Plumas,[2] and V. García,[2] Instituto de Investigaciones de Cítricos, Havana, Cuba,[1] and Instituto de Investigaciones de Sanidad Vegetal, Havana, Cuba.[2]—The applied studies and works with entomopathogenic nematodes in Cuba were initiated at the Research Institute for Citrus (Instituto de Investigaciones de Cítricos) and later joined by the Research Institute for Plant Health (Instituto de Investigaciones de Sanidad Vegetal). We present the characteristics of the participation of both centers, their exchange with other institutions at the international level, the general scheme that rules the research, and we also mention the most utilized methodologies of search, production, and application of these organisms.

PRESENT AND FUTURE OF THE USE OF ENTOMOPATHOGENIC NEMATODES IN CUBA. E. Arteaga,[1] M. Montes,[1] E. Fernández,[2] M. Pérez,[2] O. Vásquez,[2] B. Chang,[1] M. B. Márquez,[2] A. Lobaina,[2] O. Milan,[2] O. Vásquez,[1] Instituto de Investigaciones de Cítricos, Havana, Cuba,[1] and Instituto de Investigaciones de Sanidad Vegetal, Havana, Cuba.[2]—Entomopathogenic nematodes constitute one of the most promising ways to control insect pests of different crops in Cuba. In this work, the studies of susceptibility of species from 14 genera of insect pests of agricultural, clinical, and forestall importance are summarized, opening future possibilities for the use of these organisms in biological control programs and integrated pest management. Also included are results on applications of these nematodes to crops such as citrus, rice, corn, and tomato. In addition, the characteristics and organization of the laboratories where these organisms are produced, as well as the future aspects of the research, are presented.

POPULATION DYNAMICS OF *HETERODERA GLYCINES* AND ITS BACTERIAL PARASITE, *PASTEURIA* SP. N. Atibalentja, and G. R. Noel, Department of Crop Sciences, University of Illinois, and USDA, ARS, Urbana, Illinois 61801, U.S.A.—An undescribed species of *Pasteuria* infecting *Heterodera glycines* was reported in 1994. Population dynamics of second-stage juveniles (J2) races 3 and 4, the

percentage of J2 encumbered with *Pasteuria* endospores, and the number of endospores attached to each J2 were determined from soil samples obtained from naturally infested microplots. Samples were collected at 2-week intervals during the 1994 and 1995 growing seasons (April-October). Significant seasonal fluctuations in nematode population densities, percentage of endospore-laden J2, and number of endospores per J2 were observed for both races. Nematode densities were low at the beginning of each season, then increased to their highest levels in July 1994 and September 1995. The mean number of *Pasteuria* endospores per J2 per sampling date ranged from 1 to 5 in 1994, and from 1 to 20 in 1995. Overall, the percentage of infected J2 was greater than 45% throughout the study.

ON THE ROLE OF BACTERIOVORUS NEMATODES ON THE EFFECTIVENESS OF *MELOIDOGYNE* SPP. SUPPRESSIVE SOILS. M. M. B'Chir, Institut National Agronomique de Tunisie 43, Av. Charles Nicolle, 1082 Tunis-Mahrajène, Tunisia.—Stability of the effectiveness of natural enemies against root-knot nematodes was investigated in some suppressive soils. A complex but variable equilibrium was built between different microorganisms in the suppressive soils, where bacteriovorus nematodes appeared to be the key factors. Biological and ecological characteristics of a bacteriovorus species involved in the suppressive soils equilibrium was discussed.

ELECTRON MICROGRAPH IMAGE ANALYSIS OF PROTEIN INCLUSIONS PRODUCED BY DIFFERENT STRAINS OF SYMBIOTIC BACTERIA FROM ENTOMOPATHOGENIC NEMATODES. S. Baghdiguian,[1] C. Gril,[2] and N. Boemare,[1] Université Montpelier II, Pathologie Comparée INRA-CNRS (URA 1184), CP101, Place E. Bataillon, 34095 Montpellier Cedex 05, France;[1] and Université Montpellier II, Service Commun de Microscopie Electronique, Place E. Bataillon, 34095 Montpellier Cedex 05, France.[2]—Entomopathogenic bacteria of the genera *Xenorhabdus* and *Photorhabdus* produce intracellular inclusion bodies during *in vitro* culture. To study the macromolecular structure of these inclusions, a highly extensive and object-oriented system has been developed for image analysis. This system based on "rapid" and "discreet" bidimensional Fourier transforms provides a periodic representation of the crystal structure. Digital image processing is an essential step in the determination of macromolecular structure of the paracrystallin inclusions of *Xenorhabdus* and *Photorhabdus* sp. by electron microscopy. Our mathematical approach has shown that the crystal growth is precisely directed according to a repetitive design in all strains studied.

INTERACTIONS BETWEEN *RADOPHOLUS SIMILIS* AND AN ARBUSCULAR MYCORRHIZAL FUNGUS, *GLOMUS INTRARADICES*, ON BANANA ROOTS. T. J. Baker, Department of Agriculture, University of Reading, Earley Gate, PO Box 236, Reading, RG6 2AT, U.K.—Most plants colonized by arbuscular mycorrhizal fungi (a.m.f.) perform better than non-mycorrhizal ones in nutrient deficient soils. A.m.f. have also been shown to influence root herbivores and pathogens, either reducing their presence or compensating for their damage. Two experiments explored the effect that *Glomus intraradices* might have on *Radopholus similis* damage in banana roots by comparing plant growth and nematode numbers between mycorrhizal and non-mycorrhizal 'Cavendish' bananas in low phosphorus soil (available P < 10ppm). 'Dwarf Cavendish' plants were used in the first experiment and 'Grande Naine' in the second, and the nematodes were left to multiply on half the plants for 4.5 and 5 months, respectively. In the first experiment, plant growth parameters and *R. similis* damage and numbers were not significantly affected by *G. intraradices* presence or absence. In the second, the mycorrhizal plants were found to grow better than non-mycorrhizal ones, and those also inoculated with *R. similis* were found to have less nematodes per g root.

WORLDWIDE OCCURRENCE AND POTENTIAL IMPORTANCE OF *PASTEURIA PENETRANS* AS A BIOCONTROL AGENT FOR *MELOIDOGYNE* SPP. G. Bala,[1] A. Daudi,[2] J. Madulu,[3] T. Mateille,[4] C. Netscher,[5] A. Sawadago,[6] C. Trivino,[7] D. Trudgill,[8] and E. Vouyoukalou,[9] Central Experimental Station, Centano, Trinidad,[1] BRS, PO Box 5748, Limbe, Malawi,[2] RTI, PO Box 306, Tabora, Tanzania,[3] ORSTOM, BP1386, Dakar, Senegal,[4] ORSTOM, Gadjan Mada University, Sekipk 3 Yogyokarta, 55281, Indonesia,[5] LVP, BP403, Bobo-Dionlasso Burkina Faso,[6] Boliche Experiment Station, Box 7069. Guayaguil, Ecuador,[7] SCRI, Dundee, Scotland,[8] and NARF Agrokipio, Chania, Greece.[9]—A 4-year study, partly funded by the Commission of the European Communities' programme for Science and Technology for Development, aimed to assess the potential of *P. penetrans* as a biocontrol agent for *Meloidogyne* spp. on vegetables. Surveys were conducted in various countries and confirmed the wide-spread importance of *M. incognita* and *M. javanica*. However, *M. mayagnensis* also was widespread in parts of West Africa. Many *Meloidogyne* populations were infected with *P. penetrans* in those parts of Africa, Ecuador, and Trinidad surveyed, but the proportion of juveniles carrying spores was generally small. In Indonesia, detectable *P. penetrans* infections were uncommon, and it was found for the first time on the island of Crete. Field trials indicated that it was sometimes possible to increase infections of *P. penetrans* to a level where they exerted significant control of *Meloidogyne* spp. on susceptible crops such as tomato.

EFFICACY OF *AZADIRACHTA INDICA* AND *PARTHENIUM HYSTEROPHORUS* ON SPORE ATTACHABILITY OF *PASTEURIA PENETRANS* IN *MELOIDOGYNE INCOG-NITA*. G. Bala, and S. R. Gowen, Department of Plant Pathology, Central Experiment Station, Centeno Trinidad, and University of Reading, Department of Agriculture, Reading RG6 2AT, Berkshire, U.K.—Two populations of second-stage juveniles of root-knot nematodes, *Meloidogyne incognita*, were exposed in separate laboratory trials to spores of a Centeno population of *Pasteuria penetrans* at 10^5 spores/ml in 10ml water extracts of leaves of neem, *Azadirachta indica* at 4, 5, and 6 g/100 ml and whitehead, *Parthenium hysterophorus*, at 6, 8, and 12 g/100ml, and placed in 3.5-cm diam vials for 12, 24, and 36 hr. In both trials, significantly higher mean spore attachments per nematode were obtained in the control treatment of distilled water when compared to other treatments. Considerable suppression of spore attachment was recorded for all neem treatments. In treatments with whitehead, significantly higher spore attachments were obtained in mobile nematodes when compared to immobile nematodes. The results indicate that metabolites in the water extracts of neem, and to a lesser extent whitehead, adversely affect spore attachment of *P. penetrans* in root-knot nematodes.

COMPARATIVE EFFICACY OF METHYL BROMIDE, DAZOMET, AND SOLARIZATION IN CONTROL OF *MELOIDOGYNE ARENARIA*. K. R. Barker, and C. S. Echerd, Plant Pathology Department, North Carolina State University, Raleigh, North Carolina 27695, U.S.A.—A 2-year study (summers of 1994 & 1995) focused on the relative efficacy of dazomet (granular), methyl bromide, and solarization for control of *Meloidogyne arenaria* in a Fuquay coarse sand. All plots, including the untreated controls, were 1.82 m × 9.14 m. For each year, dazomet was applied at 212 kg/ha with a Gandy drill and incorporated into moist soil (11% moisture in 1995) with a "tillovator" to a depth of about 20 cm. In 1995, dazomet also was applied at 424 and 848 kg/ha. Methyl bromide was applied at 488 kg/ha, and covered with 2-mil polyethylene, as were all dazomet and solarization plots. Air temperatures during the first 2 weeks after treatment ranged from 11 to 32°C in 1994 and 18 to 35°C in 1995. Comparative efficacy of the treatments was assessed at 2 to 3 months later by soil-sample collections at depths of 0 to 7.5 cm, 7.5 to 15 cm, 15 to 30 cm, and 30 to 45 cm. These samples were assayed for second-stage juveniles by elutriation-centrifugation and infective juveniles/eggs by a tomato bioassay. Based on juvenile counts only, all treatments resulted in good nematode control at all soil depths with methyl bromide being slightly superior. However, the tomato bioassays showed that the solarization was not highly effective and had no significant impact on total inoculum potential at soil depths of 15 to 45 cm. The bioassays indicated that the efficacy of dazomet and methyl bromide was similar for the re-

spective soil depths, but efficacy diminished for both materials with increasing soil depths. Thus, dazomet may be a suitable alternative to methyl bromide under these test conditions, but solarization, even for a warm, sunny period of weeks, is a marginal alternative.

GENETIC AND MOLECULAR CHARACTERIZATION OF LINES OF WHEAT RESISTANT TO THE CEREAL CYST NEMATODE, *HETERODERA AVENAE*. D. Barloy,[1] J. Martin,[1] R. Rivoal,[2] and J. Jahier,[1] Institut National de la Recherche Agronomique,[1] and Station d'Amélioration des Plantes, Laboratoire de Zoologie, BP 29, 35 650 Le Rheu, France.[2]—*Aegilops variabilis* n°1 is one of the sources of resistance to *Heterodera avenae*. This accession displays a complete resistance to the 4 French pathotypes (Fr1=Ha 41, Fr2, Fr3=Ha11, Fr4=Ha12) and to the root-knot nematode, *Meloidogyne naasi*. This latter resistance is controlled by one dominant gene. The resistance gene(s) carried by a translocated line designated X8, derived from the addition line named X35, appear linked to the markers OpB12-1320, OpN20-1230, and OpY16-1065. These markers have been mapped close to the Rkn2 gene from the analysis of a segregating population. It is not known whether the Rkn2 gene controls the development of both species of nematodes or, if another gene linked to Rkn2, steps in the expression of resistance to *H. avenae*. The line X8 is the best line for the introgression of these 2 resistances in wheat, though it is incompletely resistant to CCN. The next steps of this study are the analysis of behavior of these lines to the Fr1 pathotype, to cumulate in a same genotype the 2 *Ae. variabilis* resistance genes to *H. avenae* as well as the resistance to *M. naasi*.

TAGGED *ARABIDOPSIS THALIANA* PROMOTER SEQUENCES INVOLVED IN NEMATODE FEEDING STRUCTURE ESTABLISHMENT. N. Barthels, M. Karimi, M. Van Montagu, and G. Gheysen, Laboratorium voor Genetica, via the Department of Genetics, affiliated to the Flanders Interuniversity Institute for Biotechnology, Universiteit Gent, K.L. Ledeganckstraat 35, B-9000 Gent, Belgium.—The sedentary endoparasitic nematode-host interaction is characterized by the formation of typical feeding structures, an absolute requirement for this parasite to complete its life cycle. In our study, syncytia and giant cells induced by the highly detrimental cyst (*Heterodera schachtii*) and root-knot (*Meloidogyne incognita*) nematodes are analyzed at the molecular level. To pursue the identification of nematode-responsive *Arabidopsis thaliana* promoters, we took advantage of a T-DNA system based on the use of a promoter trap vector engineered with a promoterless β-glucuronidase (GUS) gene. Monitoring GUS activity and assessment of its spatial specificity led to the identification of several regulatory sequences involved in nematode feeding structure establishment.The promoter trap lines NC0728 and NC1712 showed a cell type-specific GUS fusion activity pattern. For both lines, infections with cyst and root-knot nematodes resulted in a clearly blue staining inside syncytia and galls. Background expression of the transgene could be noted only for initiation sites of lateral root formation but was very weak in case of NC0728. Transgene activity is seen early in the infection cycle with the highest level at 2 and 4 days after inoculation for NC0728 and NC1712, respectively. The isolation of the tagged regulatory regions through inverse polymerase chain reaction and their potential role in this particular interaction was discussed.

SEPARATION OF SPECIES AND POPULATIONS IN CEREAL CYST NEMATODE COMPLEX INFERRED FROM RFLP IN THE RIBOSOMAL DNA. S. Bekal, and R. Rivoal, Institut National de la Recherche Agronomique, Laboratoire de Zoologie, BP 29 35650 Le Rheu, France.—The cereal cyst nematode formed a complex of at least 9 species which showed a real taxonomic problem because of the closed morphobiometrics and the frequent common host range. The study of RFLPs in the ribosomal DNA was carried out to increase our knowledge of the genetic relationships between different geographic isolates of *Heterodera avenae*, Gotland strain, *H. filipjevi*, *H. latipons*, *H. mani* and *H. schachti*, the beet cyst nematode in comparison. DNA was extracted from single white females or cysts. Two 21-meroligonucleotides primers positioned on the rDNA 18S and 26S were used to amplify the Internal Transcribed Spacers (ITS1 and ITS2) and the 5.8S gene. One DNA fragment approximately 1.2 kb

was obtained for each population and furtherly digested with 15 endonucleases. Fourteen of the restriction enzymes gave fragment length polymorphism. The RFLP matrix was computed with UPGMA analysis (PHYLIP package). The dendrogram established from the Nei distances and proved with boot strap procedure indicated clear groupings of isolates belonging to the species *H. avenae, H. filipjevi, H. latipons,* and *H. mani.* Gotland strain populations were easily differentiated from *H. avenae* and clustered to *H. filipjevi.* Restriction sites had revealed mixture of species and a possible infraspecific variation in *H. avenae.* Phylogenetic relationships in the CCN complex will be discussed.

FOLIAR SPRAYS OF *STEINERNEMA CARPOCAPSAE* AGAINST EARLY SEASON APPLE PESTS. G. Bélair, C. Vincent, and G. Chouinard. Agriculture and Agri-Food Canada, 430 Gouin Blvd., Saint-Jean-sur-Richelieu, Quebec J3B 3E6, Canada.—Persistence and field effectiveness of the entomopathogenic nematode *Steinernema carpocapsae* All strain by foliar sprays were evaluated against the apple sawfly, *Hoplocampa testudinea,* and the plum curculio, *Conctrachelus nenuphar,* two early season pests in Quebec apple orchards. From 1992 to 1995, bioassays with *Galleria mellonella* larvae were conducted to assess the persistence of *S. carpocapsae* on leaves, flower clusters, and twigs up to 4 days after evening application. *S. carpocapsae* juveniles remained infective on apple leaves 38, 42, 98, and 24 hr after application in 1992, 1993, 1994, and 1995, respectively. In bioassays, the percentage of *G. mellonella* mortality was consistently higher on leaves (average = 84%), intermediate on flower clusters (73%) and lower on twigs (43%) for all application dates. In 1992 and 1993, single nematode sprays performed every 2-3 days from early-May to mid-June on apple tree limbs reduced by 98 and 100% primary damage caused by *H. testudinea,* but all treatments were not effective in 1994. In 1993 and 1994, multiple border row sprays using a commercial hand-gun applicator against *C. nenuphar* adults were performed in an insecticide-free orchard. Plum curculio damage at harvest (PCDH) in the nematode-treated orchard reached 5 and 55% in 1993 and 1994 respectively, as compared to 80 and 85% in an adjacent insecticide-free orchard. In a second experiment performed in 1994, multiple broadcast sprays using a commercial orchard sprayer showed no significant effect on PCDH (nematode = 28%; control = 31%). Although some efficacy of canopy sprays of nematodes was detected against early season apple pests, the inconsistent results, the narrow windows, and the high application costs preclude its use as sole control tactic against these pests in commercial apple orchards.

INFLUENCE OF PHOSPHATE ON THE POPULATION DYNAMICS OF *HETERODERA SCHACHTII.* D. Bell, Federal Biological Research Centre for Agriculture and Forestry, Institute for Nematology and Vertebrate Research, Substation Elsdorf, Dürener Str. 71, D-50189 Elsdorf, Germany.—Investigations were conducted to control *Heterodera schachtii* in reclaimed soils in the Rhineland. The very low concentration of soluble phosphates in these soils (Loess) might be one reason for the extremely high abundance of the nematode in the field. Therefore, phosphate was added to several culture media (soil, sand, agar), and the population dynamics of *H. schachtii* were observed under host conditions. The addition of 5 mg P_2O_5/100 g soil reduced the number of penetrating larvae, compared to 0 or 2.5 mg P_2O_5/100 g soil. Higher amounts did not reduce larval penetration levels. Using fluid plant fertilizer, phosphate absence caused an increase of larval penetration, compared to optimal plant nutrition. Perhaps sugarbeets compensate for phosphate deficiency by producing more exudates, and they become more attractive to the nematodes. In the greenhouse using resistant oilradish, phosphate addition reduced hatching significantly. *In vitro,* phosphate inhibited hatching as well. These effects could not be detected in the field, because at the time when sugarbeets were grown on reclaimed soils (10 years after reclamation), phosphate concentration was already sufficient for the plants. Investigations on recently reclaimed soils will follow.

COMPARISON OF SELECTED AND UNSELECTED POPULATIONS OF *GLOBODERA PALLIDA* USING RAPD-PCR.-I. Bendezu,[1] K. Evans,[2] D. de Pomerai,[3] and M. Canto-Saenz,[1] Universidad Nacional Agraria - La Molina, Av. La Universidad sn. La Molina, Lima, Peru,[1] Rothamsted Experimental Station, Harpenden, Herts AL5 2JQ, U.K.,[2] and Department of Life Sciences, University of Nottingham, Nottingham NG7 2RD, U.K.[3]—RAPD-PCR patterns of British populations of the potato cyst nematode, *Globodera pallida,* reared for several generations on both resistant (*Solanum vernei* (Vt[n])[2] 62.33.3) and susceptible (Arran Banner) potato cultivars were compared. Primer Sigma gave several differences between populations, including two bands of 953 and 891 bp. between populations from Little Ouse (Pa 2) and a fragment of 492 bp. between populations from Cadishead (Pa 3). With primer Gamma, populations from Little Ouse and Cadishead each showed one additional DNA fragment of approximately 1199 and 1137 bp., respectively, to those in the unselected populations cultured on a susceptible host. These additional fragments may be linked or related to the virulence genes that allowed these populations to reproduce on the resistant cultivars.

EXPERIMENTAL HOST RANGE OF AN UNDESCRIBED ROOT-KNOT NEMATODE FROM TENNESSEE, U.S.A. E. C. Bernard, and P. L. Jennings, Department of Entomology and Plant Pathology, The University of Tennessee, PO Box 1071, Knoxville, Tennessee 37901-1071, U.S.A.—A new *Meloidogyne* sp. found in a mixed fescue-clover pasture was cultured on white clover in the greenhouse. This species resembles *M. graminicola* but has different morphometrics and perineal pattern. An extensive host range study was conducted to determine the potential pathogenicity of this nematode to crops and other cultivated plants. More than 200 plant species and cultivars in 23 families were evaluated for degree of galling. Determination of egg production was impractical because egg masses were contained wholly within the root in all plants; however, the degree of galling appeared correlated with egg production. Many Fabaceae were good hosts, including all 8 clovers (*Trifolium* spp.) tested, all 20 soybean (*Glycine max*) cultivars, including PI 423654, pea (*Pisum sativum*), and all tested *Vicia* spp. (vetch, broad bean). Peanut (*Arachis hypogaea*), garden bean (*Phaseolus vulgaris*), birdsfoot trefoil (*Lotus corniculatus*), and alfalfa (*Medicago sativa*) were poor hosts or nonhosts. Among monocotyledons, Poaceae were poor hosts except for annual ryegrass (*Lolium multiflorum*), and there was no galling of rice or wheat. Most of the species tested in Amaryllidaceae and Liliaceae were good hosts. Among non-leguminous dicotyledons, all tested Apiaceae, Brassicaceae, and Chenopodiaceae were heavily galled, while Cucurbitaceae and Solanaceae had little or no galling. Asteraceae and Lamiaceae contained both good and poor hosts.

EX-*SOLANUM SPARSIPILUM-CHACOENSE* HEAT-STABLE RESISTANCE TO *MELOIDOGYNE ARENARIA* IN *SOLANUM TUBEROSUM.* F. Berthou, P. Rousselle, and D. Mugniery, ORSTOM, UR 3c, INRA Zoologie, B.P. 29, 35700- Le Rheu, France.—*M. arenaria* juveniles caused the appearance of galls on the tubers at harvest among the inoculated susceptible potato plants growing in the summer of 1995 in the Le Rheu location. *M. arenaria* did not infest the tuber crops in inoculated-resistant Mh genotypes due to Mh gene selected in *S. sparsipilum-chacoense* × *S. tuberosum* F1 families according to the hypersensitive reactions. However, one short heat-treatment of the resistant young plants (24°C or 29°C during 4 days), just after the inoculation by juveniles in growing cabinets and before planting, induced the appearance of galls for most of Mh genotypes. This short exposure displayed the instability in some Mh genotypes, as more juveniles migrated towards more numerous feeding sites, because the hypersensitive reactions vanished and the root growth was sustained by increased temperature. But heat-stable Mh genotypes from the ex- *S. sparsipilum-chacoense* source of resistance were found to produce low numbers of females in vascular cylinders in the root and no galls in the tuber crop. Thus, in potato plant breeding for resistance against tropical root-knot nematodes, the induced change from no gall to galls in the tuber crops was used as criterion to test stability for the resistance destined for warm areas, due to the effect of heat treatment applied during the invasion of the roots. Some stable resistant Mh genotypes were found. The resistance may be studied, first in seg-

regating F1 families at moderate temperature (< 24°C) to assume the presence of the Mh gene, according to hypersensitive reactions and low developing juveniles, and secondly, on the selected genotypes, according to low numbers of developing juveniles and no tuber galling after heat shock.

ASPECTS OF BIOCHEMICAL RESISTANCE IN *MUSA* SPP. R. H. Binks, Department of Agriculture, University of Reading, Earley Gate, P.O. Box 236, Reading, Berkshire, RG6 2AT, U.K.—Sources of resistance have been identified in *Musa* spp. to the principal nematode species, *Radopholus similis* and *Pratylenchus coffeae*. Screening experiments have shown that 2 cultivars in particular show high levels of resistance/tolerance. The possible mechanisms of resistance was investigated in 2 cultivars, Yangambi. Km 5 (AAA) and Pisang Jari Buaya (AA), and to compare their lignin, suberin, and polyphenol contents with that of a known susceptible cultivar Grande Naine (AAA). To date no single mechanism of resistance has been identified, i.e specific phytoalexin production. Results of lignin content in roots from young and mature plants have indicated that there is a correlation between resistance and lignin content. The lignin is deposited in cell walls in response to damage but is also present at high levels in plant roots prior to infection, suggesting a preinfectional defence mechanism. In the case of polyphenol content in *Musa* roots, more polyphenol compounds are produced in the susceptible cultivars, and these compounds are produced as a response to nematode infection. Whether these compounds play a role in deterring nematodes from feeding will be investigated further. Future work will involve confirming which of the biochemical constituents of banana roots are associated with resistance and also to evaluate the biochemical composition of roots of wild species and cultivars, particularly those identified as less susceptible to nematodes.

MEMBRANE TRAFFIC IN GIANT CELLS INDUCED BY *MELOIDOGYNE INCOGNITA* IN ARABIDOPSIS. T. Bleve Zacheo, M. T. Melillo, L. Serna, F. Aristizabal, S. Sans-Alferez, F. Del Campo, and C. Fenoll, Instituto di Nematologia Agraria, Amendola 165/a, 70126 Bari, Italy, and Departamento de Biología Universidad Autonoma de Madrid, 28049 Madrid, Spain.—One of the genes induced at high levels by nematodes upon plant infection is the gene that codes for hydroxymethylglutaryl coenzyme A reductase (HMGRase), a key enzyme of the biosynthetic pathway that produces ubiquitous isoprenoid compounds necessary, among other processes, for membrane biogenesis. In tobacco, the HMGR gene has been reported to be induced by nematodes, likely as a part of defence response of the plant. We have found that *Meloidogyne incognita* stimulates the expression of the two Arabidopsis HMG genes, both at the level of promoter activation (in transgenic tobacco plants harboring fusions of both promoters to the reporter GUS gene) and at the level of protein accumulation (immunolocalization in Arabidopsis). Specific antibodies directed against the HMGRase revealed that the enzyme resides in ER-derived vesicles. Since crucifers like Arabidopsis do not synthesise isoprenoid phytoalexins, nematode-induced overproduction of the enzyme must have a different purpose. One possibility is that increased levels of the enzyme may be related to the synthesis of a massive amount of sterols for nematode needs. Another hypothesis is that development of giant cells requires overproduction of membrane vesicles that contributes to the process underlying giant cell differentiation, like plasmalemma and cell wall biogenesis. Support for this hypothesis is provided by immunological evidence of HMGRase involvement in the microtubule-directed routing of ER vesicles to the plasma membrane of giant cells at interphase.

GENETIC VARIATION BETWEEN AND WITHIN POPULATIONS OF POTATO CYST NEMATODE. V. C. Blok, and M. S. Phillips, SCRI, Invergowrie, Dundee DD2 5DA, Scotland.—The potato cyst nematodes (*Globodera rostochiensis* and *G. pallida*) are major pests of potato in Europe with the latter species now the major problem in the U.K. This is largely due to the absence of effective resistant cultivars. PCN was introduced into Europe from South America. Knowledge of the number of introductions and population diversity in Europe would greatly help in developing control strategies with those partially resistant cultivars that are available. Studies of genetic diversity as measured by RAPDS

on field, artificially fragmented and selected populations are shown. The range of variation released from a fragmented population was found to be comparable with the range of variation found between field populations. The analysis of populations that have been selected for virulence on different sources of quantitative resistance indicated differences in population heterogeneity and response to selection by the difference resistances.

GENE EXPRESSION IN SOYBEANS INFECTED WITH SOYBEAN CYST NEMATODE R. I. Bolla, and Y-R. Huang, Department of Biology, Saint Louis University, 3507 Laclede, St. Louis, Missouri 63103-2010, U.S.A.—Planting varieties of soybean resistant to soybean cyst nematode (*Heterodera glycines*, SCN) in combination with crop rotation is one of the most effective strategies for managing this nematode. Resistance genetics, however, remains confusing with one to several genes reported to be involved depending upon the soybean cultivar. Little is known of the molecular biology or biochemistry of the resistance response. We proposed that 2 sets of genes are involved: "resistance genes" which are induced immediately upon infection to transduce a signal eliciting a host response and "resistance-associated genes" which encode the products of the resistance response to prevent SCN from establishing. To investigate this hypothesis, we have identified genes whose transcription is induced or repressed soon after infection of resistant and susceptible soybean varieties with SCN race 3. We have isolated a gene whose transcription is induced in Hartwig 3 days after SCN infection. The gene has sequence similarity to HMGCoA reductase and appears to be a member of a small family of HMG-CoA reductase genes. Members of this gene family are constitutively expressed in Essex and Hartwig, however, at lower levels in Hartwig than in Essex. Within one day of SCN infection of Essex, transcription of this gene ceases and remains repressed for at least 5 days of infection. In SCN-infected Hartwig, on the other hand, this gene is expressed at high levels beginning 3 days after infection and declines somewhat by day 5.

NEMATODE REPRODUCTION AND PATHOGENICITY ON SUGARCANE IN LOUISIANA. J. P. Bond, E. C. McGawley, and J. W. Hoy, Department of Plant Pathology and Crop Physiology, Louisiana State University Agricultural Center, Louisiana Agricultural Experiment Station, Baton Rouge, Louisiana 70803-1720, U.S.A.—Greenhouse research evaluated nematode pathogenicity on sugarcane cultivars. Cuttings of the cultivars CP 65-357, CP 70-321, LCP 82-89, HoCP 85-845, and CP 86-454 were transplanted into 20.3-cm-diam pots and grown for 4 months. After root establishment, soil was infested with 0, 1 000, or 4 000 nematodes (7% *Tylenchorhynchus* spp., 50% *Criconemella* spp., 23% *Trichodorus* spp., and 20% *Pratylenchus* spp.) per pot. Reductions ($P \leq 0.05$) in top and root dry weight, plant height, and shoot length were observed. Across cultivars, host suitability was excellent for stunt, ring, and stubby-root nematodes; average community reproduction (R) was 75 for the 1 000 infestation level and 14 for the 4 000 level. To assess nematode pathogenicity and reproduction under simulated field conditions, a 6-month-duration microplot test was conducted. Pathogenicity was evaluated on LCP 82-89 and CP 70-321 in 45.7-cm-diam pots containing sterilized field soil with 3 nematode levels (0, 1 200, or 12 000 individuals per microplot). The community at infestation contained 40% stubby-root, 30% stunt, and 30% ring nematodes. Both cultivars again supported high nematode reproduction; final community R values were 285 and 157, and 25 and 19 for low and high infestation levels on LCP 82-89 and CP 70-321, respectively. Reductions ($P \leq 0.05$) in top and root dry weight, plant height, and shoot length were observed for LCP 82-89. Growth was not reduced in CP 70-321 even though this variety supported high nematode populations.

HOST SUITABILITY FOR *MELOIDOGYNE* SPP. OF ROTATIONAL CROPS USED IN TOBACCO CULTIVATION IN SOUTH AFRICA. M. S. Botha-Greeff, I. du Plessis, and T. Richter, Tobacco and Cotton Research Institute, Private Bag X82075, Rustenburg 0300, Republic of South Africa.—Root-knot nematodes are considered one of the most important pests of tobacco in RSA. As part of an integrated nematode control program for tobacco, possible rotational crops such as sunn hemp, oats,

wheat, certain grasses, cotton, and edible crops were evaluated at cultivar level in the greenhouse for resistance to South African populations of *M. javanica* and *M. incognita* races 2 and 4. Seedlings were established in seedtrays and inoculated separately with the 3 nematodes. A total of 104 cultivars representing 26 crops has been evaluated with respect to root mass, root gall index and reproduction factor. Results show that sunn hemp spp., *Crotalaria juncea* and *C. spectabilis* have various levels of resistance to *Meloidogyne* spp. *Tagetes erecta* and cultivars of *Eragrostis curvula, Chloris gayana, Pennisetum glaucum, Setaria sphacelata, Digitaria smutsii, Sorghum* spp., *Cenchrus ciliarus, Panicum maximum,* and *Lolium perenne* show resistance to all 3 *Meloidogyne* populations. Of *Avena* spp., the cultivars Overberg and Perdeberg have the highest level of resistance to the *Meloidogyne* populations. All cotton cultivars tested can successfully be used for rotation where *M. javanica* and *M. incognita* race 2 are present. *Glycine max* cultivars and vegetable crops showed various degrees of sensitivity towards all 3 *Meloidogyne* populations.

EFFECTS OF THE GUT OF *PONTOSCOLEX CORETHRURUS* ON SECOND-STAGE JUVENILES OF *HETERODERA SACCHARI*. J. Boyer, and G. Reversat, Laboratoire d'Ecologie des Sols Tropicaux, ORSTOM, 32 avenue Henri Varagnat, 93143 Bondy Cedex, France.—*Pontoscolex corethrurus* is a very ubiquitous geophagous earthworm of tropical areas. In field trials it has been found sometimes to improve the fertility of agricultural soil, and this could be related to an antagonistic action against plant-parasitic nematodes. In laboratory experiments, we observed that the worm was able to ingest nematodes during soil consumption. Secondly an experiment was made *in vitro* to study the effect of the gut of this earthworm on the invasive and developmental abilities of second-stage juveniles of *Heterodera sacchari*. From dissected worms and with freshly hatched juveniles of the nematode, 4 treatments of the same duration were tested: i) nematodes were kept in water (control); ii) nematodes were kept in soil; iii) nematodes incubated with the gut wall and its content of the earthworm; iv) nematodes incubated with the gut wall without its content. After treatment, 50 juveniles were inoculated into small tubes containing 1 rice seedling. Results were estimated 5 weeks later by counting adult females (white females and cysts). Compared with the control (70% of adult females), the treatments with soil and the gut wall alone were inactive (respectively 68.7% and 63% of adult females). When treated with the gut wall and its contents, number of adult females recovered were significantly reduced (47%). The possible mechanisms of this interaction were discussed.

VARIABILITY AND MORPHOLOGICAL DIFFERENTIATION BETWEEN *BURSAPHELENCHUS XYLOPHILUS* (R AND M FORM) AND *B. MUCRONATUS*. H. Braasch, Federal Biological Research Centre for Agriculture and Forestry, Office for Economic and Legal Affairs in Plant Protection, Kleinmachnow Branch, Stahnsdorfer Damm 81, D-14532 Kleinmachnow, Germany.—The shape of the female tail end is an important taxonomic feature of *Bursaphelenchus* species and the only one for morphological differentiating *B. xylophilus*-related species. The different provenances of the usually non-pathogenic species, *B. mucronatus*, differ only slightly in the shape and length of their distinct mucro, while the determination of *B. xylophilus*, a quarantine pest of the EPPO region, is more complicated due to the existence of roundtailed and mucronate forms with somewhat different host preference and pathogenicity. In inoculation experiments with roundtailed *B. xylophilus* (isolate US 15) propagated on *Botrytis cinerea*, we observed several times a change of the female tail end shape after reisolation from the coniferous seedlings. Three months after inoculating the roundtailed form on *Pinus sylvestris*, out of 108 females were only 35% roundtailed, 8% having conical tail ends, 17% with distinct mucro (largest mucro 4,5 µm), while 40% had a very small mucro (1 µm). Therefore, a morphological identification of *B. xylophilus*, isolated from wood, as a mucronate or a roundtailed form, is unreliable. Also, the observed existence of mixed populations of the 2 forms in the same tree remains questionable. Indeed, the mucronate *B. xylophilus* US 10 isolate, reared on *B. cinerea*, did not change the tail end characteristic after living over 3 months in host seedlings. It is very likely that mucronate forms of *B. xylophilus* developed by selection from roundtailed forms. A German, a French,

and a Siberian isolate of *B. mucronatus*, inoculated on seedlings of *P. sylvestris* and *Picea abies* also kept their normal female tail ends. As a rule, the female offsprings from laboratory hybridization of *B. xylophilus* (US 15) and *B. mucronatus* (Germany, Siberia) look like the females of *B. mucronatus*, while the progeny of crossings between *B. xylophilus* (US 10) and these *B. mucronatus* provenances showed females with different mucro lengths, like the parents. Crossing the isolates US 15 and US 10, half of the progeny had females with a medium sized mucro (2-2.5 µm). Morphological determination of *B. xylophilus* isolated from wood, for instance in the case of quarantine inspection, is possible only when roundtailed females are present. Mucronate females can belong to both forms of *B. xylophilus*, *B. mucronatus* or to the progeny of crossings, provided natural crossings take place. Their determination needs the use of other, preferably molecular methods.

THE IDENTITY AND DISTRIBUTION OF A SECOND PATHOTYPE OF POTATO CYST NEMATODE IN THE UNITED STATES. B. B. Brodie, USDA, ARS, Department of Plant Pathology, Cornell University, Ithaca, New York 14853, U.S.A.—A population of potato cyst nematode (PCN) was found in Steuben County, NY that multiplied freely on potato plants (cv. Hudson) with the H_1 gene that confers resistance to *Globodera rostochiensis* pathotype Ro1. This variant population of PCN was found in an area of the golden nematode research farm where Ro1 resistant cultivars had been grown repeatedly the past 8 years. Morphometric measurements, cyst color sequence, and DNA analysis identified the population as belonging to the species *G. rostochiensis* rather than *G. pallida*. Tests with differential hosts indicated that the population consisted of *G. rostochiensis* pathotype Ro2. Fields in which Ro1 resistant potato cultivars had been grown 3 or more successive years were surveyed for the presence of viable eggs of *G. rostochiensis*. Cysts with viable eggs were recovered from 4 fields in Suffolk County, NY (Long Island) and 1 additional field in Steuben County, NY. Nematodes from cysts that were recovered from 2 of the fields on Long Island were found to multiply freely on Ro1 resistant plants. Although 1 or more cysts developed on Ro1 resistant plants that had grown in soil from the other 3 fields, it was not certain if these cysts represented a pathotype of PCN that overcomes Ro1 resistance.

TRANSMISSION OF TOBACCO AND TOMATO RINGSPOT NEPOVIRUSES BY INDIVIDUAL *XIPHINEMA AMERICANUM* GROUP NEMATODES FROM 29 U.S.A. POPULATIONS. D. J. F. Brown,[1] R. T. Robbins,[2] D. Zanzinger,[3] and S. Wickizer,[2] Scottish Crop Research Institute, Invergowrie, Dundee, DD2 5DA U.K.,[1] Department of Plant Pathology, University of Arkansas, Fayetteville, Arkansas 72701, U.S.A.,[2] and 1005 Glenbrook Avenue, Meadowview, Virginia 24361, U.S.A.[3]—New vector transmission techniques involving the use of individual nematodes previously revealed *Xiphinema americanum sensu stricto*, *X. californicum*, and *X. rivesi* to be vectors of cherry rasp leaf and tobacco (TRSV) and tomato ringspot (ToRSV) nepoviruses, but *X. bricolensis* transmitted only ToRSV. In a new study, 29 populations (Pops.) of *X. americanum* group nematodes from the U.S.A. were examined for their vectoring ability with TRSV and ToRSV. Individual nematode ability to transmit the viruses was tested and the results were: i) specimens from 1 population did not transmit any virus; ii) those from 4 populations transmitted only TRSV; iii) those from 4 other populations, including 1 identified as *X. tarjanense*, transmitted only ToRSV and, iv) nematodes from 20 populations, including 4 identified as *X. intermedium*, transmitted TRSV and ToRSV. Also, when nematodes from a population were given simultaneous access to both viruses, 2 individuals transmitted only TRSV, 10 transmitted only ToRSV and 2 transmitted both viruses. These results confirm the pattern of specificity between vector nematodes and their associated viruses in Europe differs significantly from that observed in North America. Also, an examination of several populations for which there were sufficient specimens available revealed they each had only 3 juvenile stages. This is further evidence of an apparent correlation between *X. americanum* group populations having only 3 juvenile stages and the probability of nematodes from these populations being able to transmit virus.

DETECTION AND DIFFERENTIATION OF *GLOBODERA ROSTOCHIENSIS* AND *G. PALLIDA* USING SPECIES-SPECIFIC PCR PRIMERS. S. R. Bulman, and J. W. Marshall, New Zealand Institute for Crop and Food Research Limited, P.O. Box 4704, Christchurch, New Zealand.—Two species of potato cyst nematode (PCN), *G. rostochiensis* (Ro) and *G. pallida* (Pa), are present in New Zealand both as pure Ro and Pa populations and as mixed species populations. The increased use of potato cultivars with resistance to either Ro or Pa requires rapid and sensitive detection and identification of this pest and its species composition within a paddock(s). Potato cultivars with the appropriate resistance can then be grown in paddocks populated by a particular PCN. We have developed a PCR-based test that can differentiate the 2 species. The ribosomal internally transcribed spacer region (ITS) was sequenced from 5 populations of both Ro and Pa. There was a low level of sequence divergence between Ro and Pa but no difference within populations of the same species. Single, species-specific PCR primers differing by 1 and 2 bases at the 3' end were designed from the Ro-Pa differences. These primers were used in combination with a third generic primer to differentiate between the 2 species in a single reaction. The primer set has been used to detect mixtures of the 2 species in New Zealand populations.

INVESTIGATIONS INTO CAUSE OF THE DELAYED HATCH OF *GLOBODERA PALLIDA* COMPARED TO *G. ROSTOCHIENSIS*. J. Byrne, D. Walsh, K. Devine, and P. Jones, Department of Plant Science, University College, Cork, Ireland.—Under European field conditions, juveniles of the white potato cyst nematode (*G. pallida*) tend to hatch later and over a longer period than those of the golden potato cyst nematode (*G. rostochiensis*). This has implications for PCN control since in-soil breakdown of the nematostat, aldicarb, means that *G. pallida* juveniles encounter lower levels, leading to poorer chemical control of this species. More than twenty-five hatching factors (HFs) have been resolved from potato root leachate. Although both species respond to each HF, marked differences in HF-preferences were observed between *G. pallida* and *G. rostochiensis*, with *G. pallida* hatching in response to the less-polar HFs. Close agreement was recorded between the time course of hatch in soil and the hatching response of the 2 species to root leachate from potato plants of different ages. Each of 6 potato varieties produced predominantly *pallida*-preferred HFs up to day 4, then *rostochiensis*-preferred HFs to day 21, when *pallida*-preferred HFs became the principal chemicals. Application of HF preparations with different ratios of HFs to infested soil showed that juveniles of *G. pallida* could be stimulated to hatch at the same time as those of *G. rostochiensis*.

RELATIONS BETWEEN NEMATODE AND SOIL IN YAM AND TOMATO CROPS IN MARTINIQUE. P. Cadet,[2] J. Thioulouse,[1] and E. Pate,[2] Laboratoire de Biométrie, Université Lyon 1, 69622 Villeurbanne Cedex, France,[1] and Laboratoire de Nématologie, ORSTOM, B.P. 1386, DAKAR, Sénégal.[2]—The relationships between the nematode populations parasitizing two cultivars of yam (*Dioscorea cayenensis-rotundata* and *D. alata*) and physico-chemical soil parameters were studied using the co-inertia analysis method. Results show that they are significant for both cultivars. For *D. cayenensis-rotundata*, the relationship between soil parameters and nematode species composition was significant. For *D. alata* about 0.3% of random permutations gave a value of the total inertia criterion higher than the experimental one. The test is, thus still significant, but the significance is much lower. This difference is explained by the fact that the *D. cayenensis-rotundata* yam cultivar is mainly infested by *Pratylenchus coffeae*, an endemic nematode which occurrence is linked to the soil characteristics. Indeed, *P. coffeae* is mostly found in soils with higher levels of organic matter, and its abundance is influenced by pH and sodium rate. The *D. alata* cultivar is infested by *P. coffeae* or *Scutellonema bradys*, an exotic nematode species. In this case, the relationship with the soil parameters is still significant because of the infestation by *P. coffeae* and other species, but it is weakened by *S. bradys* infestation, which does not show relationships with the parameters since it is exclusively introduced by the seed tuber.

OCCURRENCE OF NEW *PASTEURIA PENETRANS* GROUP MEMBERS ON NEMATODES IN PERU. E. Carbonell Torres, and A. Ciancio, Departamento de Fitopatologia, Universidad Nacional Agraria, La Molina, Lima, Peru, and Istituto di Nematologia Agraria, Consiglio Nazionale delle Ricerche, 70126 Bari, Italy.—The occurrence of *Pasteuria* spp. bacterial spore-forming nematode parasites was surveyed in the coast and highland cultivated areas of Peru. A population of *Pasteuria* spp. parasitizing *Pratylenchus andinus* was discovered at Tingo Maria from potato cultivated soil. The endospore and central core diameters were 3.34±0.33 µm and 1.44±0.15 µm, respectively. Endospores were attached to 90% of juveniles and adults of the *P. andinus* population, and 8.3% of them was filled with endospores. This isolate appeared larger and morphologically different from *Pasteuria thornei* described from *Pratylenchus brachyurus*. A second *Pasteuria* sp.was found parasitizing *Hoplolaimus galeatus* from an oat and potato field at Juliaca. The endospore and central core measurements of this isolate were 4.52±0.43 µm and 1.91±0.22 µm, respectively. Endospores were attached to 69.2% of males, 32.8% of females, and 15.0% of juveniles. Less than 10% of the population was filled with endospores. This isolate showed similarities with a smaller spore-size parasite described from a population of *H. galeatus* found in Florida and displayed uniform spore size. Other large endospore sized *Pasteuria* spp. were found on species of *Discolaimus* and *Eudorylaimus* from Cañete and Juliaca, respectively.

NEMATICIDAL ACTIVITY OF *BACILLUS* STRAINS ON JUVENILES OF *MELOIDOGYNE JAVANICA*. R. M. D. G. Carneiro,[1] I. Souza,[2] and L. G. Freitas,[3] EMBRAPA-CPACT, P.O. Box 403, Pelotas, RS, 96001-970, Brazil,[1] Universidade Federal de Pelotas, Box 354, Pelotas, RS, 96001-970, Brazil,[2] and Entomology and Nematology Department, IFAS, University of Florida, Gainesville, FL 32611, U.S.A.[3]—Twenty-one strains of *Bacillus* spp. were tested against *Meloidogyne javanica* second-stage juveniles (J2) *in vitro* bioassays. Bacterial supernatant and whole culture broth of *B. thuringensis brasiliensis* and *B. laterosporus* killed freshly hatched J2 within 24-48 hrs, whereas the ones from *B. aizawai* and *B. circulans* caused only immobilization. Mortality was not observed when the suspension of cell-spore crystals was used in all the treatments. No effect was observed on free-living Rhabditidae under the same conditions as for *M. javanica*. Although the nature of the supernatant was not determined, the results suggested the presence of a thermostable extracellular product with strong nematicidal activity.

INFLUENCE OF A SOYBEAN PEST COMPLEX ON FEEDING AND MATURATION OF SOYBEAN LOOPER. C. H. Carter, J. S. Russin, E. C. McGawley, D. J. Boethel, and J. L. Griffin, Department of Plant Pathology and Crop Physiology, Louisiana State University Agriculture Center, Baton Rouge, Louisiana 70803, U.S.A.—Feeding and maturation by soybean looper (*Pseudoplusia includens*) was investigated on 'Davis' soybean alone and in all combinations with hemp sesbania (HS) (*Sesbania exaltata*), charcoal rot fungus (CRF) (*Macrophomina phaseolina*), and root-knot nematode (RKN) (*Meloidogyne incognita* race 2). Leaf consumption nearly doubled when soybean was stressed by HS. Leaf consumption from plants colonized by RKN or CRF alone was less than that from controls. When RKN and CRF were together, leaf consumption did not differ from controls. Foliage utilization efficiency (FUE) nearly doubled when soybean was stressed by HS. FUE was higher when soybean was colonized by RKN or CRF alone, but when RKN and CRF were both present, FUE was reduced and did not differ from controls. Moth emergence was delayed 1 week when soybean was stressed by HS. Moth emergence was accelerated when roots were colonized by CRF. This effect was absent when roots were also colonized by RKN. Moth emergence was not influenced by RKN alone. Nitrogen content of foliage decreased when soybean was stressed by HS. Nitrogen content was higher when RKN colonized soybean alone and resulted in increased final weight of larvae fed foliage from plants stressed by RKN. Analysis suggests that HS competes with soybean for available soil nitrogen, which results in smaller plants that contain lower levels of foliar nitrogen. Larvae consumed twice as much foliage from soybeans stressed by HS in order to reach body weight equivalent to that of larvae reared on foliage from soybeans grown alone and emergence was delayed.

RESPONSE OF SEVEN SELECTIONS OF *PSIDIUM GUAJAVA* AND ONE OF *PSIDIUM FRIEDRICHSTHALIANUM* TO THE NEMATODE *MELOIDOGYNE* SPP. IN ZULIA STATE, VENEZUELA. **A. M. Casassa,[1] J. Matheus,[2] R. Crozzoli,[3] Z. Suárez,[4] A. Montiel,[4] and C. Castro,[4] Universidad del Zulia, Facultad de Agronomía-IIA, Apdo. 15205, Maracaibo, Venezuela,[1] Centro Frutícola del Zulia-Corpozulia, Maracaibo, Venezuela,[2] Universidad Central de Venezuela, Facultad de Agronomía, Apdo. 4579, Maracay, Venezuela,[3] and FONAIAP-CENIAP, Maracay, Venezuela.[4]**—Seven plant selections of *P. guajava* and one of *P. friedrichsthalianum* were evaluated in the greenhouse for their reaction to the root-knot nematode, *Meloidogyne* spp. (mixed population of *M. incognita* and *M. arenaria*). Forty-five-day-old plants were inoculated with 5 000 eggs and juveniles/plant and maintained for 6 months. Uninoculated plants serves as controls. Plant height, shoot and root weights, leaf number, and shoot diam were not reduced by *Meloidogyne* spp. The maximum reproductive factor (Rf = final population/initial population) was observed on selection 5 of *P. guajava* (30.7) whereas the lowest values of reproductive factors were observed on the selection of *P. friedrichsthalianum* (0.1) and in selection 3 of *P. guajava* (0.2).

SPECIFICITY OF *MELOIDOGYNE INCOGNITA* VIRULENCE AGAINST RESISTANCE GENES FROM TOMATO AND PEPPER. **P. Castagnone-Sereno,[1] M. Bongiovanni,[1] A. Palloix[2] and A. Dalmasso,[1] INRA, Laboratoire de Biologie des Invertébrés, BP 2078, 06606 Antibes Cedex, France,[1] and INRA, Station d'Amélioration des Plantes Maraîchéres, BP 94, 84143 Montfavet Cedex, France.[2]**—Some root-knot nematode field isolates are able to develop and reproduce on crops carrying specific resistance genes. Moreover, selection of resistance-breaking (i.e. virulent) lines under controlled conditions has been demonstrated. In tomato, resistance to *Meloidogyne arenaria, M. incognita,* and *M. javanica* is controlled by the Mi gene. In pepper, the two independent genes Me1 and Me3 both confer the same resistance spectrum as Mi. To study the specificity of host-parasite relationships between *M. incognita* and these 2 solanaceous crops, experiments were designed using i) Mi-resistant tomatoes and either Me1 or Me3-resistant peppers, and ii) *M. incognita* lineages previously selected for virulence against Mi or Me3. Nematodes virulent on Mi-resistant tomatoes were not able to reproduce on Me1 nor on Me3-resistant peppers, and nematode virulent on Me3-resistant peppers were not able to reproduce on Mi-resistant tomatoes nor on Me1-resistant peppers. These results clearly demonstrated the specificity of *M. incognita* virulence against resistance genes from both different botanical species (Mi in tomato and Me1 and Me3 in pepper) or different genes in the same botanical species (Me1 and Me3 in pepper).

REACTION OF *PHASEOLUS VULGARIS* CULTIVARS TO *MELOIDOGYNE INCOGNITA* (KOFOID & WHITE) CHITWOOD IN MENDOZA, ARGENTINA. **S. A. Castellanos, M. S. del Toro, C. V. Bartucciotto, C. Reising, E. A. M. Moyano, and C. M. Bustamante, Laboratory of Plant Nematology, Faculty of Agronomy, University of Cuyo, Almirante Brown 500, 5505 Chacras de Coria, MZA, Argentina.**—The reaction of 6 bean cultivars created by the Horticultural Research Institute to *Meloidogyne incognita* were tested. The cultivars were: NEGRO FCA (Algarrobeño × Rival), VICTORIA FCA (Algarrobeña × Wade), OLLIE FCA (Beurré-O'r-Du Rhin × Madrileño), BAYO FCA (Algarrobeño × Contender), and MENDOZA FCA (Balín de Anchovera × Algarrobeño). Ten, 1-month-old bean seedlings of each cultivar were inoculated with 2 000 eggs/pot. The evaluation of resistance was for 60 days after inoculation. Root galling severity was assessed on a 1-9 scale by estimating proportion of roots galled. Egg mass production was also assessed on a 1-9 scale. A resistance index was calculated into a single value (root galling severity rating + egg mass production rating). All cultivars were susceptible.

APPLICATION OF IgY FROM YOLK OF CHICKEN EGGS IN IDENTIFICATION OF SPECIFIC ANTIGENS FROM *PASTEURIA PENETRANS* ENDOSPORES. J. Charnecki,[2] S. Y. Chen,[1] J. F. Preston,[2] D. W. Dickson,[1] J. D. Rice,[2] and T. E. Hewlett,[1] Department of Entomology and Nematology,[1] and Department of Microbiology and Cell Science, University of Florida, Gainesville, Florida 32611, U.S.A.[2]—Polyclonal IgY antibodies were raised on chicken against endospores of *Pasteuria penetrans* isolates P20 and P100. Chickens were injected with *P. penetrans* endospore suspension and IgY antibodies were extracted from egg yolk laid by the chickens 22-35 days after initial injection. The system to produce anti-*Pasteuria*-endospore IgY was productive and efficient. Enzyme-linked immunosorbance assay revealed that P20 and P100 share mostly common antigens on the surface of endospores. SDS-PAGE and immunoblot analyses suggested, however, that some difference may exist between the two isolates. Several proteins unique to *Pasteuria* endospores were detected with the IgY. The IgY inhibited attachment of *Pasteuria* endospores to its host nematode juveniles, suggesting that proteins on endospore surface may be involved in the attachment.

FUNGI COLONIZING EGGS AND CYSTS OF *HETERODERA GLYCINES* IN A MINNESOTA SOYBEAN FIELD. S. Y. Chen, University of Minnesota, Southern Experiment Station, Waseca, Minnesota 56093, U.S.A.—Fungal colonization was determined for eggs and cysts of *Heterodera glycines* in soybean rhizosphere soil collected from a Minnesota soybean field in October 1995. A total of 413 cysts were examined, and 84% were colonized by fungi. More than 11 species of fungi were isolated from the cysts. *Cylindrocarpon* spp. (mostly *C. destructans*) was the predominant fungus and colonized 40% of the cysts. *Fusarium oxysporum* and *F. solani* were also common fungi, and they colonized 12% and 6% of the nematode cysts, respectively. *Verticillium chlamydosporium, Verticillium* sp., *Phialophora*, sp., *Sporidesmium* sp., *Stilbum*, sp., *Fusarium* sp., and several unidentified species were encountered at a low frequency. The average egg-parasitic index (at a scale of 0-10, 0 = no egg colonized and 10 = 91-100% of eggs colonized) by fungi was 2.6 in the field.

SUPPRESSION OF *MELOIDOGYNE ARENARIA* WITH SOIL APPLICATION OF ENDOSPORES OF *PASTEURIA PENETRANS*. Z. X. Chen,[1] D. W. Dickson,[1] R. McSorley,[1] D. J. Mitchell,[2] and T. E. Hewlett,[1] Entomology and Nematology Department,[1] and Plant Pathology Department, Institute of Food and Agricultural Sciences, University of Florida, Gainesville, Florida 32611, U.S.A.[2].—The potential of *Pasteuria penetrans* for suppressing *Meloidogyne arenaria* race 1 on peanut (*Arachis hypogaea*) was tested over a 2-year period in a field microplot experiment. Endospores of *P. penetrans* (0, 1 000, 3 000, 10 000, and 100 000 endospores/g of soil) were inoculated in the first year only into the top 20 cm of microplots that were infested previously with *M. arenaria*. One peanut seedling was planted into each microplot. Averaged over the 2 years, root-gall indices were reduced 60-81% for 100 000 endospores/g of soil and 20-61% for 10 000 endospores/g of soil ($P \leq 0.05$). Pod galls were reduced 90-95% for 100 000 endospores/g of soil and 65-82% for 10 000 endospores/g of soil over the 2 years ($P \leq 0.05$). Pod yields were increased 94% for 100 000 endospores/g of soil and 57% for 10 000 endospores/g of soil in the second year ($P \leq 0.05$). The results from other inoculation levels were not significantly different from the control. *Pasteuria penetrans* did not reduce the final population densities of second-stage juveniles (J2) in soil over the 2-year period, but the percentage of J2 with attached endospores at harvest increased readily with increasing endospore inoculation levels ($P \leq 0.05$). Regression analyses verified the role of *P. penetrans* in the suppression of *M. arenaria*. The minimum number of endospores required for suppressing *M. arenaria* on peanut was 10 000 endospores/g of soil.

RECYCLING OF WASTE PRODUCTS FROM AGRICULTURE - AN ALTERNATIVE TO CHEMICAL CONTROL OF PARASITIC NEMATODES. B. M. Choleva, and J. I. Tzvetkov, University of Sofia, Faculty of Biology, 1421 Sofia and Complex Experimental Station, Septemvri, Bulgaria.—A new nematode control strategy in Bulgaria is based on recycling technology using different plant residues against endo-(*Meloidogyne*) and ectoparasitic (*Xiphinema index, Longidorus elongatus*) nematodes. Six

plant residues were used as substrata for vegetable growing and in grape seedlings in greenhouses as well as for mulching in vineyards. Best results were obtained from flax and ground grapevine residues. The effect of flax residue on *Meloidogyne incognita* and *M. arenaria* after 3 years of experiments showed 8 times the reduction of J2 and doubled yield of tomatoes. Significant reduction of *X. index* numbers on grape seedlings were observed. Use of ground grapevine residues for cucumber growing in soil infested with root-knot nematodes had a very favorable effect and advantage over the normal way of growing. Recycling technology is a promising method for control nematodes and other pests and has ecological and economic importance for the country.

TIME DELAYED PARASITISM AND DENSITY DEPENDENCE IN *PASTEURIA* SPP. AND HOST NEMATODE DYNAMICS. A. Ciancio, Istituto di Nematologia Agraria, Consiglio Nazionale delle Ricerche, 70126 Bari, Italy.—The population dynamics of plant-parasitic nematodes studied in Italy in association with specific isolates of *Pasteuria* spp. nematode parasites showed a relationship between parasitism frequencies and nematode density. Data from a study carried out in a citrus field infested with *Tylenchulus semipenetrans* showed that *Pasteuria* parasitism was correlated to the host juvenile density changes with a 2 month delay. A similar relationship was found for a population of *Heterodera goettingiana* and a specific *Pasteuria* sp. found in southern Italy. Infection and parasitism rates higher than 80% were frequently observed, and the parasite life-cycle was completed in *H. goettingiana* juveniles. For both populations, a simple density dependent model was matched to density and parasitism data observed in spatial sampling.The biological constants considered for modeling were the rates of nematode population growth and nematode decrease induced by the parasite and the rates of parasitism growth and *Pasteuria* endospore mortality.The model suggests that stochastic fluctuations of host densities affect the subsequent levels of parasitism and that a critical distance from the equilibrium levels can locally induce a temporary host suppression.This relationship provides a basis for the development of nematode management practices through *Pasteuria* spp. endospore treatments.

RESPONSES OF NEMATODE POPULATIONS TO GRASSLAND MANURING AND MANAGEMENT PRACTICES. R. Cook, R. Bardgett, C. S. Denton, and G. W. Yeates, Institute of Grassland and Environmental Research, Aberystywth, Dyfed SY23 3EB, U.K., School of Biological Sciences, University of Manchester, Manchester M13 9PT, U.K. and Landcare Research, Palmerston North, New Zealand.—Nematode populations were compared in soil samples from agricultural grasslands subject to different manuring and grazing management. In grass on 3 soils under conventional (regular inputs of artificial nitrogen fertilizer) or organic (no artificial N) management, there were more fungal-feeding nematodes in organic fields. Different taxa of fungal-feeders contributed to the responses on the different soils. In hill grassland, fungal-feeding nematodes were a greater proportion of the nematode population in peaty (16%) than in mineral soils (4%), and there were somewhat more in plots from which grazing livestock had been excluded (12%) than from areas continuously grazed by sheep (9%). In controlled experiments on 2 soil types, there were more *Aphelenchoides* in plots on clayey soil, and generally more fungal feeders on plots receiving no N than 280 kg N/ ha/yr on both clay and loam. In pots, *Aphelenchoides* multiplied more in the rhizosphere of grass-only treatments than in those sown with white clover. There were more *Aphelenchoides* in the rhizospheres of perennial ryegrass and meadow fescue, especially on plants with an associated endophytic fungus (*Acremonium* spp.) than in those of tall fescue, red fescue, or *Holcus mollis*. Such plant-related differences complicate the interpretation of fungal-feeding nematode populations as indicators of the slower decomposition processes which may characterize lower-input, sustainable farming systems.

PROPOSAL OF *HEMICYCLIOPHORA* SPECIES NEW SYNONIMIZATIONS. E. S. Costa-Manso, and M. Luc, EMBRAPA/CENARGEN, Caixa Postal 2372, 70849-970, Brasília/DF, Brazil, and Muséum National d'Histoire Naturelle, Lab. de Biologie Parasit., Prot., Helminth. 61, rue Buffon, 75005 Paris, France.—Although *Hemicycliophora* is a genus of easy recognition, species identification may be diffi-

cult due to similarities in morphological characters. The comparison of paratypes of *Hemicycliophora* spp., the study of morphometrical variability, and the evaluation of character stability in the genus provided a basis for the proposition of 4 new synonimizations. The morphological and morphometrical study were done by optical microscope and scanning electron microscope. *Hemicycliophora lutosoides* Loof, 1984; *H. ekdavici* Darekar and Khan, 1981; *H. karachiensis* Maqbool, Shahina and Zarina, 1986; and *H. ferrisae* Breski, 1974 are considered, respectively, junior synonyms of *H. lutosa* Loof and Heyns, 1969; *H. natalensis* Loof and Heyns, 1969; *H. typica* de Man, 1921; and *H. uniformis* Thorne, 1955.

OCURRENCE AND DISTRIBUTION OF THE CITRUS NEMATODE (*TYLENCHULUS SEMIPENETRANS*) IN VENEZUELA. R. Crozzoli,[1] D. Rivas,[1] and A. M. Casassa,[2] Universidad Central de Venezuela, Facultad de Agronomía, Instituto de Zoología Agrícola, Apdo. 4579, Maracay, Venezuela,[1] and Universidad del Zulia, Facultad de Agronomía, Maracaibo, Venezuela.[2]—Eight-hundred composite soil and root samples were taken during January 1995 and May 1995 from citrus groves in Carabobo, Yaracuy, Monagas, Aragua, and Zulia States. Field sampling revealed that the citrus nematode (*Tylenchulus semipenetrans*) was widely distributed in citrus groves in Venezuela. In Valles Bajos and Valles Altos of Carabobo State, Valles Bajos and Valles Altos of Yaracuy State, Monagas, Aragua, and Zulia States detection was 26.7, 9.3, 21.3, 17.8, 28.6, 100, and 48.6% respectively. There were different levels of nematode populations in the various geographical areas of citrus growing; rootstocks (*Citrus volkameriana* and *C. reshni*) and soil factors were found important in the situation. The highest population was found in Aragua citrus groves (> 40 000 females and juveniles/10 g root).

CHARACTERISATION OF TWO ANTIGENS FROM CEREAL CYST NEMATODE SECRETIONS USING MONOCLONAL ANTIBODIES. R. Curtis, Entomology and Nematology Department, IACR-Rothamsted, Harpenden AL5 2JQ, Herts, U.K.—Secretions of plant-parasitic nematodes, which are released into plant tissue, may play critical roles in plant-nematode interactions. The identification and characterization of these molecules are very important and may help facilitate the development of novel strategies to interfere with nematode infection of plants and decrease nematode damage to crops. An antibody-based approach is being used to isolate molecules present on the nematode surface and secretions. Monoclonal antibodies (MAbs) were produced to secretions and whole *Heterodera avenae* second-stage juveniles; several of these MAbs recognized molecules present in nematode secretions produced *in vitro*. Two of these molecules have been characterized in *H. avenae, Globodera rostochiensis, G. pallida* and *Meloidogyne incognita*. The MAb reacting with the amphids and surface of these nematodes had a different molecular weight and isoelectric point in each of the species tested. This difference in antigenicity might be related to specific functions in these nematodes. Preliminary results show that this antibody labelled parts of root tissue surrounding the giant cell induced by *M. incognita* in *Arabidopsis thaliana*.

INFLUENCE OF THE SOIL ON THE AVAILABILITY OF *PASTEURIA PENETRANS* TO PARASITIZE NEMATODES IN THE GENUS *MELOIDOGYNE*. K. R. Dabiré,[1] T. Mateille,[1] M. T. Diop,[1] S. N'diaye,[2] and R. Duponnois,[1] Laboratoire de Nématologie, ORSTOM, B.P. 1386, Dakar, Sénégal,[1] and ENSA, B.P. A296, Thiès, Sénégal.[2]—Transport of juveniles of *Meloidogyne javanica* and spores of *Pasteuria penetrans* was assayed under a water drip supply in 4 soils: a sandy soil, a clay soil and two sandy-clay soils. The sandy-clay soils, sampled in the same vegetable area, differed by sand and clay content (not by silt). For the sandy soil, 67.7% of spores of *P. penetrans* and 78% of juveniles of *M. javanica* percolated with water despite high reproduction of *M. javanica* in that soil. For the clay soil, only 0.12% of juveniles and 10.6% of spores moved downward. Only 50% of spores remained in that soil after extraction and so could not be available for attachment. Transport of juveniles of *M. javanica* and spores of *P. penetrans* was easier in the sandy-clay soil which was originally free of *P. penetrans* and contained 6% less clay than the other which was naturally infested by *P. penetrans*. A survey conducted

on vegetable crops in Senegal confirmed that juveniles of *Meloidogyne* spp. infected by *P. penetrans* were abundant in soils with an optimal content of clay. So, the availability of spores of *P. penetrans* to attach juveniles of *M. javanica* would depend on a balance of soil texture and porosity, and on the capacity of colloids to release spores absorbed to the soil matrix.

DISCOVERY IN FRANCE AND CHARACTERISTICS OF THE DUTCH VARIANT OF *MELOIDOGYNE CHITWOODI*. **S. Daher,[1] S. Gillet,[2] D. Mugniéry,[1] and H. Marzin,[2] INRA, Laboratoire de Zoologie, BP 29, 35650 France,[1] and Unité LNPV Nématologie, BP 35650 Le Rheu, France.[2]**—A *Meloidogyne* species was found infesting a Mi tomato in a greenhouse in the western part of France. Reproduction is meiotic parthenogenesis and basal number of chromosomes is 12-14. Host range includes cereals such as wheat, but not maize, potato, tomato, beet, alfalfa, artichoke, lettuce, etc. Isoenzyme patterns were tested with esterase, MDH, SOD, GPD, and GOT. No esterase was detected, whatever the support used. Typical pattern of MDH was detected, different from *M. hapla, M. arenaria, M. chitwoodi*. With SOD, complete similarity (type H1) is observed with *M. hapla* and with *M. chitwoodi*. G6PD permitted distinction of this population from *M. hapla, M. chitwoodi*, and *M. arenaria, M. incognita, M. javanica*. The GOT pattern was similar to the pattern of *M. chitwoodi* (Type H2). Amplification of ITS of the gene 5.8S from individual juvenile crude homogeneates reveals a band of 760 bp, different from *M. naasi*, but not from the other species. The digestion enzymes Alu I, and Rsa I allowed separation of this population, respectively, from *M. hapla, M. arenaria* and Dra I allowed separation of this population from *M. hapla-M. chitwoodi*. The use of an equal mixture of Rsa I and Rsa I allowed separation of this population from *M. arenaria, M. chitwoodi* and *M. hapla, M. arenaria*. All these characteristics suggest the existence of a new species, which is different from *M. chitwoodi* and which is close or identical to the variant of *M. chitwoodi* discovered in the Netherlands.

THE NATURAL INCIDENCE AND BIOLOGY OF *EMPIDOMERMIS RIOUXI* PARASITIZING SALT MARSH MOSQUITOES, *AEDES DETRITUS* (CULICIDAE), IN CAMARGUE, FRANCE. **M. A. de Doucet,[1] C. Laumond,[2] and E. Bonifassi,[2] Centro de Zoología Aplicada, CC 122, 5000 Córdoba, Argentina,[1] and Institut National de la Recherche Agronomique, 123 Bvd. F. Meilland, BP 2078, Antibes Cedex, France.[2]**—*Empidomermis riouxi* was isolated from all active stages of salt marsh mosquitoes, *Aedes detritus*, in Camargue, France. Parasitism under natural conditions was studied for 3 years in 2 different pools at Brasinvert and Salin de Badon. Infections were determined by dissecting larvae and pupae samples. Larval development was closely related to *A. detritus* development; postparasites invariably emerged from the adult, causing death. The nematode eggs and mosquitoes were located at the margins of the pool; submersion was the principal eclosion factor, rather than temperature. Parasitic index (prevalence × incidence) attained the greatest values in winter and it was always zero in summer. Multiple infections occurred often. The parasitic index and the multiple infections were related to the pool characteristics; it was always higher at Brasinvert than at Salin de Badon. The results suggest that this nematode is univoltine and *A. detritus*, an autogenic specie, was the best host within several species present in the pool.

PATHOGENICITY OF *STEINERNEMA* AND *HETERORHABDITIS* NEMATODES TO HEAD LICE, *PEDICULUS HUMANUS CAPITIS* (L) (ANOPLURA: PEDICULIDAE). **M. A. de Doucet, M. Miranda, and A. Bertolotti, Centro de Zoología Aplicada, Universidad Nacional de Córdoba, CC 122, 5000 Córdoba, Argentina.**—The action of *Steinernema rara* (Doucet), *S. glaseri* (Steiner), *Heterorhabditis bacteriophora* Poinar (isolated from Noetinger, Los Chorrillos, and Oliva Córdoba, respectively), and *H. bacteriophora* (from Rio Negro) in head lice, *Pediculus humanus capitis*, was observed. Experiments were conducted in the laboratory putting larvae of infectives nematodes (LiN) and insects in Petri dishes at 100 LiN/host concentration; the mortality was recorded after 35 hrs exposure. Adults, nymphs, and eggs of head lice were observed. All species of nematodes, excepted *S. glaseri*, killed adults and nymphs. The mortalities observed were: *H. bacteriophora* from Rio Negro, 91%; S. rara, 64%; and *H.*

bacteriophora from Oliva, 46%. Significant differences were found between isolates of *H. bacteriophora*. *S. rara* as well as *H. bacteriophora* from Rio Negro equally killed adults and nymphs, and the isolate of *H. bacteriophora* from Oliva was more aggressive in adults. No infection was found in eggs. Apparently, penetration into the lice body took place through the spiracles and anus; the body size of the LiN is probably limiting for nematode infection. This is the first report of parasitism in head lice by entomogenous nematodes.

STUDIES ON THREE SPECIES OF *GASTROMERMIS* MICOLETZKY 1923 (MERMITHIDAE). MORPHOMETRIC VARIABILITY. M. A. de Doucet and S. Cagnolo, Centro de Zoología Aplicada, F.C.E.F. y Naturales. U.N.C. C.C. 122. 5000 Córdoba, Argentina.—*Gastromermis fidelis, G. kolleonis,* and *Gastromermis* sp. were analyzed taking into account morphological and morphometric characters; the of variability morphometric characters was evaluated. The morphometric variability was determinate according to Stanuszek's criterion on the basis of coefficient of variation values < 10 = low; 10-20 = medium; > 20 = high. Results showed that all species differ in the mouth position and in the combination and presence or absence of distinct characters. Significant differences were detected among mean values of all measurements considered, except the size of amphids (width in females and length in males) V ratio, and the width of spicule ($P \leq 0.05$). Variability appears low in length head to nerve ring, body diam at anus, and V ratio; in the other characters the variability was low, medium, or high depending of the character and sex. The results suggest that *Gastromermis* sp. could be a new species in regard to the differences detected. Although a high level of variability was expected since mermithids frequently show pluriparasitism and epigenetic sex determinism, little variability was registered in the species studied.

A SANDWICH METHOD TO STUDY HOST FINDING BY PLANT-PARASITIC NEMATODES. A. de Heij and F. C. Zoon, DLO Research Institute for Plant Protection, PO Box 9060, NL-6700 GW Wageningen, The Netherlands.—The efficiency of host finding by plant-parasitic nematodes is expected to be an important determinant of the damage to the host plant, especially in the case of small plants which are vulnerable to massive attack or virus transmission by nematodes. Yet, little is known about the processes and stimuli of host finding. A sandwich method was developed to study nematode activation and attraction towards plant roots. The system consists of two or three stacked layers of soil, contained in sieves of 2-cm-high with a nylon mesh bottom which prevents the passage of plant roots. The bottom layer contains soil with nematode inoculum which is quiescent to some extent. For Trichodoridae, natural populations in field soil were suitable. These nematodes appear to be activated by the extraction procedure, and thus nematode suspensions may cause artifacts. Survival stages may act as inoculum for Heteroderinae and other taxa. Attractive seedlings or test plants are planted in the top layer. In the two-layer setup, the attractiveness of plant species can be compared. In the three-layer setup, the effect of either crops or soils can be studied. In the latter case, the central layer contains the soil which has to be tested with respect to its relative resistance to host finding. The layers are moistened, stacked, and incubated at a constant soil moisture level. After a few days of incubation, the layers are separated and nematode populations are extracted from each layer and analyzed. When using *Paratrichodorus teres* in this system, little migration was observed in treatments without plants, whereas with sugarbeet seedlings the majority of the nematodes moved upwards to the root compartment within 4 days. After another 4 days, many well-fed nematodes had moved back to the central layer. With less attractive crops, the whole process was less and slower. A central layer of soil amended with 2% organic household waste compost inhibited attraction of *P. teres* by 70%, apparently due to interaction of the soil with plant root signals.

NEW INSIGHT INTO THE SYNERGISTIC INTERACTION BETWEEN NEMATODES AND *PSEUDOMONAS SOLANACEARUM* (RACE 1) IN TOMATO. P. Deberdt, P. Quénéhervé, and P. Prior, Laboratoire de Nématologie ORSTOM-INRA, Centre ORSTOM, BI 8006, 97259 Fort-de-France Cedex, Martinique, and Laboratoire de Phytobactériologie, INRA-URPV, BP 515, 97165 Pointe-à-Pitre Cedex, Guadeloupe (F.W.I.).—*Pseudomonas solanacearum* is co-distributed with polyspecific nematode populations in tropical and sub-tropical areas. Synergistic effects between these microorganisms on wilt symptoms are widely recognized, especially on tomato. Additional wounds to the root system caused by nematodes are generally reported as the factor responsible for synergistic effects, due to the increase of bacterial pathogen entries. Bacterial wilt incidence in a controlled environment was assessed on susceptible tomato cv 'Floradel' and polygenically resistant 'Caraibo' and 'Carmido' (isogenic but introgressing Mi gene), following cross-infection between strain GMI 8217 (race 1, Bvl) and two different plant-parasitic nematodes: *Meloidogyne incognita* (RKN, root-knot nematode) and *Rotylenchulus reniformis* (RN, reniform nematode). At low temperatures (22-27 C), GMI 8217 was slightly pathogenic to all tomato lines, except 'Floradel' co-infected by RKN. At higher temperatures (27-32 C), RKN increased both bacterial colonization at midstem and wilt severity in 'Floradel' while RN only increased bacterial colonization. RKN significantly increased wilting in the resistant cultivar 'Caraibo'. Galling was observed rarely on the entire root system. 'Carmido' was the best host plant for the RN compared to 'Floradel' but without increased bacterial colonization or wilting. Consequently, galling was considered the primary synergistic determinant increasing bacterial wilt severity. In addition, hypotheses were presented on the possible close association between the Mi gene and a gene conferring partial temperature-dependent resistance to bacterial wilt. Both genes are located on tomato chromosome 6.

REVIEW OF DESMOSCOLECIDS FROM NON-MARINE HABITATS, WITH DISCUSSION OF A *DESMOSCOLEX (DESMOLORENZENIA) MONTANA* SPECIES COMPLEX. W. Decraemer, Koninklijk Belgisch Instituut voor Natuurwetenschappen, Brussels, Belgium.—Desmoscolecida are essentially marine nematodes from sublittoral or deep-sea habitats. Records from non-marine habitats are rare and currently, mainly regarding members of the subfamily Desmoscolecinae (sensu Decraemer, 1985). Species composition of the Desmoscolecidae in salt marshes is rather diverse and in that way comparable with marine habitats rather than with terrestrial and freshwater habitats. Some of these species occur also in other brackish habitats. Records from freshwater habitats are restricted to 4 *Desmoscolex* species which display a similar habitus showing close resemblance with terrestrial *Desmoscolex* (*Desmolorenzenia*) species from the circumtropical region mainly. Currently, terrestrial Desmoscolecidae are represented by 1 genus (*Desmoscolex*) and 2 subgenera (*Desmoscolex* and *Desmolorenzenia*). The *Desmolorenzenia* species are closely related, differing in minor features from *D. (D.) montana*, the first species described. This relationship is confirmed by SEM studies of the lip region revealing the same basic pattern, clearly different from the patterns described of marine desmoscolecids. The terrestrial *Desmolorenzenia* species are distributed worldwide among similar habitats which apparently is reflected in a similar habitus.

OBTAINING *DITYLENCHUS DIPSACI*-FREE GARLIC SEED IN MENDOZA, ARGENTINA. M. S. del Toro, S. J. Castellanos and E. A. M. Moyano, Laboratory of Plant Nematology, Faculty of Agronomy, University of Cuyo, Almirante Brown 500, 5505 Chacras de Coria, MZA, ARG.—The main area of garlic production in Argentina is Mendoza. In 1994/95 6 300 ha were grown in garlic. The principal garlic pest is *Ditylenchus dipsaci*. Since 1989, a regulatory process was started to obtain garlic seed free of nematodes. A sample of 1 000 g of cloves of garlic was taken from 1 000 kg of garlic bulbs of each grower registered in the garlic seed program. The percentage of lots rejected due to *D. dipsaci* was: 1989/90, 25%; 1990/91, 10%; 1991/92, 70%; 1992/93, 23%; 1993/94, 50%; 1994/95, 34%. In 1994/95, certificated garlic seed free of *D. dipsaci* was obtained.

GRAPEVINE FANLEAF VIRUS (GFLV) DETECTION IN *XIPHINEMA INDEX* BY ELISA AND RT-PCR METHODS. G. Demangeat,[1] D. Esmenjaud,[2] L. Pinck,[3] and B. Walter,[1] INRA, 68021 Colmar, France,[1] and INRA, 06600, Antibes, France,[2] and IBMP-CNRS, 67084 Strasbourg, France.[3]—Biotin-streptavidin DAS-ELISA and RT-PCR methods have been adapted for the detection of GFLV in *X. index*. The ELISA method required a minimum of 10-20 nematodes for virus detection. RT-PCR detection has been obtained from single individuals using 25-mer primers corresponding to a region of the viral coat protein sequence. The ELISA method was first used to evaluate the viral infectious potential (VIP) of diverse nematode populations sampled in French vineyards. The field population Mesnil from Champagne with a high ELISA response was chosen to study the nematode VIP evolution in the absence of the host plants. Soil samples from this diseased grapevine were stored at 20°C and 7°C. Lots of 30 adult and J4 nematodes were extracted after different storage times (0, 6 and 12 months) for ELISA tests. Comparison of field nematodes with virus-free control nematodes showed an important reduction of VIP after 6 and 12 months at 20°C. Nevertheless, the virus was still detected in lots of 5 nematodes for each date. After 12 months storage at 7°C, ELISA values were intermediate between those of initial (0 month) and 12 month-20°C values.

EFFECTS OF CROP ROTATIONS ON POPULATIONS OF *MELOIDOGYNE JAVANICA* AND *PASTEURIA PENETRANS*. M. T. Diop,[1] T. Mateille,[1] S. N'diaye,[2] K. R. Dabiré,[1] and R. Duponnois,[1] Laboratoire de Nématologie, ORSTOM, B.P. 1386, Dakar, Sénégal,[1] and ENSA, B.P. A296, Thiès, Sénégal.[2]—Effects of crop rotations on the development of a population of *Meloidogyne javanica* infected by *Pasteuria penetrans* were compared. During the first crop (dry season) on both tomato and susceptible intercropping weeds (*Amaranthus* sp. and *Boerhavia* sp.), the percentage of infected juveniles decreased during the first month resulting from a lack of juveniles which penetrated the roots. Then it increased until the end of the crop, following the multiplication of the juveniles. During the second crop (rainy season), the evolution of the percentage of infected juveniles was the same on a susceptible leguminous crop (*Vigna sinensis*) as it was on tomato and on weeds during the first crop. On a poor host (onion), a nonhost (sorghum) and a trap host (peanut), the proportion of infected juveniles increased during the first month and then decreased. During the third crop (dry season), the proportion was the same on tomato as on the previous crops. The proportions of infected juveniles showed optimal thresholds which depended on different total populations of juveniles according to the crops and the previous crops. Thus, the development of *P. penetrans* seems to be influenced by the development of *M. javanica* and by the host plant independently of nematode development.

CHARACTERISATION OF *HETERORHABDITIS* ISOLATES FROM HUNGARY AND DENMARK. I. Dix, C. T. Griffin, S. A. Joyce, A. M. Burnell, and M. J. Downes, Biology Department, St. Patrick's College, Maynooth, Co. Kildare, Ireland.—Three biological species of *Heterorhabditis* have been recognized in Europe: *Heterorhabditis* from southern and central regions are interfertile with *Heterorhabditis* HP88, a member of the *H. bacteriophora* species complex; members of the NW European *Heterorhabditis* species (NWE) occur in coastal regions of the Netherlands, Germany, Poland, and the south of England, and the Irish species type occurs in Ireland and the British Isles. Members of these 3 species can be distinguished by their reproductive isolation in crossbreeding tests and by the distinctive restriction fragment profiles obtained from PCR amplified DNA from the rDNA ITS region. In November 1991, soil samples were collected in Denmark (Sjaelland region) and Hungary (Kecskemet and Debrecen regions). *Heterorhabditis* was recovered from 10 of the 26 sites sampled in Denmark and 12 of the 46 sites sampled Hungary. Restriction enzyme analysis of the rDNA ITS region of these isolates revealed that isolates from 10 of the 12 Hungarian sites displayed a HP88 type profile and those from 2 sites displayed a restriction profile characteristic of the Irish species. Four Danish sites had only NWE type isolates,4 sites had only Irish type isolates, and at one site both Irish and NWE species type restriction profiles were recorded. A second sampling programme confirmed the occurrence of the Irish species type in Denmark and Hungary. The Hungarian and Danish isolates which display an

Irish type profile are interfertile with each other and with the Irish type isolate K122. Although the ITS restriction profile of 14 strains of Hungarian origin are identical to that of *Heterorhabditis* sp. HP88, the Hungarian isolates represent a distinct biological species from HP88 since a fertile hybrid line could not be established from crosses between these Hungarian isolates and the HP88 strain.

IDENTIFICATION OF DNA MARKERS LINKED TO THE ME3 GENE CONTROLLING RESISTANCE TO ROOT-KNOT NEMATODES IN PEPPER (*CAPSICUM ANNUUM* L.). C. Djian-Caporalino, L. Pijarowski, A. Januel, A. Palloix, V. Lefebvre, and T. Phally, INRA 06600 Antibes, and INRA, 84143 Montfavet, France.—Several genes control the resistance to *Meloidogyne* spp. in some lines of *Capsicum annuum* L. Some of them are found specifically active against one *Meloidogyne* population, others have a broad spectrum of action. The resistance of PM687 line (issued from PI 322719) is determined by a single dominant gene Me3 controlling resistance to several *Meloidogyne* species. To confirm and complete those results, 75 haplodiploid progenies (HD), obtained in France by androgenesis from the F1 hybrid (PM687 × 'Yolo Wonder', cultivated pepper), have been tested for their reaction to different *Meloidogyne* populations. The segregation ratio in the HD is 43 resistant: 32 susceptible. It is not significantly different from the 1: 1 ratio (P = 20.4%) suggesting that effectively a single gene controls this resistance. Fifty new HD lines of the same progeny will complete those results. Me3 also confers resistance to nematodes at 40°C temperature at which Mi (resistant gene in tomato) is not effective. Bulked-segregant analysis with resistant and susceptible DNA pools was employed to identify RAPD and AFLP markers linked to this resistant gene. Decamer oligonucleotide primers (530) have been screened for RAPD polymorphisms and 4 markers identified. The 4 markers do not recombine in the 75 HD progenies, but percentage of recombination with the resistant gene, indicate that they are still far away from the Me3 gene. More rapid and efficacious results were obtained with the AFLP technics: 160 HindxMse oligonucleotide primer combinations have been screened on DNA pools and 6 markers identified. The polymorphic bands of each of them are in search in the HD progenies. One of them is linked with the 4 RAPD markers. Our aim is to localize the Me3 gene with those markers on the pepper map and to clone the gene.

GENETIC ANALYSIS OF *HETERODERA GLYCINES* PARASITISM. K. Dong and C. H. Opperman, Department of Plant Pathology, North Carolina State University, Raleigh, North Carolina, U.S.A.—The soybean cyst nematode (SCN), *Heterodera glycines*, is a sedentary endoparasite that causes substantial economic damage to soybean on a worldwide scale. Although management via the use of resistant soybean cultivars may be achieved, there are numerous genotypes (races) of SCN that may evade host resistance responses. Because SCN maintains an intimate parasitic relationship with its host, it is suspected that numerous nematode genes are involved in parasitic abilities. In order to identify how many nematode genes are involved and to isolate these genes, we have taken a genetic approach. To attain this goal, we have constructed a linkage map of the *H. glycines* genome. This map may be utilized to identify numerous genetic loci related to nematode parasitism traits, including host preference and feeding site establishment. Controlled crosses have been performed between several SCN strains. Segregation analyses have revealed that inheritance of parasitic ability is Mendelian in nature. The genes controlling parasitic ability on a specific host differential appear to be unlinked loci. The SCN gene controlling ability to parasitize PI 88,788 appears to be inherited as a dominant allele, whereas the genes conferring parasitic ability on PI 90,763 and Pickett are recessive. We have screened approximately 1 000 RAPD 10mer primers against 3 SCN inbred parental strains and 1 600 progeny lines. We have mapped parasitism loci using a RAPD-PCR approach with a modified bulk segregant analysis. Specifically, we have identified 2 DNA markers tightly linked to the PI 88788 parasitism locus. We have tentatively mapped the parasitism genes for PI 88788, PI 90763, and Peking to different linkage groups (chromosomes).

INTRAPOPULATION VARIABILITY OF ENZYME PHENOTYPES IN *NACOBBUS ABERRANS* FROM ARGENTINA. M. E. Doucet,[1] E. Montamat,[2] and A. Giayetto,[3] Fac. Cs. Agropecuarias, C.C. 509, (5000) Córdoba,[1] Fac. Cs. Médicas, C.C. 35, (5016) Córdoba,[2] Fac. Cs. E.F. y Naturales, Univ. Nac. Córdoba, Av. Vélez Sársfiel 299, (5000) Córdoba, Argentina.[3]—Electrophoresis on polyacrilamide gels were made on individual *Nacobbus aberrans* swollen females from a south Córdoba, Argentina population. Nematodes were obtained from roots of *Chenopodium album* L. and roots of *Lycopersicon esculentum* Mill. Fifteen enzymes were developed. The results showed that the enzyme phenotypes were independent of the host on which the nematode developed. Esterases appeared as the most variable enzyme showing 8 different phenotypes. Lactate dehydrogenase and β hydroxybutyrate dehydrogenase were monomorphic. Glucose-phosphate isomerase and malate dehydrogenase showed 3 different phenotypes. Malic enzyme and mannose-phosphate-isomerase presented some variability that needs to be confirmed due to the low activity of those enzymes. The rest of the enzymes (hexokinase, catalase, glucose-6 phosphate dehydrogenase, phosphoglucomutase, isocitrate dehydrogenase, alcohol dehydrogenase NADP + dependent, aspartate aminotransferase, and phosphatase acid) did not show any activity. These preliminary results suggest the existence of a large isoenzymatic variability in the population studied.

INDUCING RESISTANCE AND TOLERANCE OF TOMATOES TO *GLOBODERA ROSTOCHIENSIS*. A. Dowe, H. Decker, and D. Hassan, University of Rostock, FB Agrarökologie (FG Phytomedizin), Satower Str. 48, 18059 Rostock (Germany) and Agricult. Fakulty, University of Khartoum (Sudan).—Pot experiments were conducted in greenhouses over a 4-year period with a combination of simultaneous and staggered inoculations (Pi = 500 or 2 000 larvae per 100 cm^2 soil, respectively) with *G. rostochiensis* (Gr) and *Heterodera schachtii* (Hs). In the case of the simultaneous inoculation of the 2 nematode species, the reproduction rate (RR) of Gr fell noticeably. In the case of inoculation with Hs 3 weeks before Gr, this inhibiting effect was even more marked. With initial Hs inoculation, the longitudinal growth of plants was not reduced, in contrast to initial Gr inoculation with 2 000 E+L. After 6- 8 weeks, the differences in longitudinal growth had to a large extent levelled out but differences in yield became apparent later. In additional two- year pot experiments on the influence of urea doses (7.5 ppm, 4× at weekly intervals) on the population development of Gr in tomatoes, RR was significantly reduced at low Pi. Apart from the greater weight of individual fruits in all treated variants, yield was higher only in the urea variant with low Pi. The lower RR of Gr observed in several variants in all the experiments, together with the prevention of growth inhibition can be interpreted as induced resistance and tolerance in the tomato plants.

THE COMBINED EFFECT OF *PASTEURIA PENETRANS* AND RHODES GRASS ON POPULATIONS OF *MELOIDOGYNE JAVANICA*. B. N. Dube, University of Zimbabwe, Department of Biological Sciences, P.O. Box MP 167, Mt. Pleasant, Harare, Zimbabwe.—The combined effect of *Pasteuria penetrans* (Pp) and Katambora Rhodes grass (KRG) on populations of *Meloidogyne javanica* (Mj) was tested for 3 seasons (1993/4, 1994/5, 1995/6) at 3 field research sites: Henderson Experimental Station (HES), Horticultural Research Centre (HRC), and Makoholi Experimental Station (MES). Soil types at the 3 sites were clay, loam, and sand, respectively. At each site, 20 (5× 4.5 m) microplots infested with Mj were used. *P. penetrans* was initially inoculated at the rate of 500mg/L soil and KRG grown as a winter cover crop in between the growing seasons. Field beans cv Natal sugar was the nematode host. Population densities of Mj (P1, P2, P3,and P4)taken every 28 days and final yields were recorded. Results of nematode counts at the 3 sites showed that population densities of Mj in Pp infested plots that did not have the winter grass cover crop had been significantly reduced by 23% in sandy soils (MES), 16% in clay soils (HES), and 20% in loam soils (HRC). However, reduction of Mj population densities in plots that had the winter grass cover crop was even higher (30% in sandy soils, 24% in clay soils, and 22% in loam soils). Although results of bean yield showed a similar trend, generally higher yields were realized in loam soils for plots that had or had not received the winter grass

cover crop. Also at the end of the 3 seasons, there was a significant increase in the bacterial spore counts in soils from all the plots at the 3 field sites (HES, HRC, MES) in which Pp had been initially applied.

A PUTATIVE NEMATODE AUXIN BINDING PROTEIN FROM THE POTATO CYST NEMATODE *GLOBODERA PALLIDA*. L. H. Duncan,[1] W. M. Robertson,[2] J. R. Kusel,[1] and M. S. Phillips,[2] Division of Biochemistry and Molecular Biology, University of Glasgow,[1] and Scottish Crop Research Institute,[2] Invergowrie, Dundee DD2 5DA, Scotland, U.K.—Previous work has shown that incubation of *Globodera pallida* with the phytohormone auxin results in the induction of oesophageal gland secretions, as well as changes that occur at the surface of the nematode. Auxin thus appears to be acting as a host cue to the nematode, raising the possibility that the nematode may possess a receptor for auxin. Using anti-serum D16, which is raised against the conserved 16 amino acid region embracing the auxin binding domain of maize auxin binding protein, it has been possible to identify a 45kDa protein in *Globodera pallida* that may represent a putative auxin binding protein/receptor. Preliminary immunogold localization studies indicate that D16 localizes to structures at the head of the nematode, possibly the amphids. Further work aims to purify the protein in question using ion exchange chromatography followed by FPLC.

***PASTEURIA PENETRANS* HELPER BACTERIA (PHB): EFFECTS OF TWO BACTERIAL STRAINS ON THE RELATIONSHIPS BETWEEN *P. PENETRANS* AND *MELOIDOGYNE INCOGNITA*. R. Duponnois, and T. Mateille, Laboratoire de Nématologie, ORSTOM, B.P. 1386, Dakar, Sénégal.**—This study investigates the presence of biological agents stimulating the infection of the root-knot nematode, *Meloidogyne* spp., by the actinomycete, *Pasteuria penetrans*. A soil infested with *M. javanica* and *P. penetrans* was sampled from a tomato crop at an experimental station near Thies, Sénégal. Two bacterial strains (*Bacillus cloacea* and *Pseudomonas mendocina*) were isolated from the tomato rhizosphere. These strains were inoculated with *M. incognita* and *P. penetrans* either *in vitro* or into a disinfected soil in the presence of a tomato plant. Both bacterial strains enhanced attachment of *P. penetrans* to juveniles *in vitro*, and increased tomato growth and the multiplication of *P. penetrans*. These bacteria were referred to as *P. penetrans* helper bacteria (PHB). The absence of *P. penetrans* in a field plot near those sampled for this study might have resulted from a lack of the PHB. Their potential and the mechanisms involved in these stimulating effects are discussed.

TELONE C-17 AS AN ALTERNATIVE TO METHYL BROMIDE IN FLORIDA MULCHED VEGETABLE PRODUCTION. J. E. Eger, and R. M. Huckaba, DowElanco, Indianapolis, Indiana, 46268, U.S.A.—Telone C-17 (77.9% 1, 3-dichloropropene + 16.5% chloropicrin) was evaluated in more than 20 small plot trials in Florida during 1993-1994 as an alternative to methyl bromide/chloropicrin mixtures now being used. Trials focused on Florida plastic-mulched vegetable production, of which tomatoes, peppers, and strawberries constitute 80+% of the fumigated acreage. Efficacy of these products was evaluated on nematodes (primarily root-knot nematodes, *Meloidogyne* spp.), several diseases (primarily fusarium wilt, *Fusarium oxysporum* f.sp. *lycopersici*, and fusarium crown and root rot, *Fusarium oxysporum* f.sp. *radicis-lycopersici*), and weeds (primarily yellow nutsedge, *Cyperus esculentus*, and purple nutsedge, *Cyperus rotundus*). Overall, Telone C-17 was generally equivalent to methyl bromide/chloropicrin for control of root-knot nematodes and diseases. Telone C-17 did not provide equivalent control of nutsedge and a herbicide will be necessary for weed control where nutsedge is a problem. Total yields in 1994 pepper and tomato plots treated with Telone C-17 ranged from 76-128% of those in plots treated with methyl bromide/chloropicrin mixtures, with an average of ca. 95%. In these small plot trials, Telone C-17, in combination with an effective herbicide, provided nematode, weed, and soilborne disease control and yields that were usually equivalent to those obtained with methyl bromide/chloropicrin mixtures.

MODE OF ACTION OF SOME SOIL AMENDMENTS IN NEMATODE CONTROL. O. A. Egunjobi, and J. O. Olaitan, Zoology Department, Ondo State University, Ado-Ekiti, Nigeria, and Sotonwa, Department of Crop Production and Protection, University of Ibadan, Nigeria.—Two soil amendments, CPH (pod husks of *Theobroma cacao*) and CASP (tuber peels of *Manihot utilissima*) were investigated for their mode of action on *Meloidogyne* pests of cowpea (*Vigna unguiculata*). Chemical analysis with a hexane-acetone mixture showed that CPH and CASP contained unsaturated and saturated fatty acid derivatives, respectively, indicating a direct effect through the lipid anti-oxidants inherent in them. CPH and CASP may also be acting indirectly by boosting the numbers of soil nematophagous fungi and arthropods by about 2×/3.9× and 2.7×/3.1×, respectively.

MOLECULAR IDENTIFICATION OF *PHOTORHABDUS LUMINESCENS*, BACTERIAL SYMBIONTS OF ENTOMOPATHOGENIC NEMATODES OF *HETERORHABDITIS* SPP. R.-U. Ehlers, I. Niemann, and U. Wyss, Institute for Phythopathology, Christian-Albrechts-University, Kiel, Germany.—The 16S ribosomal RNA of bacterial symbionts of *Heterorhabditis megidis* (strain HSH2) and *H. bacteriophora* (strain New Jersey RS 120) were sequenced. Highly variable regions were identified and used to synthesize strain specific primers. Together with a primer based on a conservative region of the 16S rRNA common to all procaryotes, they were used to amplify DNA by the Polymerase Chain Reaction. Stringent annealing temperature conditions, identified experimentally, assure a PCR product only when complementary DNA templates of the specific primer regions are present. The primers were tested with 35 different isolates of *P. luminescens*. Several Dutch, Irish, German, one Swiss, and one Finnish isolate from nematodes of the North-West European and Irish group of *H. megidis* gave positive results with the primer developed for the HSH2 strain. These isolates most probably belong to a yet undescribed species of the genus *Photorhabdus*. Isolates from the U.S.A., the Azores, Spain, Argentina, Germany and the symbiont of the original *H. megidis* nematode from Ohio were positive with the primer developed for the RS 120 strain. With a primer complementary to the type strain ATCC 29999 none of the *P. luminescens* strains, except the type strain, gave a positive reaction. Isolates from Italy (IH127), the U.S.A. (strains California, Hawaii, ATCC 29304, WX), Poland (Sie), Australia (D1, V16), Germany (HD01, HK6), Moldavia, and Israel (IS-4) were negative for all 3 primers. The results indicate that *H. bacteriophora* is probably associated with more than one *Photorhabdus* species.

FINE STRUCTURE OF INFECTIVE LARVAE (L3) OF *ONCHOCERCA VOLVULUS* DEVELOPED IN *SIMULIUM YAHENSE* IN LIBERIA. B. Y. Endo, and M. Trpis, Nematology Laboratory, Plant Sciences Institute, USDA, Beltsville, Maryland 20705, U.S.A., and The Johns Hopkins University, Department of Molecular Microbiology and Immunology, Baltimore, Maryland 21205, U.S.A.—Third-stage infective larvae of *Onchocerca volvulus* were examined to elucidate the ultrastructure and the interrelations of the stoma, esophagus, intestine, and nervous system. The alimentary canal involves a cuticularized stoma with a triradiate lumen that is continuous with a similar triradiate lumen in the muscular region of the esophagus. The lumen wall may be laterally appressed or opened 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 evaginations of the apical membranes of the intestinal epithelium. In cross section, groups of 5 radiating epithelial cells are joined near the lumen surface by junctional complexes. The alimentary canal terminates via a rectal valve and channel supported by somatic and neural cells. The central nervous system consists of a nerve ring that surrounds the muscular region of the esophagus. Related neurons support chemo- and tactoreceptors of sensilla and the extensive coelomyarian-meromyarian somatic muscles. Extensive accumulations of glycogen rosettes are present in many of the muscle and hypodermal cells.

INFLUENCE OF SOYBEAN GENOTYPE ON COMPETITION BETWEEN *MELOIDOGYNE INCOGNITA* AND *ROTYLENCHULUS RENIFORMIS*. **S. R. Erwin, E. C. McGawley, and J. S. Russin. Department of Plant Pathology and Crop Physiology, Louisiana State University Agricultural Center, Louisiana Agricultural Experiment Station, Baton Rouge, Louisiana 70803-1720, U.S.A.**—The effect of soybean genotype on competition between *Meloidogyne incognita* race 2 (MI) and *Rotylenchulus reniformis* (RR) was evaluated in greenhouse and microplot replacement series experiments. Soil in pots containing seedlings of 'Davis' (susceptible to both nematodes) or 'Buckshot 66' (tolerant to both nematodes) was infested with 1 000 vermiform individuals in the following MI:RR ratios: 100:0, 75:25, 50:50, 25:75, and 0:100. After 91 days, the relative yield (number in mixed culture/number in single culture) of each species was calculated based on the numbers of vermiform individuals in soil per g of dry root tissue. To define the relationship between the two species, calculated relative yields were compared with a theoretical noncompetition model using lack-of-fit regression. In the greenhouse, MI populations on Davis were stimulated in the presence of RR. In microplots, low MI and RR populations likely resulted from severe galling and destruction of feeder roots which probably occurred early in the season. On Buckshot 66, MI populations in microplots were not influenced by RR while in the greenhouse they were inhibited at MI:RR ratios of 50:50 and 25:75. With the single exception noted for Davis in the microplot, RR populations were not influenced by competition with MI.

CHARACTERIZATION OF *MELOIDOGYNE MAYAGUENSIS* AND ITS RELATIONSHIP TO OTHER TROPICAL ROOT-KNOT NEMATODES. **M. Fargette,[1] R. Duponnois,[2] T. Mateille,[2] and V. C. Blok,[3] Laboratoire de Nématologie, CIRAD/ORSTOM, BP 5035, 34032 Montpellier Cedex, France,[1] ORSTOM, Laboratoire de Nématologie, B.P. 1386, Dakar, Sénégal,[2] and SCRI, Invergowrie, Dundee DD2 5DA, Scotland.[3]**—Populations of *M. mayaguensis* have been identified in West Africa which overcome the resistance of cultivars as diverse as tomato cv. Rossol, sweet potato cv. CDH, and soybean cv. Forrest. Information on their occurrence, fertility, virulence in relation to resistant cultivars, biochemical and molecular relationships to the other major tropical root-knot nematode species, and surface specificity with *Pasteuria penetrans* were presented. A particular focus on its distribution in Senegal and its possible spread throughout the country stresses and exemplifies the threat posed by this nematode. The problems related to its control have relevance to future management or quarantine measures which may be required.

ANALYSIS OF T-DNA TAGGED *ARABIDOPSIS THALIANA* LINES FOR NEMATODE RESPONSIVE PROMOTORS. **B. Favery, N. Gil, C. Bihet, and P. Abad, INRA, Laboratoire de Biologie des Invertébrés, BP 2078, 06600 Antibes, France.**—*Meloidogyne* spp. are obligate plant parasites which have evolved a highly specialized and complex feeding relationship with their hosts. They induce a redifferenciation process of root cells (repeated mitosis without cytokinesis) that leads to the formation of multinucleated feeding cells on which they depend for the completion of their life cycle. These "giant cells" appear to be metabolically active and act as transfer cells between nematodes and the vascular system of plants. Promoter tagging strategy can provide access to plant genes involved in induction and maintenance of giant cells. Based on transformation *in planta* and random integration of a promoterless gus gene construct binary vector into *Arabidopsis thaliana*, this method allows screening for nematode feeding site (NFS) specific for up and down-regulated promotors. A collection of 50 000 - 100 000 T-DNA insertion mutants is being generated. From the first 2 000 T-DNA tagged lines tested for gus expression patterns after *Meloidogyne* infection, several transgenic lines have been found with increased gus expression inside the giant cells. From the latter, two lines were selected which display gus activity in giant cells and the root apex, likely meristematic tissue. These lines have been tested in detail for gus induction or decrease time course in NFS after *Meloidogyne* infection, and for tissue specificity during plant development and inducibility. Segregation analysis (2:1) and Southern blot of one of these lines suggest a single locus integration of tandem copies of T-DNA and an embryonic lethal

homozygous mutant. Interestingly, the promoter can now be isolated by plasmid rescue. These results were discussed in relation to plant-nematode interaction.

ALGINATE FILMS FOR PRESERVATION AND INOCULUM DELIVERY OF *PRATYLENCHUS* SPECIES UNDER *IN VIVO* AND *IN VITRO* CONDITIONS. C. Fernández,[1] J. Pinochet,[1] and R. Rodríguez-Kábana,[2] Departamento de Patología Vegetal, Institut de Recerca i Tecnologia Agroalimentàries, Crta. de Cabrils s/n, 08348 Cabrils, Barcelona, Spain,[1] and Department of Plant Pathology, Auburn University, Auburn Alabama 36849, U.S.A.[2]—A method for inoculum preservation and delivery of *Pratylenchus* species was developed using alginate films. Nematodes were surface sterilized, suspended in 3% (w/v) sodium alginate solution applied to polyvinyl chloride coated fiberglass screens of 0.4 thickness and gelled by dipping in 0.25 M $CaCl_2$. Films were kept in moist condition at 4°C until needed. Inocula containing all life stages of the nematodes were used. In a first *in vitro* experiment, the comparative reproduction of *P. vulnus* on carrot disks was assessed 110 days after inoculation with inoculum previously kept at 1, 45, and 160 days in storage in sterile water suspension at 4°C or alginate films. There were no differences in nematode buildup between water and alginate film treatments kept 1 and 45 days in storage. At 160 days storage, nematodes maintained in water suspension reproduced well while those in alginate film barely survived. In a second *in vitro* experiment, the reproduction of *P. vulnus, P. scibneri, P. agilis, P. goodeyi,* and *P. thornei* was compared on carrot disks at 110 days following inoculation with alginate films containing 30 nematodes per culture. All the species reproduced, but *P. vulnus* and *P. thornei* multiplied significantly more than the other species. In a third experiment with 'Conference' pear, reproduction of *P. vulnus* was assessed 90 days after inoculation. Plants inoculated with *P. vulnus* inoculum in alginate films reached higher nematode root densities than those inoculated with water suspension.

INTEGRATED NEMATODE MANAGEMENT OF BANANA IN CUBA. E. Fernandez, O. Acosta, A. Perez, H. Gandarilla, M. Basterrehea, J. M. Draguiche, N. Olivares, V. Garcia, and M. Lopez, Instituto de Investigaciones de Sanidad Vegetal, Calle 110 #516 entre B y 5 F, Miramar, Playa, Ciudad Habana, Cuba.—The banana parasitic nematodes are among the most damaging pests of the crop around the world, including Cuba where they cause different types of damage. *Radopholus similis, Meloidogyne* spp., and *Pratylenchus coffeae* are the main species in Cuban banana plantations, but *Helicotylenchus multicinctus* and *Rotylenchulus reniformis* occasionally appear in some places. An integrated nematode management system (INMS) was designed using basic studies on biological and ecological characteristics of the main species, the assessment of crop losses, and the evaluation of different control methods such as quarantine, physical, chemical and biological control and use of some cultural practices. The fungus *Paecilomyces lilacinus* was employed for the first time in Cuba. The INMS showed good efficacy in more than 50% of Cuban provinces and also has been included at conferences, field demonstrations, and distribution of a bulletin about nematodes. This work has shown the possibilities of controlling nematodes in banana without massive use of pesticides, and optimizing non-chemical approaches to control including biological agents. The same team of Cuban nematologists are working in other INMS.

THE CURRENT STATUS OF *HETERODERA GLYCINES* ICHINOHE ON SOYBEAN IN BRAZIL. S. Ferraz, and L. A. C. do Valle, Universidade Federal de Viçosa, Departamento de Fitopatologia, 36571-000, Viçosa, MG, Brazil.—Brazil ranks second in soybean production in the world. In a total area of 12 million ha, 25 million tons were produced in 1994/95. *Heterodera glycines* was found in Brazil during the soybean growing season of 1991/92. Initially detected in 4 States (Mato Grosso, Mato Grosso do Sul, Goiás, and Minas Gerais), it was later found in the States of São Paulo, Rio Grande do Sul, and Paraná, comprising around 50 counties. It has been estimated that more than one million ha have already been infested and the losses up to now could be higher than 150 million dollars. More than 250 soybean lines and cultivars were evaluated for resistance and only the cv. Ipagro 21, adapted to south-

ern areas of Brazil, was found resistant. Breeding programs are underway and seed of resistant varieties will be available to farmers in the next 3 years. Six races of *H. glycines* have already been found (2, 3, 4, 5, 10, and 14), race 3 being considered the most widespread. The only efficient method of control now in use has been 2-year crop rotation with non-host plants, mainly corn. Velvet bean (*Mucuna aterrima*) has been evaluated in crop rotation schemes and the results are very promising. This plant stimulates egg hatching, when compared with soybean. When inoculated with 4 000 eggs of *H. glycines*, no females were found up to 45 days later, even though juveniles and males were found inside the roots.

PHYLOGENETIC RELATIONSHIPS OF "GOETTINGIANA GROUP" HETERODERID CYST NEMATODES, BASED ON RIBOSOMAL DNA SEQUENCES. V. R. Ferris, J. M. Ferris, and J. Faghihi, Department of Entomology, Purdue University, West Lafayette, Indiana 47907-1158, U.S.A.—Sequence data were compared for ribosomal DNA (rDNA) of U.S. isolates of 3 taxa usually assigned to the "Goettingiana group" of plant-parasitic cyst nematodes in the genus *Heterodera*. The rDNA sequences included the complete transcribed spacer regions, ITS1 and ITS2, the 5.8S rRNA gene, and small portions of the 18S and 28S genes. The taxa used were pea cyst nematode (PEA), *H. goettingiana*, from WA; the cabbage cyst nematode (CAB), *H. cruciferae*, from CA; and the carrot cyst nematode (CAR), *H. carotae*, from MI. PEA and CAB were 94% similar in their rDNA sequence, but each was only about 70% similar to CAR (which was 98% similar to members of the Schachtii group of *Heterodera*). In phylogenetic analyses of the rDNA data, PEA and CAB always clustered together, whereas CAR clustered with the Schachtii group taxa. The PEA + CAB cluster appeared to be only distantly related phylogenetically to other heteroderids for which similar data were available. Depending on the particular outgroup used for the analysis, the PEA + CAB cluster grouped most closely with either a *Globodera/Cactodera* group, or with a *Heterodera* group (comprised of Schachtii group species plus *Bidera* species).

***IN VITRO* EFFECTS OF CYST-ASSOCIATED BACTERIA ON GROWTH OF THE FUNGAL HYPHOMYCETE (ARF 18), A BIOCONTROL AGENT FOR *HETERODERA GLYCINES*. T. K. Field, R. D. Riggs, and R. T. Robbins, Department of Plant Pathology, University of Arkansas, Fayetteville, Arkansas 72701, U.S.A.**—Isolates A and B of ARF 18 have been shown to parasitize eggs and juveniles of the soybean cyst nematode (SCN), *Heterodera glycines*, in greenhouse and field plot soil experiments. Over 130 bacteria, isolated from cysts extracted from various native soils in Arkansas, were tested *in vitro* for their ability to affect the growth of ARF 18. Each bacterium was seeded on agar containing a 1:1 mixture of tryptic soy agar (TSA) and potato dextrose agar (PDA)/2 in Petri dishes. A 3-mm cube of each fungal isolate was placed on each bacterial lawn and incubated in the dark at 28°C for 5-7 days. Each bacterial and fungal isolate was rated on growth and characterized by having normal growth (+), reduced growth (+/-), or no growth (-). Zones of inhibition, if present, were measured in mm. Each experiment was replicated 3-5 times. Results indicated several bacteria grew successfully in the presence of the fungus. Many of the bacterial isolates inhibited the growth of one or both isolates of ARF 18. This may negatively effect control of SCN by ARF 18. At least two bacteria increased the growth of both fungal isolates when compared to control dishes containing only fungus. Bacteria that stimulate growth of ARF 18, in conjunction with fungal field applications, may increase biocontrol effectiveness of SCN and therefore warrant further investigation.

CHARACTERIZATION AND PRELIMINARY STUDY OF THE DIVERSITY OF SYMBIOTIC BACTERIA ASSOCIATED WITH TROPICAL ENTOMOPATHOGENIC NEMATODES. M. Fischer,[1] H. Mauléon,[2] and N. Boemare,[1] Université Montpellier II, URA n°1184, INRA-CNRS, Laboratoire de Pathologie comparée, C.P. 101, Place Eugène Bataillon, 34095 Montpellier Cedex 05, France,[1] and INRA, Unité de Recherche en Production Végétale, Laboratoire de Nématologie, B.P.515, 97165 Pointe à Pitre Cedex, Guadeloupe (FWI).[2]—Symbiotic bacteria from 6 *Steinernema*, and 11 *Heterorhabditis* entomopathogenic nematodes collected around the Caribbean basin were isolated by the hang-

ing drop method. Two ways of characterization have been used: a phenotypic method including 177 morphological, biochemical, and physiological characters, and a genotypic method using RFLP of the 16S rRNA genes. The amplified products were digested by 4 restriction enzymes. Several restricted patterns were obtained. A software program allows us to construct a dendrogram by using the unweighted pair group method (UPGMA). This method has discriminated, among our isolates, the 2 bacterial genera *Xenorhabdus* and *Photorhabdus* (respectively associated with *Steinernema* and *Heterorhabditis* nematodes), which were additionally recognized by their phenotypic patterns. Two isolates (CUB19 et FL1) show a genotypic identity with the reference strains belonging to the species *X. nematophilus* and *X. bovienii*. Since JAM26 is out of the dendrogram, this strain is considered an atypical strain and further investigations are required. The most interesting result of this study is the close relatedness between taxonomic structure of the bacterial isolates and their associated nematodes. A phenomenon of co-speciation between these organisms is confirmed.

POTATO CYST NEMATODE DIAGNOSTICS USING ALLELE SPECIFIC AMPLIFICATION. C. C. Fleming,[1] S. J. Turner,[1] V. Mulholland,[2] K. J. O'Donnell,[2] and T. O. Powers,[3] Department of Agriculture for N. Ireland, Newforge Lane, Belfast BT9 5PX, U.K.,[1] SASA, East Craigs, Edinburgh EH12 8NJ, U.K.,[2] and Department of Plant Pathology, University of Nebraska, Lincoln, Nebraska, U.S.A.[3]—
Globodera rostochiensis and *G. pallida* continue to be targets for phytosanitory and advisory programmes in many parts of the world. Rapid and accurate diagnostics are crucial to the management of these important potato pests. A polymerase chain reaction (PCR) technique was developed based on allele specific amplification (ASA) enabling identification of these species. Ribosomal ITS1 and 5.8s DNA sequences were obtained using PCN isolates from South America and Europe and a species specific ITS1 primer designed for each species. Each of these primers mismatches with the other species at the 3' end of ITS1. A third "universal" primer, which binds to both species is located downstream from the ITS1 primers in the 5.8s gene. Amplification of *G. pallida* extracts result in a 391 base pair (bp) product, while *G. rostochiensis* produces a 238bp product. Species mixtures will produce both PCR products. Because ASA uses all 3 primers in a single PCR reaction tube, this procedure incorporates an internal control, as at least one PCR product should always be amplified. ASA is rapid (a result is obtained in less than 3 hrs), very sensitive, and more economical than other PCR based diagnostic tests.

FATTY ACID COMPOSITION OF LIPIDS OF BACTERIAL ASSOCIATES OF ENTOMOPATHOGENIC NEMATODES. E. Fodor,[1] E. Szállás,[2] Z. Kiss,[2] A. Fodor,[2] D. J. Chitwood,[3] and T. Farkas,[1] Institute of Biochemistry, Biological Research Centre, P.O. Box 521, H-6701Szeged, Hungary,[1] Institute of Genetics, Eötvös Loránd University, Múzeum krt. 4/A, H-1088 Budapest, Hungary,[2] and USDA ARS, Nematology Laboratory, Building 011A, BARC-West, Beltsville, Maryland 20705-2350, U.S.A.[3]—
The entomopathogenic nematodes, *Heterorhabditis* spp. and *Steinernema carpocapsae*, contain the insect-killing bacterial symbionts, *Photorhabdus luminescens* and *Xenorhabdus nematophilus*, respectively. Primary and secondary phase variants of *P. luminescens* Hm and *X. nematophilus* N2-4 were grown at 18°C and 28°C from 24 to 96 hrs. At each temperature and culture period, the fatty acid compositions were determined by gas-liquid chromatography. The proportions of the fatty acids 16:1 (carbon atoms: number of double bonds) and 18:1 generally were higher in primary phase variants of *P. luminescens* grown at 18°C than at 28°C. Prolonged culture at 18°C caused the level of 18:1 to fall and reach that observed at 28°C. The ratio of saturated to unsaturated fatty acids rose with prolonged culture times in both phase variants of each species. When grown at 18°C, the proportion of 16:1 in *X. nematophilus* was lower than in *P. luminescens*; the patterns of temperature-induced changes were similar in each species. *X. nematophilus* contained a greater percentage of short-chain fatty acids (i.e., chain length < 14.0) than *P. luminescens*. The results reflect substantial differences in lipid metabolism between the 2 bacterial symbiotes.

GENOTYPING POTATO CYST NEMATODES (PCN): A SEARCH AFTER THE INITIAL INTRODUCTIONS. R. T. Folkertsma,[1] J. N. A. M. Rouppe van der Voort,[1] M. Armstrong,[2] B. Harrower,[2] M. Thiéry,[3] M. J. Morena da Cunha,[4] A. Fullaondo,[5] J. Helder,[1] M. S. Phillips,[2] V. Blok,[2] D. L. Trudgill,[2] M. Bossis,[3] D. Mugniery,[3] M. S. A. de Santos,[4] I. M. O. Abrantes,[4] E. Ritter,[5] A. Salazar,[5] and F. J. Gommers,[1] Department of Nematology, Wageningen Agricultural University, P.O. Box 8123, 6700 ES Wageningen, The Netherlands,[1] Scottish Crop Research Institute, Invergrowie, Dundee DD2 5DA, Scotland,[2] Laboratoire de Recherche de la Chair de Zoologie, INRA, Le Rheu 55650, France,[3] Departamentado de Zoologia da Universidade de Coimbra, 3000 Coimbra, Portugal,[4] and Centro de Investigacion y Mejora Agraria, Apartado 46, 01080 Vitoria, Spain.[5]—The genetic variation of populations of PCN in Europe is a consequence of the genetic structure of the introductions from South America and subsequent genetic drift and gene flow. Molecular studies, to identify these introductions, together with the assessment of virulence will allow a better targeting of resistance breeding and the improvement of integrated control strategies. For *G. rostochiensis,* a consensus is found between the molecular clustering of populations (using 2DGE, RAPD's, AFLP's and variations in mtDNA and rDNA) and a pathotype scheme. With *G. pallida* populations, the molecular clustering and the pathotype designation were incongruous. For reliable discrimination of virulence groups, molecular and virulence data should be integrated. The risks of introducing genes for (a)virulence into European PCN from other round cyst nematodes are also being evaluated. Viable hybrids were obtained between *G. pallida* and *G. mexicana* with the possibility of novel combinations of (a)virulence genes.

COLORED MULCHES AFFECT ROOT-KNOT OF TOBACCO. B. A. Fortnum, J. Rideout, and D. Decoteau, Clemson University, Pee Dee Research and Education Center, Florence, South Carolina 29501-9603, U.S.A.—The effect of different colored polyethylene mulches on quantity and spectra of reflected light, plant morphology, and root-knot disease was studied in flue-cured tobacco (1994 and 1995). Tobacco transplants were planted into field plots treated with chlorpyrifos, fenamiphos, 1,3-dichloropropene + chloropicrin (C17) or methyl bromide (Mbr) and grown over white, red, or black mulch or noncovered soil. Experimental design was a RCB, 2 factor factorial with main effects as mulch color and subplot effects as nematicides. The field site was naturally infested with *Meloidogyne arenaria.* White mulch reflected more total light, more blue light and a lower far-red to red ratio than red mulch, whereas black mulch reflected less than five percent of any color. Soil temperatures were warmer under black and red than under white. Mulch color and nematicide application altered stem, root, and leaf weights ($P \leq 0.01$). A mulch color × nematicide interaction was not observed. Plants grown over red mulch had greater leaf weights than plants grown over black mulch or non mulched soil ($P \leq 0.05$). C17 or Mbr reduced root-galling ($P \leq 0.05$) when compared to an untreated control.

HOST SUITABILITY OF SOUTH AFRICAN SOYBEAN CULTIVARS TO ROOT-KNOT NEMATODES. H. Fourie, and A. H. McDonald, Grain Crops Institute, Agricultural Research Council, Private Bag X1251, Potchefstroom, 2520, South Africa.—Thirty commercial soybean cultivars were screened in the greenhouse for host suitability to *Meloidogyne javanica* and *M. incognita* race 2. Based on number of egg masses and number of eggs per plant, the cultivars Highveld Top, Wilge, Knap, Hutcheson, Hennops, and Prima were good hosts for both species. A5409, Zebra, Gazelle, Talana, A7119, SCSI, PAN 494, Nyala, SNK 60, and PAN 812 gave reproduction factor (RF) values lower than one and are consequently regarded as poor hosts for *M. javanica.* The RF values of *M. incognita* race 2 were higher than one on all cultivars screened, with Hutton giving the lowest RF value of 1.1. All cultivars were good hosts for *M. incognita* race 2 based on RF values.

ENDOSPORE ATTACHMENT TO MALES OF *MELOIDOGYNE ARENARIA* BY *PASTEURIA PENETRANS*. **L. G. Freitas,[1] R. M. D. Carneiro,[2] and D. W. Dickson,[1] Entomology and Nematology Department, IFAS, University of Forida, Gainesville, Florida 32611, U.S.A.,[1] and EMBRAPA-CPACT, P.O. Box 403, Pelotas, RS, 96001-970, Brazil.[2]**—The attachment of 7 isolates of *Pasteuria penetrans* from Florida on males of *Meloidogyne arenaria* race 1 was evaluated *in vitro*. The ability of the endospores to attach was first evaluated on freshly-hatched juveniles in an aqueous suspension of 10^6 endospores/ml for 24 hrs. All seven *P. penetrans* isolates were adhesive to the juveniles of *M. arenaria*. The attachment on males was tried using the same procedure as that for juveniles except they were exposed to endospores for 48 hrs and submitted to centrifugation at 6 500g for 5 min. No attachment of endospores was observed on males for any of the isolates. These results suggests that males found in the soil with their bodies filled with endospores of *P. penetrans* were infected as second-stage juveniles.

CORRELATION BETWEEN SALT MARSH VEGETATION AND NEMATODE NUMBERS IN A SOUTH AFRICAN ESTUARY. **J. P. Furstenberg, Department of Zoology, University of Port Elizabeth, Box 1600, Port Elizabeth 6000, South Africa.**—Extensive intertidal salt marshes consisting of five plant species, viz. *Spartina maritima, Triglochin bulbosum, Sarcocornia perennis, Chenolea diffusa,* and *Limonium linifolium*, occur in an open estuary near Port Elizabeth, South Africa. These species are subject to regular inundation of seawater for varying periods. Nine sampling sites were chosen of which eight were characterized by dense marsh beds and zoned distribution of salt marsh vegetation on muddy flats. Site one had no vegetation; sites two to four were dominated by *Spartina* with a varying degree of abundance and sites five to nine were covered by two or more of the remaining four plant species. Total nematode numbers at sites one to four were always below 1 000 per 250 cm^3 sediment sample. Sites five to nine, which contained no *Spartina*, yielded between 20 000 and 70 000 nematodes per sample. All sites were numerically dominated by two nematode species, viz. *Helicotylenchus californicus* and an unknown species of *Tylenchus*. On the average, 34% and 50% of all nematodes in sites one to four were numerically dominated by *Tylenchus* sp. and *H. californicus*, respectively. The most abundant genus at sites five to nine was *Helicotylenchus*, making up on average 81% of total numbers. Second most abundant was *Tylenchus* (14%). Eighty-three percent of the total variation in *Helicotylenchus* numbers at all sites can be explained by a linear regression ($r^2 = 0.83$) of abundance of this species against vegetation cover of *Triglochin* and *Chenolea*. The numbers of *Tylenchus* behaved very similarly to the numbers of *Helicotylenchus*, but at a lower level. A steady decline in numbers occurred from sites seven to nine. Fifty-six percent of the variation in numbers could be explained by linear regression ($r^2 = 0.56$) of abundance of this species against cover of *Triglochin*.

MOLECULAR TAXONOMY OF PLANT-PARASITIC NEMATODES. **N. J. Galway, F. Driver, and J. Curran, Cooperative Research Centre for Plant Science, Australian National University, Canberra, ACT, 2601, Australia.**—To test current hypotheses on evolutionary relationships and address conflicts with the current taxonomy, DNA sequence data was analyzed for key taxa from the order Tylenchida. A 300bp fragment of a nuclear ribosomal RNA gene was amplified using universal primers and sequenced for several Tylenchids, including species of *Pratylenchus, Radopholus, Globodera, Heterodera, Meloidogyne, Helicotylenchus, Rotylenchulus,* and *Ditylenchus*. Secondary structure models were used to assist in the alignment of the sequence. There was considerable sequence variation in this region among *Pratylenchus* species compared to other plant-parasitic nematode genera. A phylogenetic analysis of sequence data resolved the populations of *Pratylenchus* into major groupings in concordance with published taxonomies of this genus. The use of molecular data in phylogenetic reconstructions was discussed.

***STEINERNEMA KRAUSSEI* (STEINER, 1923) (RHABDITA: STEINERNEMATIDAE) FROM SPAIN.** **F. García del Pino, and A. Palomo, Departamento Biología Animal, Facultad de Ciencias, Universidad Autónoma de Barcelona, 08193 Bellatera. Barcelona, Spain.**—*Steinernema kraussei* (Steiner, 1923) was described from larval sawflies, *Cephaleia abietis* (Pamphilidae, Hymenoptera) in Germany. In this study

we present a new strain of *S. kraussei* from Spain. We have isolated this nematode from soil samples ("Galleria trap" method) of a pine-forest of the Catalonian Pyrenees (Spain). The nematodes were propagated in *Galleria mellonella* larvae. Infective juveniles, males, and females of both generations were killed in warm water, fixed and mounted for observations in light and scanning electron microscopy. The nematodes had been characterized by morphological studies, and DNA techniques (RFLP analysis). The morphological characters of this Spanish strain have been discussed. Finally, the isolation of this new strain of *S. kraussei* from Spain extends the geographical range of this species to southern Europe.

PRESENCE OF *STEINERNEMA BICORNUTUM* (TALLOSI, PETER AND EHLERS, 1995) (RHABDITIDA: STEINERNEMATIDAE) IN THE CANARY ISLANDS (SPAIN). F. García del Pino, and A. Palomo, Departamento Biología Animal, Facultad de Ciencias, Universidad Autónoma de Barcelona, 08193 Bellaterra, Barcelona, Spain.—*Steinernema bicornutum* has been recently described from Yugoslavian soils. The presence of a double horn-like papillae on the lip region of the dauer juveniles of this species is a distinct and unusual character which distinguish this species from all other described *Steinernema* spp. Until now, *S. bicornutum* has been reported only from Yugoslavia and Germany. In this study we present the morphometric characters of the dauer juveniles of a population of *S. bicornutum* isolated in the subtropical island of Tenerife (Canary Islands, Spain). The isolation of this new strain of *S. bicornutum* extends the biogeographical range of this species to the Subtropics.

HEAD PATTERNS IN THE HETERODERIDAE AS COMPARED TO THE PATTERNS IN REMAINING TYLENCHOIDEA. E. Geraert, Gent University, Institute of Zoology, K.L. Ledeganckstraat 35, B-9000 Gent, Belgium.—The end-on views of the head can be used to characterize the families within the tylenchids. *Meloidogyne* has a front very similar to those found in the Pratylenchidae, while *Globodera* is similar to those found in Hoplolaimidae. *Heterodera* often shows a fusion of the labial disc with the submedial lip sectors as in Pratylenchidae, but with a different result. Moreover, the inner labial sensilla never open on the front. Therefore, *Heterodera* is considered with *Globodera* and related to the Hoplolaimidae.

MODELLING LIMITS OF *GLOBODERA* SPP. POPULATIONS DYNAMICS. A. Ghariani, and M. M. B'Chir, Institut National Agronomique de Tunisie 43, Av. Charles Nicolle, 1082 Tunis-Mahrajène, Tunisia.—In the southern part of the Mediterranean region, unevenness of some environmental conditions induced a large fluctuation in potato yield and economic importance of cyst nematodes. The main factors explaining yield variability due to nematodes were assessed under field conditions. In an attempt to optimize economic returns from potato cyst nematode control, a predictive model of the population dynamics has been corrected to take into account egg viability and juvenile hatching rates. The validation of this adjusted model was presented and discussed.

THE DEVELOPMENT OF *PASTEURIA PENETRANS* AS AFFECTED BY THE AGE OF SPORES. I. O. Giannakou, Department of Agriculture, University of Reading, Earley Gate P.O. Box 236, Reading RG6 2AT, U.K.– Factors that cause poor levels of spore attachment include unfavorable environmental conditions and incompatibility of spore and nematode cuticle. This poster describes, for the first time, the influence of the age of *Pasteuria penetrans* spores on their ability to attach to the host nematode cuticle, and subsequently to germinate and proliferate within the nematode body. The root-knot nematode, *Meloidogyne javanica* (an isolate from Malawi), was reared in a greenhouse on tomato (*Lycopersicum esculentum* cv. Tiny Tim). The population of *P. penetrans* originated from South Africa (Pp3). A comparison was made between spores of Pp3 prepared in a powdered form from tomato roots and stored in a cupboard at room temperature for 8 years, and a similar preparation that was derived from the same population 5 months previously. Storage significantly reduced the ability of spores to create infection despite the relatively high level of attachment to active *M. javanica* juveniles.

CHARACTERIZATION OF *MELOIDOGYNE* JUVENILE CUTICULAR SURFACE BY USING POLYCLONALS: VARIATIONS BETWEEN AND WITHIN SPECIES. L. Gimeno,[1] M. Fargette,[1] and J. M. S. Forrest,[2] Laboratoire de Nématologie ORSTOM, CIRAD, BP 5035, 34032 Montpellier Cedex 1, France,[1] and SCRI, Invergowrie, DD2 5DA Dundee, U.K.[2]—Two polyclonal sera have been raised towards single egg mass lines of *Meloidogyne, M. javanica* and *M. mayaguensis*, respectively, by inoculating rabbits with J2 of each line. A protocol which enhances the immune reaction was used. These two polyclonals have been used to compare *in situ* labelling of the cuticular surface of live J2 belonging to 60 single egg mass lines of *Meloidogyne*. Some of the lines represent 6 identified species and some lines are as yet unidentified. Based on their immunological responses, surfaces have been characterized for their relationships with *M. javanica* and *M. mayaguensis* - surface types. The labelling pattern of lines belonging to the same species look similar to each other except for *M. arenaria*. In this case, the surface of J2 shows variation in the specific labelling by the 2 polyclonals.

EFFICACY OF DITERA BIOLOGICAL NEMATICIDE FOR ROOT-KNOT NEMATODE SUPPRESSION ON CARROT. P. A. Grau, R. Hopkins, J. D. Radewald, and P. Warrior, Abbott Laboratories, 1401 Sheridan Road, North Chicago, Illinois 60064, U.S.A., and University of California, Riverside, California 92557, U.S.A.—DiTera® Biological Nematicide is produced by fermentation of the fungus, *Myrothecium* spp. We have studied the effect of 3 forms of the product (a technical powder, emulsifiable suspension and granular) for control of root-knot nematode, *Meloidogyne incognita* on carrot. Field trials were conducted in California, U.S.A., within commercial plantings. Rate studies indicated that maximum control is achieved in the range of 12 to 50 kg/ha when applied to the surface of the seed bed, depending on soil type and growing conditions. Applications just prior to germination were better than those prior to, or at the time of seeding, due to the practice of applying large quantities of water by sprinkler irrigation and the mobility of the active ingredient. Earliness in maturity and greater carrot size were noted. Yield parameters including number, weight and quality of carrots show that DiTera can provide commercially acceptable suppression of root-knot nematodes concurrent with economical benefit to the grower. Data from 1994-95 field trials are summarized.

GIANT CELLS IN *ARABIDOPSIS THALIANA* ROOTS EXPRESSING *MELOIDOGYNE* SURFACE ASSOCIATED MOLECULES. M. J. Gravato Nobre,[1] L. Dolan,[2] G. Calder,[2] M. A. McClure,[3] K. G. Davies,[1] N. von Mende,[1] B. Mulligan,[4] and K. Evans,[1] Entomology and Nematology Department, IACR Rothamsted, Hertfordshire, AL5 2JQ, U.K.,[1] Department of Cell Biology, The John Innes Centre, Colney Lane, Norwich NR4 7UH, U.K.,[2] Department of Plant Pathology, University of Arizona, College of Agriculture, Tucson Arizona 85721, U.S.A.,[3] and Department of Life Science, University of Nottingham, Nottingham, NG7 2RD, U.K.[4]—The surface coat of *Meloidogyne incognita* juveniles is shed during their migration in *Arabidopsis thaliana* roots. Antigen deposits, probed with an anti-surface coat monoclonal antibody (MISC), accumulate on plant cell walls in the vicinity of the migratory nematode and are left behind as the juvenile progresses within the root. The pattern of expression of these molecules changes at the feeding site, where the antigen is highly expressed not only on the cell wall ingrowths, but also in the cytoplasm of the giant cells. This resembles some animal parasitic nematode systems, in which surface associated antigens are found in "nurse cells". Moreover, phloem elements transport the antigens from the feeding site to a distal position. The importance of these shed surface materials is discussed in the context of pathogenicity. A variety of immunolabelling methods was used and a technique based on whole-mount roots was developed, providing a new and useful way of examining candidate antigens involved in host-parasite interactions.

STUDY OF MARINER-LIKE TRANSPOSABLE ELEMENTS IN THE ENTOMOPATHOGENIC NEMATODE *HETERORHABDITIS BACTERIOPHORA*. E. Grenier, and M. Abadon, INRA, Laboratoire de Biologie des Invertébrés, BP 2078, 06606 Antibes Cedex, France.—The "mariner" transposable element is a small member of the short inverted terminal repeat class thought to transpose via a

DNA intermediate. Originally described in *Drosophia mauritiana*, it also has been identified in many other insect species and more recently in the planarian *Dugesia tigrina* and the nematode *Caenorhabditis elegans*. Using a polymerase chain reaction strategy, we have been able to detect the presence of "mariner-like" elements (MLE) in the nematode, *Heterohabditis bacteriophora*. These MLE appear to be either deleted forms or full-sized elements of 1279 bp. In a full-sized element, the transposase part codes for 358 amino acids. None of these sequences has an open reading frame encoding for a putative transposase. Interestingly, the *H. bacteriophora* transposase shares more similarities with insect MLE transposase than with the *C. elegans* one. Among the conserved *H. bacteriophora* amino acids, only 14% are typical *C. elegans* amino acids while 28% are typical insect ones. When analyzed with the Genbank data system, the most important similarity has been found with a *Carpelimus* sp. "mariner". The *H. bacteriophora* MLE is 73% similar at the nucleotidic level to this coleopteran MLE, while it is only 52% similar to a *C. elegans* mariner. The distribution of *H. bacteriophora* MLE has been studied by Southern blot analysis. All the tested *H. bacteriophora, H. megidis* and *H. zealandica* isolates contain MLE, while they seem to be absent from *H. indicus* and the *Steinernema* genus. These results, together with the fact that *H. bacteriophora* is an entomoparasitic rhabditid, strongly suggest a case of trans-phyla horizontal transfer.

DEVELOPMENT OF THE FIRST BIOLOGICAL PRODUCT FOR THE SUPPRESSION OF PLANT-PARASITIC NEMATODE POPULATIONS. P. S. Grewal, R. W. Miller, W. R. Martin, and R. Georgis, biosys Inc., Columbia, Maryland 21046, U.S.A.—Control of plant-parasitic nematodes is vital to agriculture. Crop damage caused by plant-parasitic nematodes accounts for approximately 12% of the annual loss of food and fiber production worldwide. Current dissatisfaction with chemical nematicides, due to safety issues, environmental concerns, and loss of availability, has stimulated interest in developing alternative nematode control strategies. We report the development of a biological product based on the use of entomopathogenic nematodes to suppress field populations of plant-parasitic nematodes in professional turfgrass. Numerous field trials and laboratory experiments have shown that entomopathogenic nematodes, *Steinernema carpocapsae, S. riobravis*, and *S. feltiae*, reduce populations of root-knot (*Meloidogyne* spp.), sting (*Belonolaimus* sp.), and ring (*Criconemella* sp.) nematodes well below economic threshold levels. While studies are continuing, the data suggest a number of possible mechanisms of the nematode-nematode interaction.

A THIRD LEVEL OF SPECIFICITY OF ASSOCIATION BETWEEN ENTOMOPATHOGENIC NEMATODES AND BACTERIA. P. S. Grewal, biosys Inc., Columbia, Maryland 21046, U.S.A.—Entomopathogenic nematodes in Steinernematidae possess a mutualistic association with *Xenorhabdus* bacteria (Enterobacteriacae). Each nematode species has a specific natural association with only one *Xenorhabdus* sp. although some *Steinernema* spp. share the same bacteria. The specificity of this association has been postulated to operate at 2 levels: the provision of essential nutrients by bacteria and retention of bacteria within the intestine of the infective juvenile nematodes. With the use of 'novel' nematode-bacteria hybrids, we found a third level of specificity of this association. We discovered that infective juvenile nematodes exit from 'dauer' phase only in the presence of their natural symbiotic bacteria. This recognition of bacteria by the infective juveniles is highly specific and has important ecological implications. For example, the symbiont specific exit from 'dauer' phase would prevent wastage of the infective stages in the rhizosphere where some soil bacteria may be nutritionally suitable for the development of nematodes. Results suggest that the specific recognition of bacteria by infective juvenile nematodes may be the most important factor governing bacteria-nematode specificity.

STUDIES OF STEM NEMATODE (*DITYLENCHUS DIPSACI*) POPULATION DEVELOPMENT IN WHITE CLOVER (*TRIFOLIUM REPENS*) STOLONS. G. S. Griffith, R. Cook, and K. A. Mizen, Institute of Grassland and Environmental Research, Aberystwyth, Dyfed SY23 3EB, U.K.—Rates of development of the white clover (*Trifolium repens*) stem nematode, *Ditylenchus dipsaci*, were found to be a

linear function of temperature over the range 0 to 25°C. The generation time was ca. 380 accumulated day degrees above a minimal develop-mental temperature of ca. 3°C (DD_3). Rates of egg-laying also were temperature dependent, with ca. 0.3 eggs being laid per female per DD_3. Within stolons of inoculated plants, developing nematode populations were confined to limited volumes of host tissue (the infestation locus). The linear dimensions of an infestation locus increased at a rate that was directly proportional to temperature and independent of the number of nematodes it contained (ca. 10 $\mu m.DD_3^{-1}$ longitudinally and ca. 5 $\mu m.DD_3^{-1}$ radially). At the same time, the nematode population within loci increased exponentially leading to a build-up of population pressure. At high population density, rates of egg production by adults declined and fourth stage juveniles (J4) did not molt. In this way, population growth was brought in line with the rate of locus expansion and the proportion of J4 in the population gradually increased. Symptom development, including internode shortening, was shown to occur only when infestation loci within 20 mm of terminal meristems developed population levels exceeding 100 nematodes. Because internode growth is limited by low light levels during winter and early spring, increases in mean winter/spring temperatures will lead to more widespread symptom development (damage) in infested white clover swards. A thermal time simulation model of the white clover/stem nematode host/pest system has been developed. The model realistically simulates stem nematode population development within individual infestation loci.

A FLUORESCENT *IN SITU* HYBRIDISATION STUDY ON THE NUCLEAR rDNA ARRAYS IN *GLOBODERA PALLIDA*. M. E. S. Grisi,[1,2] S. Bauwens,[3] P. R. Burrows,[1] and R. N.Perry,[1] Entomology and Nematology Department, IACR-Rothamsted, Hertfordshire, AL5 2JQ, U.K.,[1] Federal University of Paraiba, Brazil,[2] and Genetic Laboratory, Faculty of Sciences, University of Gent 35, 900 Gent, Belgium.[3]—As part of a project to study the genome of the potato cyst nematode, *G. pallida*, the structure and organization of the nematode nuclear rDNA was examined. Fluorescence *in situ* hybridization was used to determine the number of chromosomal locations of the rRNA cistron arrays. A *G. pallida* rDNA fragment probe, labelled with either biotin or digoxigenin, was hybridized to slide preparations of whole mount gonads dissected from young *G. pallida* females. The resulting hybridizations were analyzed using a Confocal Scanning Laser microscope. The results of this analysis showed that *G. pallida* has just one chromosomal location for the rDNA repeats. This is in line with many other eukaryotes and the great majority of the *Caenorhabditis elegans* strains examined.

EFFICACY OF TWO FORMULATIONS OF ABG-9008, A BIOLOGICAL NEMATICIDE, ON SUGARBEETS GROWN IN SOIL INFESTED WITH *HETERODERA SCHACHTII*. S. L. Hafez, G. C. Weiser, P. A. Grau, and P. Warrior, University of Idaho, Parma, Idaho 83660, U.S.A, and Abbott Laboratories, 6131 RFD Oakwood Road, Long Grove, Illinois 60047, U.S.A.—Commercial sugarbeet (*Beta vulgaris*) production has been terminated in many areas of the U.S.A because of crop damage or failure due to *Heterodera schachtii*. Management with chemical nematicides is limited because of environmental risk. This study tested the efficacy of 2 formulations of a biological, fungal-derived nematicide, ABG-9008, in reducing nematode reproduction under greenhouse conditions at rates between 0 and 224 kg/ha and dilutions of a single rate from 2:1 to 16:1 (H_2O:material v/v). Sugarbeet seeds ('WS PM-9') were planted in pots containing 500 cm of nematode infested soil (average of 3 *H. schachtii* eggs and larvae per cm^3) with 6 replications. No obvious influence of the nematicide was noticed with regard to overall plant growth or vigor. The number of cysts from subsequent generations of the initial inoculum in the soil-root systems after 45 days of growth decreased by 57% compared to the untreated control with application of 224 kg/ha oil-based formulation. Similarly, 56 and 112 kg/ha applications of a dry powder formulation reduced final cyst populations by 16 and 33%, respectively. Linear reductions were observed in both experiments over the rates tested. Dilutions of the oil-based formulation from 2:1 to 8:1 reduced final cyst populations by an average of 18% compared with the control. Cyst numbers were reduced by only 6% in soil-root systems receiving the 16:1 dilution, indicating that the most effective working dilution probably lies between 2:1 and 8:1.

***LONGIDORUS* AND *XIPHINEMA* SPP. WITH THREE JUVENILE DEVELOPMENTAL STAGES. J. M. Halbrendt,[1] D. J. F. Brown,[2] R. T. Robbins,[3] and T. C. Vrain,[4] Pennsylvania State University, Fruit Research Laboratory, P.O. Box 309, Biglerville, Pennsylvania 17307-0309, U.S.A.,[1] Scottish Crop Research Institute, Invergowrie, Dundee, DD2 5DA, U.K.,[2] Department of Plant Pathology, University of Arkansas, Fayetteville, Arkansas 72701, U.S.A.[3], Agriculture and Agri-Food Canada, Summerland Research Centre, Summerland, British Columbia V0H 1Z0, Canada.[4]**—It is generally assumed that the Longidoridae, in common with other Nematoda, have 4 juvenile developmental stages (JDS) but several authors provide data which distinguish only 3 JDS. In the genus *Longidorus*, 8 species present in Afro-Asia (AA) and New Zealand have only 3 JDS and 5 of these species have ancestral characters such as a posteriorly situated guide ring (near mid-odontostyle) and a swollen odontophore base. Within the genus *Xiphinema*, members of the *X. americanum* group present in North America (NA), *X. chambersi* (NA and in Japan where it was probably introduced from NA) and *X. hygrophilum* (West Africa (WA)) have only 3 JDS. The anterior genital branch is absent in *X. chambersi* and is reduced in *X. hygrophilum*, also both species have elongate tails, an ancestral character. The *X. americanum* group has a world-wide distribution with almost half the species reported from NA and is relatively homogeneous, its members being distinguished only by minor morphological and morphometrical differences. The patterns of geographical distribution and presence of ancestral characters in several of these species suggests that development of 3 JDS occurred early in the evolution of *Longidorus*, in AA possibly during the early Jurassic period, and in Xiphinema in WA and NA possibly during the late Jurassic period.

NEMATODE INTERACTIONS WITH ENDOPHYTES I: ISOLATION OF ENDOPHYTIC MICROORGANISMS. J. Hallmann, A. Quadt-Hallmann, J. W. Kloepper, and R. Rodríguez-Kábana, Department of Plant Pathology, Auburn University, Auburn, Alabama 36849, U.S.A.—Endoparasitic nematodes are the most devastating plant-parasitic nematode group causing severe economical losses every year. Biological control of these organisms is often difficult due to their protected occurrence inside the root tissue. The antagonistic potential of soil and rhizosphere microorganisms is usually limited to egg parasitism or inhibition of juvenile attraction and penetration. Endophytic microorganisms colonizing the root tissue may have the potential to control endoparasitic nematodes after they enter roots. Isolation of endophytic microorganisms is sometimes complicated, and sterility checks must be included to avoid isolating microorganisms from the root surface. This report compared isolation following surface disinfestation with a pressure bomb technique for recovering endophytic bacteria. With surface disinfestation we achieved the best results for cotton, tomato, bean and cucumber by incubating 2-4 week-old roots for 60 sec in 1.05% NaOCl followed by 3 rinses in 0.02 N sterile potassium phosphate buffer (PB) (pH 7.0). The roots were imprinted on tryptic soy agar (TSA) as a sterility check. If bacterial growth occurred within 48 hrs the sample was discarded. In addition, cytological and immunological studies of embedded surface-disinfested cotton roots suggested that this method completely excluded rhizoplane microorganisms. Roots were weighed and triturated in 5 times (w/v) PB, followed by serial dilution of the triturate and plating on TSA. For the pressure bomb technique, freshly harvested roots were placed into a pressure chamber with the stem base sticking out. Pressure (compressed nitrogen) was released up to 15 bar, which is equivalent to a medium level water stress. Plant sap appearing at the stem cutting was collected, measured and plated on TSA. Overall, surface disinfestation resulted in higher numbers of total cfu/g root when compared with the pressure bomb technique. Nevertheless, richness and diversity of endophyte species was greater when samples were extracted with the pressure bomb technique.

NEMATODE INTERACTIONS WITH ENDOPHYTES III: SCREENING DIFFERENT SOURCES OF ENDOPHYTIC BACTERIA FOR CONTROL OF *MELOIDOGYNE INCOGNITA* ON COTTON AND CUCUMBER. J. Hallmann, J. W. Kloepper, and R. Rodríguez-Kábana, Department of Plant Pathology, Auburn University, Auburn, Alabama 36849, U.S.A.—Sedentary endoparasites like cyst (*Heterodera* spp., *Globodera* spp.) and root-knot nematodes (*Meloidogyne* spp.) are well protected by the

surrounding plant tissue and are therefore difficult to control. Endophytic bacteria with anti-nematode potential might increase overall nematode control, especially when used in combination with other biocontrol methods. We tested 3 sources of endophytic bacteria: i) biocontrol agents of fungal, bacterial and viral diseases including inducers of systemic resistance, ii) endophytes isolated from cotton grown in nematode-suppressive and conducive soil, and iii) endophytes with chitinolytic activity. These endophytic bacteria were applied as seed treatments on cotton and cucumber. Seed was planted in heat-treated sand, and the experiment was set up under greenhouse conditions. Ten days after planting, 1 500 J2 of *M. incognita* were inoculated per plant, and the experiment was terminated 6 weeks after planting. Plant growth was recorded, number of galls and egg masses were counted, and the gall index was determined on a scale from 0-10 (0 = no galls, 10 = severe galling). Seventy-two strains were tested on both cotton and cucumber. All strains were tested for hydrolytic enzymes, i.e. chitinase, collagenase, and protease. In addition, the bacterial culture filtrates were screened for *in vitro* inhibition of *M. incognita* juveniles. The results indicate that the percentage of endophytes with biocontrol potential depended on sources of isolation. Biocontrol activity was not always associated with *in vitro* inhibition or hydrolytic enzyme activity.

NEMATODE INTERACTIONS WITH ENDOPHYTES IV: EFFECT OF NEMATODE-SUPPRESSIVE SOIL ON BACTERIAL DIVERSITY OF COTTON ENDOPHYTES. J. Hallmann, R. Rodríguez-Kábana, and J. W. Kloepper, Department of Plant Pathology, Auburn University, Auburn, Alabama 36849, U.S.A.—Nematode-suppressive soil is often characterized by a specific microbial community antagonistic to plant-parasitic nematodes. We hypothesize that the endophytic community of the host plant is also modified when grown in suppressive soil and therefore partly contributes to the observed suppressiveness. To test this hypothesis, we mixed 1% chitin into field soil to increase suppressiveness. Chitin-treated soil was allowed to decompose for 3 weeks prior to planting with cotton cv. 'Rowden'. The cotton plants were harvested 5 weeks after planting. Plant growth as well as nematode suppressiveness was examined. Additionally, at days 0, 21, and 54, the following parameters were assessed: soil-pH, nematode populations, total populations of bacteria, fungi, chitinolytic microorganisms, and bacterial diversity within the rhizosphere. At the last sampling 54 days after chitin amendment, the endophytic flora of surface disinfested cotton roots were evaluated for total populations of bacteria, fungi and chitinolytic microorganisms as well as bacterial diversity. The soil treatment with 1% chitin was sufficient for complete suppression of plant-parasitic nematodes.The chitin treatment did not affect stem weight, but for cotton grown in chitin-treated soil, root weight was reduced. Total numbers of bacteria increased in chitin-treated soil from 2.5×10^6 to 2.0×10^8 cfu/g within 3 weeks and dropped to 1.1×10^7 cfu/g at day 54. In contrast, fungal populations of chitin-treated soil continuously increased, starting at 5.5×10^3, and reaching 3.9×10^4 cfu/g after 21 days and 1.7×10^6 cfu/g after 54 days, respectively. Total populations of chitinolytic organisms in chitin-treated soil increased from 1.9×10^3 to 1.7×10^7 within 21 days and dropped to 3.7×10^6 cfu/g at 54 days after treatment. The chitin treatment almost eliminated *Arthrobacter* spp. and markedly reduced *Bacillus* spp. In contrast, chitin amendment favored the genera *Aureobacterium, Clavibacter, Corynebacterium,* and *Rathayibacter* and in particular promoted *Burkholderia cepacia.* Focusing on bacterial endophytes, cotton plants grown in chitin-treated soil were dominated by *Burkholderia* spp. (71% of identified bacteria). *Phyllobacterium* (69%) was the dominant genus isolated from cotton grown in nontreated soil. The results demonstrate that suppressive soil influences the indigenous endophytic microflora which, therefore, needs to be considered in biocontrol approaches.

MOLECULAR ANALYSIS OF DIFFERENT *MELOIDOGYNE INCOGNITA* POPULATIONS, ISOLATED FROM EGYPT, BY USING POLYMERASE CHAIN REACTION METHOD. S. A. Haroon, and P. Karlovsky, Cairo University-Branch (Fayoum), Plant Protection Department, Cairo, Egypt, and Universität Hohenheim (360), Otto-Sander-Str.5, D-70599, Stuttgart, Germany.—Samples of *Meloidogyne incognita* were isolated from 13 fields with 3 different crop plants in Egypt. For a comparison, two

samples of German origin were included in the analysis. DNA was analyzed by PCR with primers No. 28, 29, 53, and 58. Each isolate produced 3 to 10 scorable bands with each primer. Amplicons produced by randomly primed PCR were rather long, particularly with Primer No. 29; 90% of scorable bands migrated slower than a standard fragment of 1 000 bp. The difference among banding patterns of different populations demonstrates a high degree of genetic heterogeneity. Both German populations produced identical patterns with all primers tested. They did not reveal any similarity with Egyptian populations. For the quantitative evaluation of the results, the values of genetic similarity were calculated using all scorable bands and all 4 primers. The distance matrix was constructed and cluster analysis made using the UPGMA method. In order to address the question of the correlation between geographical distance and genetic similarity, the matrix of genetic distances was compared with geographical distances using a statistical method. The lack of correlation between the geographical distance and genetic similarity was a surprising and most significant finding of our study. Although there is much genetic diversity between populations, some similarities were found. Similar band patterns shared even by very remote populations (e.g. Nos. 9, 10, 11, and 12) demonstrate that successful genotypes can spread fast over long distances, probably due to farmer activities (e.g. use of contaminated fertilizer, seedlings, or seed). Both the large genetic variability present in *M. incognita* in Egypt and the ability of genotypes to spread over long distances have implications for plant protection policy. Pathogen species with large genetic polymorphism are expected to possess a potential to overcome resistances integrated into new varieties by breeding, to develop resistances against nematicides and also to produce races that overcome modern crop protection strategies based on plants with repellent or attractant effects. Further work will focus on the question of genetic diversity within *M. incognita* populations.

ORYZACYSTATIN EXPRESSION IN TRANSGENIC PLANTS: AN ALTERNATIVE TO NEMATICIDES. P. Havstad, A. Larson, J. Randall, J. Gilroy, L. Higgins, D. Sutton, and J. D. Kemp, New Mexico State University, PGEL, Las Cruces, New Mexico 88003, U.S.A.—A protease inhibitor gene, oryzacystatin-I (Oc-I), was isolated from rice genomic DNA and placed as both the gene (containing its intron) and an engineered form (minus intron) behind the 35S constitutive promoter. Transgenic plants were developed to determine if this gene would provide protection against plant pests, including nematodes. Expression of both forms of Oc-I in tobacco plants was tested via Northern assays. While transcription of the "plus intron" form resulted in RNA in which the intron sequence was not processed out, the "minus-intron" form was transcribed into the expected size of approximately 500 bp. Enzyme activity in the leaf tissue of plants transformed with the "minus-intron" form was assayed using the BANA-substrate technique. Several independent transformants showed protease inhibitor activity. Studies to determine expression in roots and efficacy against nematodes are in progress. Additional oryzacystatin constructs with tissue and stimulus specific promoters are also being tested for effectiveness in plants against parasitic nematodes.

***MELOIDOGYNE FALLAX* AND *MELOIDOGYNE CHITWOODI*: EFFECTS OF TRAP CROPS GROWN AS A ROTATIONAL SET ASIDE. W. Heijbroek, and L. J. P. C. Swinkels, Institute of Sugar Beet Research (IRS), 4600 AA Bergen op Zoom, The Netherlands.**—Resistant cruciferous green manure crops can suppress the population of *Heterodera schachtii* and *H. trifolii* f.sp.*betae* considerably, when drilled after mid-May and grown as a rotational set aside. However in some sugar beet growing areas on sandy and light silty soil, *Meloidogyne fallax* and *M. chitwoodi* constitute an increasing problem that can not be controlled by nematicides sufficiently. In particular, fodder radish and to a lesser extent also white mustard varieties show differences in susceptibility to both root-knot nematode species. In field trials, a number of existing cultivars and resistant selections and some other potential trap crops were tested for their effect on *M. fallax* and *M. chitwoodi* as compared to bare fallow. Some of these cultivars caused a reduction in the population of both nematodes which was comparable to a reduction under bare fallow (circa 85-95%). These effects could be confirmed by growing a sugar-beet test

crop the following year. However, fodder radish selected for resistance against *M. hapla* did not suppress the population development of *M. hapla, M. fallax,* or *M. chitwoodi* more than some existing cultivars. This was caused by the fact that in a greenhouse test, a maximum of 40% of the plant population showed resistance. Differences in reactions between *M. fallax* and *M. chitwoodi* were discussed. New selections showing combined resistance against *H. schachtii, M. hapla,* and *M. chitwoodi* were tested in greenhouse trials.

USE OF A HIGH PRESSURE WATER-JET METHOD FOR EXTRACTING *GLOBODERA* CYSTS. G. Hendrickx, and M. Moens, Centrum voor Landbouwkundig Onderzoek, 9820 Merelbeke, Belgium.—A pressurized fine jet of water injected for 5 min into a beaker (1 litre) filled with 200 g of air-dried soil disperses and agitates the soil. As a result, the cysts float on the soil suspension. The water jet is generated by an injector with a 0.4 mm aperture and a pressure of 10 kg/cm^2 which produces a flow rate of 500 ml/min. The excess water, along with the cysts and the light soil and organic fraction, is drained off and caught on a set of sieves (850 µm and 250 µm). At the end of the extraction, the collar of the beaker and the remaining floating fraction are cleaned with a sprayer attached to the system. The total consumption of water for one extraction is about 3 L. A series of 5 units of this device delivers an extract every min. The device is easy to build since materials are available in the laboratory, with the exception of the pressure creating equipment. This extraction method is simple and needs little water and few human laborers. Extractions are reproducible and reliable, and their efficiency is even higher than the Fenwick extraction method.

ENZYMATIC CHARACTERIZATION AND REPRODUCTIVE FITNESS ON COFFEE OF ROOT-KNOT NEMATODE POPULATIONS FROM CENTRAL AMERICA. A. Hernandez, M. Fargette, V. Molinier, H. Ramenason, B. Decazy, and J. L. Sarah. Laboratoire de nématologie, CIRAD-ORSTOM, BP 5035, 34032 Montpellier, France.—Thirty-three populations of *Meloidogyne* spp. collected in Central America have been characterized by their electrophoretic isozyme patterns:Esterase (EST), Malate Deshydrogenase (MDH), Superoxyde Dismutase (SOD), and Glutamate Oxaloacetate Transaminase (GOT). *M. exigua* was identified in 3 populations from Honduras, 4 from Nicaragua, and 4 from Costa Rica; *M. arenaria* in 3 populations from El Salvador; and an atypical *M. incognita* (EST 'S1') in 8 populations from Costa Rica. *M. javanica* was found once in Costa Rica, and *M. mayaguensis* once in Guatemala; 6 other populations from Guatemala showed the 'F1' Esterase phenotype. Two new types have been encountered, the 'Salvador' type, and the 'Costa Rica' type, each one found once in their respective country. Thirteen of these populations, representing every species or phenotype, have been studied for their pathogenicity on *Coffea arabica* seedlings. None of the populations of *M. javanica, M. incognita* (EST 'S1'), and *M. mayaguensis* were able to develop on Catuai and ET-15 varieties. The Ethiopian variety ET-15 was resistant to *Meloidogyne* sp. (EST 'F1') the most widespread root-knot nematode in Guatemala coffee plantations and to the 'Costa Rica' type. In contrast, this variety was not resistant to *M. arenaria* and *M. exigua.* The 'Salvador' type and two populations of *M. exigua* from Costa Rica multiplied on ET-15 roots at significantly higher rates than any other population.

THE USE OF TANNINS FOR CONTROL OF ROOT-KNOT NEMATODES. T. E. Hewlett, and D. W. Dickson, Department of Entomology and Nematology, University of Florida, Gainesville, Florida 32611, U.S.A.—Tannic acid was recently identified in laboratory tests as an attractant of root-knot nematodes. Tests were conducted to determine the efficacy of tannic acid on *Meloidogyne arenaria* race 1 in microplots and whether commercially produced tannins contained root-knot nematode attractive properties. The efficacy study was done with powdered and pelleted formulations of tannic acid applied at different rates and application times. The attractive qualities of sumac, querbracho, wattle, sweet chestnut, and tara tannins were compared to tannic acid on water agar in petri plates. Preplant applications of tannic acid pellets (1.0 g/6 300 cm^3 soil 10 cm deep) resulted in lower numbers of galls and increased plant height of tomato compared to the untreated control ($P \leq 0.1$). Second-stage ju-

veniles of *M. arenaria* were attracted to tannic acid and querbracho tannin and were repelled by sweet chestnut and sumac tannins ($P \leq 0.5$). Wattle and tara tannins did not repel or attract second-stage juveniles, however, there was an orientation behavior change when they came near these materials.

EVALUATION OF SOYBEAN BREEDING LINES FOR RESISTANCE TO SOYBEAN CYST NEMATODE *HETERODERA GLYCINES* IN BRAZIL. D. M. Hiromoto,[1] M. da S. Assunção,[1] R. A. de S. Kiihl,[1] L. A. de Almeida,[1] J. F. V. Silva,[1] W. H. Higashi[2] and C. Takeda,[2] EMBRAPA-CNPSo, C.P. 231, 86001-970 Londrina, PR, Brazil,[1] and Fundação MT. Rua Pernambuco, 1267, 78705-040 Rondonópolis, MT, Brazil.[2]—A total of 24 680 soybean breeding lines were evaluated for reaction to the soybean cyst nematode at 2 locations: Primavera do Leste, state of Mato Grosso, and Chapadão do Céu, state of Goiás. Plastic bags filled with homogenized naturally infested soil were used. The soil was collected in areas containing high cyst counts. The initial population of each batch of soil was determined in 100 cm^3 samples. Plants were thinned to one per bag. The evaluation was performed 30 days after emergence by counting the white females and using the following scale (percent number of cysts in the cultivar 'Cristalina' used as check). Grade: 1. resistant: 0 to 10%; 2. moderately resistant: 11 to 30%; 3. moderately susceptible: 31 to 60%; and 4. susceptible: >60%. Lines with low grades were retested so that some breeding lines had 4 replications. The race was determined for each location. Race 1 was identified at Primavera do Leste, and race 14 at Chapadão do Céu. All the 24 680 lines also were evaluated at Primavera do Leste, and 2 898 lines were evaluated in Chapadão do Céu. According to the results, 'Centennial', 'Gordon', 'Sharkey', 'Cordell', and 'Hartwig' were good ancestors for resistance to race 1. For race 14, the best lines had 'Leflore', 'Cordell,' or 'Hartwig' as parents. Lines resistant to both races were identified and had 'Cordell' or 'Hartwig' as source of resistance.

SIGNIFICANT INTERCEPTIONS AND OTHER NEW OR UNUSUAL PLANT-PARASITIC NEMATODES RECORDED IN ENGLAND 1994/1995. S. Hockland, Central Science Laboratory (CSL), MAFF, Hatching Green, Harpenden, Herts, AL5 2BD, England.—The Invertebrate Identification Team in the Plant Health Group, CSL, identifies plant-parasitic nematodes and other invertebrate pests for the Plant Health and Seeds Inspectorate, pest control consultants, and growers. A selection of notable nematode identifications made on imported material is summarized here. The increased trade in ornamental plants, especially from the Far East, has resulted in a wide variety of plant-parasitic nematodes being intercepted, some for the first time in the U.K. *Helicotylenchus* spp. (mainly *H. dihystera*) were the most common species found associated with such plants followed by *Tylenchorhynchus* spp. (mainly *T. crassicaudatus* and *T. leviterminalis*). Other species included *Aphelenchoides besseyi, Hirschmanniella* spp., *Meloidogyne* spp., *Rotylenchulus reniformis*, and *Xiphinema* spp. Nematodes also were spread by soil residues associated with consignments of plant products, such as potato tubers, and agricultural or construction machinery. Some of the species intercepted, namely *Helicotylenchus* spp., *Longidorus* cf. *paraelongatus, L. vineacola, Pratylenchus* cf. *mediterraneus, P. penetrans, P. thornei, R. macrosomus*, and *Tylenchorhynchus* sp., might not usually be expected to survive.

NEMATODE AND SOILBORNE DISEASE CONTROL IN VEGETABLES WITH COMBINATIONS OF 1,3-DICHLOROPROPENE PLUS CHLOROPICRIN. R. M. Huckaba, J. E. Eger, M. W. Melichar, and J. P. Mueller, DowElanco, Indianapolis, Indiana 46268, U.S.A.—Field tests were conducted in Florida, California, and North Carolina comparing 3 formulations of 1,3-dichloropropene (1,3-D) plus chloropicrin (Telone C-17, Telone C-25, and Telone C-35) to methyl bromide for nematode and soil-borne disease control in 1995. In the tests, the 1,3-D rate was held constant while the chloropicrin rate varied. Treatments were applied in the manner that vegetable growers apply methyl bromide. Measurements on nematode counts, root injury, disease incidence and severity, and yields showed that Telone C-35 performed similarly to methyl bromide and generally was superior to Telone C-17 and Telone C-25.

NEMATICIDAL EFFECTIVENESS OF THREE HOUSEHOLD DISINFECTANTS. D. G. Hutton, Faculty of Agriculture Unit, The University of the West Indies, Mona, Kingston 7, Jamaica.—Chloroxylenol (Dettol), Jeyes Fluid, and bleach were as or more lethal than oxamyl to *Rotylenchulus reniformis, Meloidogyne incognita, Pratylenchus coffeae, Radopholus similis, Helicotylenchus* spp, *Tylenchorhynchus* sp., other plant-parasitic nematodes, and free living nematodes in *in vitro* tests. The disinfectants also suppressed or eliminated *R. reniformis, M. incognita,* and *Helicotylenchus* spp. populations in soil, and produced better growth of tomato (*Lycopersicon esculentum*), cucumber (*Cucumis sativus*), red kidney bean (*Phaseolus vulgaris*), or red sorrel (*Hibiscus sabdariffa*). However, at least 3 weeks had to elapse between drenching the soil and planting, otherwise the disinfectants were phytotoxic. *P. coffeae*-infested yellow yam (*Dioscorea cayenensis*) planting material (heads) disinfested in chloroxylenol or Jeyes Fluid solutions sprouted earlier and bore more vigorous vines than oxamyl-dipped or control heads in greenhouse trials, and in addition, produced greater weights of tubers in field trials. The disinfectants were phytotoxic to tomato and cucumber transplants dipped in them. These disinfectants appear to be good alternatives to traditional nematicides and other nematode control strategies under certain conditions, with the significant advantage of their low mammalian toxicity, but their effects on the environment require investigation.

APPROACH TO A NEW TRANSGENIC PLANT SYSTEM FOR NEMATODE-INDUCIBLE PROTECTION. T. Irdani, P. Bogani, and M. Buiatti, Dipartimento di Biologia Animale e Genetica Universita' di Firenze, Istituto Sperimentale per la Zoologia Agraria, Firenze, Italy.—The presence of various steroid hormones has been detected in a range of nematodes including the following genera: *Panagrellus, Aphelenchus, Haemonchus, Dirofilaria, Ascaris,* and *Caenorabditis.* Plant-parasitic nematodes account for severe crop losses worldwide on a yearly basis. Genetic host resistance is the most effective cost and environmentally sound method for management of plant-parasitic nematodes. Plants genetically engineered to resist systemic infection from virus and insect pests have already been developed. However, constitutive expression of any toxin in a host species places strong selective pressure on the pest population and may lead to some problems related to biosafety of the plant products. Regulating expression of foreign genes conferring pest resistance might overcome some of these disadvantages. Nematode-inducible expression of a protein toxic to nematodes would have the advantage that the toxin production would be limited to particular tissue or to specific stages of nematode infection. A new transgenic plant system for potential nematode-inducible protection is presented. A glucocorticoid inducible expression cassette has been introduced in *Nicotiana langsdorffii* by *Agrobacterium tumefaciens* cotransformations. As nematodes produce steroids for moulting processes, such a system carrying genes coding for peptides toxic to nematodes could be induced at the infection stage.

***APHELENCHUS AVENAE*: REVIVING AN EFFECTIVE BIOLOGICAL CONTROL AGENT AGAINST SOILBORNE FUNGAL DISEASES. N. Ishibashi, Department of Applied Biological Sciences, Saga University, Saga 840, Japan.**—*A. avenae* has numerous advantages in biological control. It is applicable to a wide spectrum of soil-borne fungal diseases, although an isolate or strain of this nematode has its own host preference. Self perpetuation can be expected after the soil application and the nematode is established. Mixed-application with steinernematid nematodes is possible to manage soil pests. Additionally, interference by these beneficial nematodes of root invasion by plant-parasitic nematodes may occur. Storage is easy for a long time under ambient temperatures and disadvantages may be improved by further studies. Disadvantages are: somewhat injurious to germination of plant seeds in sterilized soil with inundative application without any other concurrent organisms; many isolates or strains, which should be established from the pathological or physiological viewpoints and decline of multiplication after repeated subculture on the same fungus species.

EFFECT OF MAINTENANCE TEMPERATURE ON THE DISTRIBUTION PATTERNS OF ISOZYMES IN STEINERNEMATIDS. G. B. Jagdale,[1] R. Gordon,[1] and T. C. Vrain,[2] Memorial University of Newfoundland, St. John's, Newfoundland A1B 3X9,[1] and Agriculture Canada Research Station, Vancouver, British Columbia V6T 1X2, Canada.[2]—Cellulose acetate electrophoresis was used to determine the degree to which isozyme banding patterns differed among isolates and laboratory colonies of steinernematid nematodes that had been maintained at different temperatures. Isozymes of 8 enzymes were separated in infective juveniles of 5 steinernematid isolates: *Steinernema feltiae* Umeå strain, *S. feltiae* L1C strain, *S. carpocapsae* All strain, *S. riobravis* TX strain and *S. feltiae* NF strain, recently isolated from soil in Newfoundland, Canada. The composite isozyme banding patterns were diagnostic for each of the isolates maintained at 25°C. Arginine kinase yielded zymograms that were distinctive for each of the isolates, except for the Umeå and NF strains of *S. feltiae*. Four other metabolic enzymes yielded isozyme banding patterns that were characteristic for each isolate, except for the L1C and NF strains of *S. feltiae*. Aspartate amino transferase and glycerol-3-phosphate dehydrogenase yielded zymograms that permitted *S. carpocapsae* All strain to be discriminated from the other 4 isolates, while mannose-6-phosphate isomerase was discriminatory for *S. riobravis* TX strain. Isozyme banding patterns of certain enzymes were affected by maintenance temperature and new isozymes were synthesized in response to low temperature (5 and 10°C). Such synthesis of isozymes may constitute a physiological strategy for cold adaptation.

INTRA-SPECIFIC VARIATION OF *MELOIDOGYNE* SPP. FOR RESISTANCE IN WILD TUBER-BEARING *SOLANUM* SPP. AND ITS IMPLICATIONS FOR POTATO BREEDING. G. J. W. Janssen, A. van Norel, and R. Janssen, CPRO-DLO, PO Box 16, 6700 AA Wageningen, The Netherlands.—Present potato cultivars have no resistance to root-knot nematodes. In search for resistance to *Meloidogyne hapla, M. chitwoodi*, and *M. fallax*, extensive screening trials have revealed numerous resistant genotypes from various wild *Solanum* spp. Experiments were conducted in the greenhouse using several field populations of *Meloidogyne* spp. to verify the resistance. In the case of the North American species, *S. bulbocastanum, S. hougasii, S. fendleri*, and *S. cardiophyllum* resistant genotypes were effective against all tested populations of *M. chitwoodi* and *M. fallax*. In the South-American species, *S. chacoense* resistance was found against *M. fallax* but not against *M. chitwoodi*. Genotypes of *S. bulbocastanum, S. hougasii*, and *S. chacoense*, which were resistant to a standard used population of *M. hapla*, showed moderate to high reproduction of some other *M. hapla* populations. The finding that the resistance from these species is not effective against all *M. hapla* populations emphasizes the need for introduction of resistance from various *Solanum* sources into new potato cultivars in order to avoid a high selection of virulence and devaluation of the resistance.

INHERITANCE OF ROOT-KNOT NEMATODE RESISTANCE IN PEACH-ALMOND HYBRIDS IN SPAIN. B. Jáuregui, J. Pinochet, R. Messeguer, A. Felipe, and C. Fernández, Departamento de Patología Vegetal, Institut de Recerca i Tecnologia Agroalimentaries (IRTA), Crta. de Cabrils s/n, 08348 Cabrils, Barcelona, Spain.—Root-knot nematodes can become an important limitation in peach production in Mediterranean environments. Little information is available in relation to the genetic control of this trait on peach-almond hybrids. Crosses between 2 root-knot nematode resistant inbreds derived from susceptible Garfi almond female parent and resistant Nemared peach male parent were studied in F2 generations to determine the heritability to the nematode resistant character. Cuttings from 88 clones in F2 generations were evaluated for their reaction to a virulent isolate of *Meloidogyne incognita* (Mi VR-ES) based on number of galls per root system and number of nematode per g of root in a greenhouse experiment. Resistance to *M. incognita* is transmitted from Nemared to its offspring in a 3:1 ratio and may segregate in the F2 population as a monogenic trait. Pedigree formed by nematode resistant Nemared, Nemaguard and F1 hybrids G × N No 9, G × N No 15 and G × N No 22 also resulted immune to the same *M. incognita* isolate in further evaluations. When tested against a mixture of 20 isolates comprising 5 root-knot species (*M. incognita, M. javanica, M. hapla, M. arenaria*, and

M. hispanica), resistant pedigree maintained a similar response to that of Mi VR-ES isolate, suggesting that this isolate is representative of the main root-knot species (broad resistance).

ENDOTOKIA MATRICIDA IN HERMAPHRODITES OF THE ENTOMOPATHOGENIC NEMATODE *HETERORHABDITIS BACTERIOPHORA*. S.-A. Johnigk, and R.-U. Ehlers, Department for Biotechnology and Biological Control, Institute for Phythopathology, Christian-Albrechts-University Kiel, Germany.—In suitable media, dauer juveniles (DJs) of the entomopathogenic nematode *H. bacteriophora* (Rhabditida) develop to self-fertilizing hermaphrodites. At first, eggs are layed into the surrounding medium. Together with deteriorating nutritional conditions the rate of egg production of the hermaphrodite decreases and the first stage juveniles (J1) hatch inside the uterus. Eight to 12 hrs later, egg production in the ovary ceases due to the destructive activity of the J1. They first feed on spermatocytes and unfertilized eggs in the ovary and later on the decaying body content of the hermaphrodite. It is not yet clear whether the destruction of the hermaphrodite tissues is caused by the mechanical and/or enzymatic activities of the offspring. When the pre-dauer stages (J2d) occur, only the cuticle and pharynx of the hermaphrodite is left. Pumping until its death, the hermaphrodite provides cells of the symbiotic bacterium *Photorhabdus luminescens*, which are embedded inside the intestine of the J2d. Readily developed DJs emigrate from the maternal carcase leaving behind a few late offspring arrested in the J1 or J2 stage, unable to finish development inside the hermaphrodite due to the lack of food.

CHARACTERISATION OF A cDNA CLONE CODING FOR A MOLECULE PRODUCED IN THE SUBVENTRAL GLAND CELLS OF *GLOBODERA PALLIDA*. J. T. Jones, P. R. Burrows, P. J. Wightman, J. M. S. Forrest, and W. M. Robertson, Nematology Department, SCRI, Invergowrie, Dundee DD2 5DA, Scotland, and Department of Entomology and Nematology, AFRC IACR Rothamsted, Harpenden, Herts AL5 2JQ, U.K.—Secretions of the various gland cells of plant-parasitic nematodes have been suggested to have a role in the induction of feeding sites in the roots of their hosts. In order to investigate this phenomenon, we have raised an antibody which recognizes secretory granules in the SVG cells of *Globodera rostochiensis* and *G. pallida*. The antibody recognizes a protein with a molecular weight of 48KDa on western blots. We have used this antibody to isolate a cDNA clone from a mixed stage *G. pallida* expression library. The clone codes for a protein with homology to secreted proteins from other nematodes. Northern blotting revealed that this gene is expressed specifically in second-stage juveniles of *G. pallida*. Although the results suggest the molecule is not involved in granule transport, the role of the molecule if any, in the host-parasite relationship remains uncertain and is currently being investigated.

DIFFERENTIAL MICROEVOLUTION OF *HETERODERA SCHACHTII* ISOLATES IN RESPONSE TO HOST SELECTION PRESSURE. M. Kaplan, and E. P. Caswell-Chen, Department of Nematology, University of California, Davis, California 95616, U.S.A.—Preliminary data show phenotypic differences among 3 geographic isolates of *Heterodera schachtii* on oilseed radish, white mustard, cabbage, and sugarbeet. Differences are manifested with respect to cyst production, clutch size, male to female ratios, and morphometrics. Selection pressures imposed by these crops have resulted in changes in aggressiveness on subsequent hosts and changes in genetic markers. Fewer cysts and eggs per cyst were produced on oilseed radish and white mustard than on cabbage or sugarbeet ($P \leq 0.001$). Differences were observed among *H. schachtii* isolates in the number of cysts produced on the white mustard ($P \leq 0.05$). There was no difference in the total number of cysts among the 3 isolates on cabbage. However, there were differences among isolates with respect to the number of eggs per cyst produced ($P \leq 0.06$). The ratio of males to females was greater on oilseed radish than on cabbage or sugarbeet ($P \leq 0.05$). The bodies of pre-infective J2 from white mustard were longer than those from the other crops ($P \leq 0.001$), and J2 from sugarbeet were narrower ($P \leq 0.001$). The results thus far emphasize the need to understand the dynamics among hosts and nematode populations to determine appro-

priate nematode management schemes based on host/non-host/nematode interactions such as crop rotation or the use of trap crops.

MOLECULAR ANALYSIS OF NEMATODE-PLANT GENE INTERACTION USING *IN VIVO* β-GLUCURONIDASE FUSIONS. M. Karimi, N. Barthels, and G. Gheysen, Laboratorium voor Genetica, affiliated to the Flanders Interuniversity Institute for Biotechnology, Universiteit Gent, K.L. Ledeganckstraat 35, B-9000 Gent, Belgium.—We have used the promoter tagging approach to identify root-knot nematode-induced genes in *Arabidopsis thaliana*. Several thousand *Arabidopsis* transgenic lines harboring one or more promoterless β-glucuronidase (gus) inserts were produced. So far, 4 lines (ARM1, ARM2, ARM3, and ARM4) were identified as promising lines for nematode-induced gus expression. Microscopical examination of galls from ARM1 indicated that the gus expression is mainly inside giant cells. In ARM1, the nematode-induced gus expression pattern is linked to 1 T-DNA locus containing 2 T-DNAs in an inverted repeat array. The T-DNA-flanking regions were isolated by using inverse polymerase chain reaction. The isolated regions were used to screen a genomic library of *Arabidopsis*. Sequence analysis indicated that the T-DNAs were inserted in an open reading frame of the *Arabidopsis* genome. Insertion did not cause deletion of plant DNA but complex repeats were generated at the insertion site. The putative promoter from one T-DNA-flanking region has been fused to gus, and subsequently, it has been introduced back into *Arabidopsis* C24. Analysis of some transformant plants show the same gus pattern as in the original ARM1 line. In the ARM2 and ARM3 lines, gus is mostly expressed in galls and high gus activity is observed 4 days after infection. Southern analysis of these lines showed that both contain one T-DNA. In the ARM4 line, gus activity can be detected 1 week after infection and is limited to galls. Southern analysis of ARM4 shows that this line has 2 T-DNAs.

TAXONOMY OF ROOT-KNOT NEMATODES DESCRIBED FROM EUROPE: THE SECOND-STAGE JUVENILES. G. Karssen, Plant Protection Service, P.O. Box 9102, 6700 HC, Wageningen, The Netherlands.—A small number of 19 nominal species of the genus *Meloidogyne* Goeldi, 1892 have been reported occurring in Europe. This number includes 12 species that have been described from a European type locality in the last 35 years. Type slides and live cultures were collected and used for a morphological study of the second-stage juveniles (J2). The available J2 types of *M. ardenensis, M. artiellia, M. hispanica, M. kralli, M. lusitanica,* and *M. naasi* are in agreement with the published species descriptions. Types of *M. megriensis*, deposited in Armenia, are not (yet) available and J2 types of *M. kirjanovae* have never been prepared. A translation of the original Russian description of *M. kirjanovae* from 1965 suggests that the juveniles are closely related to *M. incognita*. J2 types of *M. maritima*, including the holotype, are in a very poor condition and inappropriate for morphological studies. The preparation of new types of *M. maritima* is proposed. *M. litoralis* juveniles strongly resemble J2 of *M. ardenensis*. *M. deconincki* J2 paratypes contain a mixture of 2 different species: *M. ardenensis* and *M. hapla*. A key based on total body length, tail shape/length, hyaline tail length and hemizonid position relative to the excretory pore is prepared for J2 of the European root-knot nematodes.

SUPPRESSION OF ROOT LESION NEMATODES IN POTATOES ON PRINCE EDWARD ISLAND, CANADA, WITH FOSTHIAZATE. J. Kimpinski, W. J. Arsenault and J. B. Sanderson, Agriculture and Agri-Food Canada Research Centre, Charlottetown, Prince Edward Island, C1A 7M8, Canada.—The potato (*Solanum tuberosum*) crop on Prince Edward Island in 1995 exceeded 1 250 000 MT produced on about 43 000 ha. Figures for 1995 are not yet available, but in 1994, the crop value was 120 million $US. The most important nematode pest in the region is the root lesion nematode (*Pratylenchus penetrans*). Control of nematodes with fumigation is usually not economical, and systemic granular nematicides such as aldicarb are no longer used. We investigated the potential of the systemic organophosphorus compound, fosthiazate, during 4 growing seasons (May to October), 1991-1994. Root lesion nematode populations were reduced significantly in plots where the emulsifiable concentrate was applied at levels ranging from 0.7 to 13 kg a.i./ha, and total tuber yields were increased by

an average of 8%, 18%, and 40% in 1991, 1993, and 1994, respectively, in comparison to untreated plots. There was no effect on yield in 1992. Our data suggest that fosthiazate may be effective in the control of root lesion nematodes in potato, especially when combined with other management strategies such as selection of rotational crops that are poor hosts for nematodes, and growing of potato varieties that can tolerate moderate populations of root lesion nematodes.

ASSESSING THE POTENTIAL OF SESAME (*SESAMUM INDICUM*) AS A CASH CROP IN ALABAMA. P. S. King, R. Rodríguez-Kábana, and D. G. Robertson, Department of Plant Pathology, Auburn University, Alabama 36849, U.S.A.—Sesame (*Sesamum indicum*) has become an alternative cash crop in certain areas of the United States. The potential of this crop in Alabama has never been assessed. The objective of this study was to evaluate 6 commercially available sesame cultivars [KB-01(S-19), KB-05(S-18), KB-08(S-17), KB-13(S-16), KB-15(S-11), KB-24(S-20)] for effects on nematode populations and yield. These 6 sesame cultivars were planted in a field in north Alabama that had been in cotton monoculture for 5 years. Nematode samples from this field in previous years showed that cotton sustained high population densities of *Scutellonema brachyurum*, while root-knot (*Meloidogyne* spp.) and lesion (*Pratylenchus* spp.) nematodes were present in much lower numbers. Each sesame cultivar was planted in 8-row plots replicated 6 times in a randomized complete block design. Deltapine-51 cotton was planted in 8-row strips adjacent to each sesame plot as a check to determine nematode populations on a known susceptible host. Nematode populations and yield were determined at harvest. Deltapine-51 cotton continued to support high levels (approximately 500/100 cm^3 soil) of *S. brachyurum* while sesame proved to be a non-host for this nematode pathogen. Yields obtained from sesame ranged from 806 kg/ha for KB-01(S-19) to 1 271 kg/ha for KB-13(S-6). These were comparable to yields obtained in other regions of the U.S. where sesame has been grown for a number of years. This preliminary data suggests that sesame has potential as an alternative cash crop in this region of Alabama.

RATES OF 1,3-DICHLOROPROPENE (1,3-D) AND ALDICARB TO MANAGE *MELOIDOGYNE INCOGNITA* IN COTTON. R. A. Kinloch,[1] J. R. Rich,[2] and S. K. Barber,[2] University of Florida, AREC, Route 3, Box 575, Jay, FL 32565, U.S.A.,[1] and University of Florida, NFREC, Route 3, Box 4370, Quincy, FL 32351, U.S.A.[2]—Two field trials were conducted in northwest Florida. Treatments in both consisted of 4 rates each of aldicarb (0.5, 1.0, 1.5, and 2.0 kg a.i./ha) and 1,3-D (16.0, 32.0, 48.0, and 64.0 kg a.i./ha) and a control. The 1,3-D treatments were applied with a single chisel in the row 2-3 weeks prior to planting. All aldicarb treatments were applied in the furrow at planting. Phorate was applied in the furrow at 1.1 kg a.i./ha to the control and 1,3-D treatments at planting to reduce thrip (*Frankliniella fusca*) levels, since aldicarb has activity on this insect pest. Plots were 4 rows wide (0.91 m/row) × 15 m long, and treatments were replicated 6 times in a randomized complete block design. Six preplant and postharvest soil cores (2.5-cm-diam) were taken from each plot to 24-cm-deep and composited. Nematodes were extracted from a 100 cm^3 subsample by the centrifugation-sugar flotation technique. Lint yield was determined by harvesting the center 2 rows of each plot. Stalk weight was determined from 1 m of each plot row. Highest yields, averaging 696 kg/ha, and stalk weights, averaging 4 969 kg/ha, were found in the 1,3-D treatments. They were significantly ($P \leq 0.05$) higher than from the controls which averaged 521 kg/ha lint and 4 219 kg/ha stalk weight. No significant differences ($P \leq 0.05$) in lint yield or stalk weight were found among the aldicarb treatments and only few were significantly greater than the control. Plant stunting with the 1.5 and 2.0 kg a.i./ha rates was observed. This may have impacted cotton yield. Few differences in nematode populations were found among soil samples taken postharvest.

EFFECTS OF NEMATICIDE TREATMENT ON THE ROOT-KNOT NEMATODE/FUSARIUM WILT DISEASE COMPLEX IN COTTON. T. L. Kirkpatrick, P. D. Colyer, and W. D. Caldwell, University of Arkansas-Southwest Research and Extension Center, Hope, Arkansas 71801, U.S.A., and Louisiana State University-Red River Research Station, Bossier City, Louisiana 71113, U.S.A.—Eight

cotton cultivars grown extensively in the southern U.S. were evaluated in a site infested by both *Meloidogyne incognita* race 3 and *Fusarium oxysporum* f. sp. *vasinfectum* during 1994 and 1995 on the Louisiana State University Red River Research Station. Cotton yield components, nematode population density and root galling, and severity of vascular discoloration due to *F. oxysporum* f. sp. *vasinfectum* were evaluated with an at-planting application of the nematicide aldicarb (Temik 15 G) at 1.2 kg a.i/ha and without aldicarb. Experimental design was a randomized complete block, split-plot where main plots were cotton cultivars and subplots were presence or absence of aldicarb. Four replications of each treatment were included in the test each year. Plots that did not receive aldicarb were treated with the insecticide disulfoton (Di-Syston 15 G) at 1.1 kg a.i/ha to aid in control of early-season insects. Application of aldicarb increased lint yield and boll weight and decreased root gall ratings and wilt ratings both years. Lint percentage was increased with aldicarb in 1994 only, while fiber micronaire and strength were not affected by the nematicide either year. Fiber length differed among cultivars and was greater following aldicarb treatment. *M. incognita* population densities did not differ among cotton cultivars but were generally lower at mid-season (July) following aldicarb. No differences in final (November) population density were found for either cultivar or nematicide treatment either year. These results indicate that control of *M. incognita* with nematicides in situations where both the nematode and *F. oxysporum* f. sp. *vasinfectum* are present may improve cotton performance, possibly by moderating the effects of the disease complex.

MOLECULAR AND CELLULAR CHARACTERIZATION OF RESISTANCE RESPONSE TO *HETERODERA SCHACHTII* IN SUGAR BEET. M. Kleine,[1] S. Lange,[2] O. Oberschmidt,[1] B. Holtmann,[2] and F. M. W. Grundler,[2] Institut für Pflanzenzüchtung,[1] and Institut für Phytopathologie, Universitaet Kiel, Germany.[2]—A monogenic resistance against *Heterodera schachtii* deriving from *Beta procumbens* is available in translocation lines of sugar beet. During reproduction, the translocation can be lost. In this way nearly isogenic lines, with and without the translocation, can be used to characterize the mechanism of resistance. Resistant and susceptible plants were invaded by infective juveniles to the same degree without a detectable resistance response. Syncytia are induced in both resistant and susceptible lines. In resistant lines, the majority of the invaded juveniles stagnated as J3. As detected by 2D electrophoresis, resistant lines express a root specific protein of 18 kDA and a pI of 6.5. The protein, an ideal molecular marker for resistance, was partially sequenced and cDNA and genomic clones were picked with deduced probes. They are currently analyzed. The mRNAs that differentially transcribed during the first week after infection can be detected with the aid of "the differential display" technique. A number of clones were identified which are now analyzed for their relevance to the resistance response.

BIOLOGY AND ULTRASTRUCTURE OF *PRATYLENCHUS CRENATUS*. M. Knosel, and U. Zunke, University of Hamburg, Institute of Applied Botany, 20355 Hamburg, Germany.—Within the plant-parasitic nematodes in agriculture, *Pratylenchus* is the second most important genus and attacks a wide range of host plants e.g. corn, coffee, bananas, vegetables, and ornamentals. The biology and ultrastructural anatomy of adult females of the hermaphroditic specie, *P. crenatus*, was examined and compared to *P. penetrans*. Mass rearing on different host plants was investigated in climate chambers where a maximum reproduction level was achieved on carrot roots (cv. Rotin) at 24 - 26°C. In contrast to *P. penetrans*, the lowest level of reproduction was observed on *Arabidopsis thaliana*. Similar to *P. penetrans*, *P. crenatus* fed as an ectoparasite on root hairs and an endoparasite within roots. Ultrastructural anatomy was observed using transmission (TEM) and scanning electron microscopy (SEM) with emphasis on the anatomy of the esophagus, intestine, and reproductive system. The lateral field began on the seventh body annule as 2 bands and divided into 3 bands near the excretory pore. SEM observations showed phasmids near the tail tip in the middle of the lateral field. Two subventral glands were clearly separated by cell walls but differed slightly in form and shape compared to *P. penetrans*. Secretory granules in the dorsal and subventral glands were different in size and number. Similar to *P. penetrans*,

the vaginal cuticle of *P. crenatus* formed a flat contoured channel which was convoluted near the vulva and continuous with the body cuticle. Two pairs of muscles with longitudinal to tangential orientations were attached to the cuticular walls of the vulva.

CHANGES IN NEMATODE COMMUNITY STRUCTURE AND COTTON PRODUCTIVITY AS AFFECTED BY POULTRY-LITTER AMENDMENTS. S. R. Koenning,[1] K. R. Barker,[1] and K. L. Edmisten,[2] Plant Pathology Department[1], and Crop Science Department, North Carolina State University, Raleigh, North Carolina 27695, U.S.A.[2]—Experiments conducted at 2 locations focused on the impact of poultry-litter amendments on cotton yield and the population densities of plant-parasitic, fungivorous, bacterivorous, omnivorous, and predacious nematodes in cotton fields infested with the Columbia lance nematode, *Hoplolaimus columbus*. Plots were arranged in a split-plot design with 4 levels of poultry litter (0, 6.7, 13.4, and 20.1 MT/ha) as whole plots and growth-regulator treatments (PIX) as subplots. Poultry litter was added to the soil surface and incorporated 2-4 weeks before cotton was planted in May. Soil samples for nematode assays were taken prior to the addition of poultry litter, at midseason, and at cotton harvest. Growth-regulator treatments generally did not affect nematode numbers in this study. Midseason population densities of Columbia lance nematodes decreased linearly with increasing levels of poultry litter ($P = 0.10$) at 1 location. Numbers of bacterivorous nematodes at midseason were positively related ($P = 0.10$) to the amount of poultry litter applied in the spring at both locations, but not numbers of fungivorous, omnivorous or predaceous nematodes. *Helicotylenchus dihystera* population densities generally were not affected by pre-season litter applications. Only fungivorous nematodes were significantly greater ($P = 0.10$) in plots amended with poultry litter at cotton harvest. Application of poultry litter effected significant cotton-yield increases at both locations.

EFFECTS OF SWITCHGRASS ROTATIONS WITH PEANUT AND COTTON ON NEMATODES AND SOIL MICROFLORA. N. Kokalis-Burelle, R. Rodríguez-Kábana, J. W. Kloepper, W. F. Mahaffee, and K. L. Bowen, Department of Plant Pathology, Auburn University, Auburn, Alabama 36849-5409, U.S.A.—Peanut production in the southeastern United States is limited by damage from the root-knot nematode (*Meloidogyne arenaria*), southern blight (*Sclerotium rolfsii*), and aflatoxigenic fungi (*Aspergillus* spp.). Nematodes often interact synergistically with these soilborne fungal pathogens. Shifts in nematode and microbial populations and species diversity were assessed to determine environmental impacts and sustainability of forage grass rotations for disease control. Microplot results indicated that switchgrass (*Panicum virgatum*) reduced egg viability and juvenile emergence, increased the number of parasitized eggs, and reduced the number of root-knot nematode juveniles in soil compared to peanut or cotton. In field trials, switchgrass and cotton did not support populations of *M. arenaria*. Switchgrass supported higher populations of nonparasitic (nonstylet-bearing) nematodes than cotton. Switchgrass supported lower numbers of rhizosphere fungi than peanut throughout the season, and a distinctly different bacterial microflora compared to continuous peanut and peanut following switchgrass. Previous crop and nematicide treatment did not have a consistently significant effect on the incidence of pods infected with *Aspergillus*; however, pod invasion by *A. flavus* was highest in plots previously planted to peanut and to which nematicide had not been applied. In 1995, peanut yield increased significantly in plots previously planted with switchgrass.

APPLICATION OF ALGINATE FILMS FOR EVALUATION OF NEMATODE-TRAPPING FUNGI AS BIOLOGICAL CONTROL AGENTS. N. Kokalis-Burelle, and R. Rodríguez-Kábana, Department of Plant Pathology, Auburn University, Auburn, Alabama 36849-5409, U.S.A.—Alginate films were used in greenhouse experiments to assess the effects of commercial formulations of the nematode-trapping fungi *Arthrobotrys oligospora*, *A. botryospora*, and *Dactylaria brochopaga* on egg parasitism, emergence, and survival of *Meloidogyne incognita* juveniles. In the first experiment, soybean was planted in sterile sand treated with the formulation of trapping fungi and inoculated with films containing nem-

atode eggs. Sand treated with fungi reduced egg viability and juvenile emergence, increased the number of parasitized eggs, and reduced the number of *M. incognita* juveniles compared to sterile sand. In the second experiment, field soil naturally infested with *M. incognita* and *Heterodera glycines* was treated with nematode trapping fungi and planted with soybean. Populations of root-knot juveniles and non-parasitic (non-stylet-bearing) nematodes were reduced in soils treated with formulations of fungi at 0.45 kg/ha. Root galling also was reduced at 0.45 kg/ha compared to the untreated control. Alginate films placed in soil indicated an increase in the number of eggs parasitized by fungi and a decrease in the number of viable *M. incognita* juveniles migrating out of screens placed in soil treated with the formulation of trapping fungi.

ISOLATION AND CHARACTERIZATION OF THE *MELOIDOGYNE JAVANICA* FIRST COLLAGEN GENE. H. Koltai,[1] N. Chejanovsky,[2] B. Raccah,[3] and Y. Spiegel,[1] Departments of Nematology,[1] Entomology,[2] and Virology,[3] ARO, The Volcani Center, Bet-Dagan 50-250 Israel.—The surface of a nematode is covered by a multilayered cuticle, which consists mainly of collagen proteins. We propose to identify and characterize the root-knot nematode *Meloidogyne javanica* cuticle collagen genes and their developmental expression, as a prelude to investigate the potential of managing plant-parasitic nematodes by targeting and disrupting their cuticle. This novel approach would involve no risk to non-target soil organisms or to human health. We have identified, cloned and characterized the first *M. javanica* cuticular collagen gene, mjcol-1. The gene putatively encodes a 32 kDa collagen protein, including a propeptide which possesses a subtilisin-like protease cleavage site. Sequence of mjcol-1 gene upstream (5') region revealed two regulatory elements. Downstream (3') to the gene a putative poly-A site was observed. Transcription of the gene was detected in developing eggs and to a lesser extent in infective juveniles. The basic structure of the predicted protein sequence of the root-knot nematode cuticle collagen mjcol-1 was found to be primarily similar to *Caenorhabditis elegans* dpy-7 gene, possessing 52% homology between the two gene sequences and up to 100% homology at the conserved regions. One of the non-conserved features of the mjcol-1 gene was found to be the number and position of the tyrosine residues. Since triple-helical conformation are formed through covalent tyrosine-tyrosine bonds, this non-conserved feature may indicate different architecture of collagens binding in *M. javanica* cuticle, and hence, a unique root-knot nematode cuticle structure.

RELATIONSHIPS BETWEEN NEMATODES AND FUSARIUM FUNGI AFFECTING CONIFER SEEDLINGS. O. A. Kulinich, Institute of Parasitology, Russian Academy of Sciences, 33 Leninsky prospect, Moscow 117071, Russia.—Species of *Fusarium* cause some of the most important forest nursery diseases, especially of sown seeds and young seedlings during their first month of growth. A 2-year study in a forest nursery revealed a significant ($P \leq 0.0001$) relationship between nematode numbers and severity of *Fusarium*-caused disease of conifer seedlings (*Pinus sylvestris, Larix sibirica*). The mycophagous nematode *Aphelenchus avenae* accounted for 90% of the nematodes while the numbers of other nematodes in the roots and soil appeared not to affect disease severity. Laboratory inoculations with *A. avenae* in combination with pathogenic isolates of *F. graminearum* or *F. oxysporum* resulted in a noticeable decrease in *Fusarium*-caused disease of seeds and seedlings of Scots pine.

DISTRIBUTION OF RUSSIAN SPECIES OF LONG-HORN BEETLES (*MONOCHAMUS* SPP.) RELATED TO THEIR POTENTIAL AS VECTORS OF THE PINEWOOD NEMATODE. O. A. Kulinich,[1] P. D. Orlinski,[2] and N. V. Kolossova,[1] Institute of Parasitology, Russian Academy of Sciences, 33 Leninsky prospect, Moscow 11707, Russia,[1] and All Russian Institute of Plant Quarantine, 32 Pogranichnaya str., Bykovo, Moscow Region 140150, Russia.[2]—The pinewood nematode (*Bursaphelenchus xylophilus*) causes pine wilt disease and is a quarantine pest for Russia since it is a potential threat to conifers over most of the country. Consequently, analysis was made for the nematode and its primary insect vectors, *Monochamus* spp., across Russia. The results showed that *M. galloprovincialis, M. saltuarius, M. sutor, M. urussovi* and *M. impulviatus* are widespread throughout Russia. The

M. nitens beetles are present in Sakhalin Island, China, and Japan. Although a nematode closely related to the pinewood nematode, *B. mucronatus*, was present, no pinewood nematodes were found. However, this does not lessen the threat to Russian forests since *B. xylophilus* causes massive destruction of conifer forests. Based on the distribution of *Monochamus* spp. in Russia, we believe that either *M. nitens* and/or *M. saltuarius* pose the greatest threat of vectoring the pinewood nematode to forests in the Russian Far East from China and Japan.

USE OF SPECIES-SPECIFIC SATELLITE DNAs AS DIAGNOSTIC PROBES IN THE IDENTIFICATION OF HETERORHABDITIDAE ENTOMOPATHOGENIC NEMATODES. C. Laumond, E. Bonifassi, and E. Grenier, Laboratoire de Biologie des Invertébrés, BP 2078, 06606 Antibes Cedex, France.—The entomopathogenic nematodes of the genus *Heterorhabditis* contain highly tandemly repeated sequences known as satellite DNAs. We have now cloned in *Heterorhabditis bacteriophora* and *H. indicus* satellite DNA sequences that appear always to be specific of the species from which they were isolated. Using these cloned sequences as probes, we are able to clearly identify, in simple squashed nematode experiments, *H. bacteriophora* and *H. indicus* isolates as a "yes"/"no" result. This last procedure is effective even on a single infective juvenile, with the main advantage of avoiding time-consuming DNA extraction. Using these tools, we have analyzed the entire laboratory collection of geographically various *Heterorhabditis* and showed that among the 22 unassigned isolates tested, 17 were recognized as *H. bacteriophora* while the remaining 5 were *H. indicus* (*H. zealandica* and *H. megidis* were used as negative controls). The predominance of the *H. bacteriophora* species with a world geographic distribution seems to be a reality. Since the experimental procedure is very easy, our satellite sequences could be developed into specific non-radioactive probes. It should be possible to introduce them in field work without the need of a well-equipped laboratory.

MANAGEMENT OF RENIFORM NEMATODE IN MISSISSIPPI WITH ALDICARB AND OXAMYL. G. W. Lawrence, and K. S. McLean, Department of Entomology and Plant Pathology, Mississippi State University, Mississippi State, Mississippi, 39762, and Department of Agriculture, Northeast Louisiana University, Monroe, LA 71209, U.S.A.—Management of the reniform nematode (*Rotylenchulus reniformis*) on Sure-Grow 125 cotton with oxamyl and aldicarb was examined. Treatments included oxamyl applied at 0.3, 0.6, and 1.2 L a.i./ha in combination with aldicarb (0.6 kg a.i/ha), aldicarb at 0.6 kg a.i./ha alone and a control. Aldicarb was applied in the seed furrow at planting. Oxamyl was applied as a foliar spray at 37 (6th true leaf stage) and 58 days after planing in a total volume of 94.63 L/ha. Reniform numbers averaged over the growing season were lower in the plots which received oxamyl compared with aldicarb and the control. Cotton boll position, number of open bolls, and open boll weights were recorded at harvest. Cotton bolls were produced lower on plants which received a nematicide. The first open bolls were produced at node 5 on all nematicide-treated plants compared to node 11 on the control. A larger number of open bolls and bolls with a greater weight were recorded from the nematicide-treated plots. An average of 36, 31, and 21 open cotton bolls were produced in the oxamyl + aldicarb, aldicarb alone, and control plots, respectively. Average seed cotton weights were 162, 134, and 78 g/plant in the treatments which received oxamyl + aldicarb, aldicarb alone, and the control, respectively. Two foliar applications of oxamyl applied with aldicarb improved seed cotton yields an average of 28 g/plant over aldicarb alone, and 84 g/plant over the control.

LETHAL AND SUBLETHAL EFFECTS OF THE ENTOMOGENOUS NEMATODE, *STEINERNEMA CARPOCAPSAE*, ON THE COCCINELLID *HARMONIA AXYRIDIS*. S. Lemire,[1] D. Coderre,[1] C. Vincent,[2] and G. Bélair,[2] Université du Québec à Montréal, Dépt. Sc. Biologiques, C.P. 8888, Succ. Centreville, Montréal, Québec H3C 3P8, Canada,[1] Agriculture Canada, 430 Gouin, St-Jean-sur-Richelieu, Québec J3B 3E6, Canada.[2]—The Coccinellid beetle, *H. axyridis*, is an important predator of many insect pests and could be used simultaneously with *Steinernema carpocapsae* in many agroecosystems. Experiments were conducted in 9-cm Petri dishes using 5 concentrations of the nematode

against the beetle (10, 50, 100, 400, 800/1.3 ml/insect). The highest rate of mortality was obtained with 400 nematodes where 55% of the coccinellids died. Mortalities of 10, 20, 21.7, and 33% were found at concentrations of 10, 50, 100 and 800 nematodes. The nematodes could develop and reproduce in their hosts. Neuromotor disorder and paralysis also were observed in 23.3 to 50% of the beetles at the concentrations tested. Unlike other susceptible insects, which usually die within a 48 to 72 hr period, the mortality or effects were expressed gradually from the second to the tenth day after exposure. Furthermore, some beetles which were completely or partially paralyzed were observed recovering. In other experiments and under high concentrations, *H. axyrids* could react to the presence of the nematode when applied directly on the insect. Our results suggest that simultaneous utilization of the nematode and the coccinellid in IPM programs could be deleterious. However, the capacity of the predator to perceive the presence of the nematode may allow them to escape.

SENSITIVE AND SPECIFIC INDENTIFICATION OF *MELOIDOGYNE HAPLA* BY SATELLITE DNA PCR-AMPLIFICATION. F. Leroy,[1] G. Esparrago,[2] and P. Castagnone-Serrano,[1] INRA, Laboratoire de Biologie des Invertébrésd, BP 2078, 06606 Antibes Cedex, France,[1] and Consejeria de Agricultura y Comercio, Departamento de Fitopatologia, 06080 Badajoz, Spain.[2]—Satellite DNA (satDNA) is constituted of tandem-repetitive noncoding DNA sequences and has been characterized in a number of plant and animal species, including nematodes. Recently, a StyI satDNA was cloned from the root-knot nematode, *Meloidogyne hapla*, and proved to be specific to this species when used as a probe in Southern blot experiments. We report here the development of a simple and efficient method to identify *M. hapla* isolates, based upon the amplification of this satDNA through PCR. From the sequence of the monomeric unit, two oligonucleotides (MhS1 and MhS2) were designed that could readily anneal to its borders. As expected, ladder patterns of monomers and multimers of an approximate 150-170-bp repeat were amplified from purified genomic DNA of all the *M. hapla* isolates studied, while no amplification was detected with the 5 other *Meloidogyne* species tested. In further experiments, DNA was extracted from single females, males, juveniles, or eggs according to a simple procedure and used as a template in PCR assays. Amplification products were obtained, whose electrophoretic patterns were always identical to those from *M. hapla* genomic DNA. These results demonstrated the specificity and sensitivity of the method, thus showing its potential as a diagnostic tool for nematode identification.

BIOASSAY SCREENING OF SALT BARRIERS AGAINST *MELOIDOGNE INCOGNITA* FOR TOMATO PLANT-PROTECTION. R. Le Saux, and P. Quénéhervé, Laboratoire de Nématologie ORSTOM-INRA, BP 8006, 97259 Fort-de-France Cedex, Martinique, F.W.I.—A preliminary experiment was conducted in Martinique to evaluate possible plant protection against the root-knot nematode *Meloidogyne incognita* of ten selected salts, $CaCl_2$, KCl, NaCl, NH_4Cl, $CaNO_3$, KNO_3, $NaNO_3$, NH_4NO_3, Na_2SO_4, $(NH_4)_2SO_4$, used as salt barriers in micro-vials. First, we demonstrated that no absolute protection was achieved with any salt. Reproductive factors at 45 days ranked from 1.6 ($CaNO_3$) to 20 (control). Based on the number of nematodes extracted from both roots and soil, 3 groups can be distinguished showing different protection levels. The best protection (86-91%, $P \leq 0.05$) was given by 3 nitrate salts [$Ca(NO_3)_2$, KNO_3, NH_4NO_3] and a sulfate salt [$(NH_4)_2SO_4$]. Intermediate protection (64-68%, $0.05 < P \leq 0.02$) was obtained with 3 chloride salts (KCl, NaCl and NH_4Cl), and no or very low protection was observed with $CaCl_2$, $NaNO_3$ and Na_2SO_4. In our experimental conditions, nitrate salts (except $NaNO_3$ which gave an unexpected result) seemed to be good candidates to insure relative protection of young tomato plants against *M. incognita*. Further laboratory and field experiments would be necessary to evaluate the feasibility and use conditions of these salts already known as fertilizers in order to improve their protective potential against nematodes.

DISTRIBUTION OF THE GENUS *NYGOLAIMUS*, 1913 (NEMATODA: DORYLAIMIDA) IN ANDALUCÍA ORIENTAL, SPAIN. G. Liébanas, and R. Peña Santiago, Departamento de Biología Animal, Vegetal y Ecología, Universidad de Jaén, Virgen de la Cabeza nº 4, 23008-Jaén, Spain.—Eleven species belonging to the genus *Nygolaimus* have been recorded mainly in natural areas from Andalucía Oriental, southeastern Spain. These are *N. anneckei, N. brachyuris, N. sp. cf. N. gobabiensis, N. parabrachyuris, N. paratenuis, N. tenuis*, and another 5 previously undescribed. The distribution of this genus in 10 habitat types (aquatics, oak forests, coniferous forests, high altitude mediterranean brushwood, low altitude mediterranean brushwood, grasslands and pastures, riverside groves, other wild trees, dry field crops, and fruit orchards) is established and the relationships between the nematodes and the habitat types are studied. *N. brachyuris* is the most widely distributed species. Eight species show significant association (analyzed by chi square test) with 1 of the habitat types, but only 3 habitat types (mediterranean forests, low altitude mediterranean brushwood, and grasslands and pastures) present some significantly associated species.

CONTROL OF *MELOIDOGYNE JAVANICA* BY DIFFERENT ISOLATES OF *ARTHROBOTRYS* SPP. UNDER GREENHOUSE CONDITIONS. R. D. Lima, and S. Ferraz, Universidade Federal de Viçosa, Departamento de Fitopatologia, 36571-000, Viçosa, MG, Brazil.—Seven isolates of *Arthrobotrys* from Brazilian soils (*A. oligospora* 1, *A. superba* 2, and *A. musiformis* 4) and 1 isolate from France (*A. superba*) were selected from previous *in vitro* tests as potential agents for biological control of *Meloidogyne javanica*. Fungus inoculum was introduced through infected *Panagrellus* sp. (PG), in the proportion of 1 infected PG /g of soil. Simultaneously, *M. javanica* eggs were introduced in the same proportion and seedlings of lettuce cv. Regina de Verão were transplanted 7 days later. In 2 crop cycles (ca. 45 days each), fungus survival, plant development, and *M. javanica* behavior were evaluated. The test was arranged in random blocks using a factorial scheme ($2\times8+2^2$). In all treatments with *Arthrobotrys*, the fungus could be isolated from the rhizoplane and the rhizosphere at the end of the experiment. Number of leaves, as well as dry and fresh weight of the aerial parts, did not show significant differences. In the second crop cycle, there were significant differences among the treatments in number of galls and egg masses/root system and number of juveniles of *M. javanica*/100 cm^3 of soil. The isolate A-183 of *A. oligospora* showed a better efficiency as a biocontrol agent than the other isolates.

FURTHER OBSERVATIONS ON THE EFFECT OF LYSINE ON THE EMBRIOLOGY AND HATCHING OF *PRATYLENCHUS ZEAE* EGGS. G. C. Loots, and S. Steenkamp, Department of Zoology, PU For CHE, Potchefstroom 2520, South Africa.—Fifty eggs in the one-cell stage were exposed to each of the following concentrations of lysine: 0, 0.25, 0.5, 0.75 and 1.0 µmol. Observations were made of the duration for each of the developmental stages, viz. 1-cell stage to 1-blastula, 1-blastula to blastula, blastula to gastrula, gastrula to gastrula differentiation stage, emergence of 1st juvenile stage, emergence of 2nd juvenile stage, hatching. The different concentrations of lysine had no influence on the duration of any of the stages. The highest concentration (1.0 µmol) caused a high mortality of embryos in the stages before the gastrula. The concentration of 0.25 had the highest hatching rate.

EFFECTS OF THE INTERACTION OF MYCORRHIZAL FUNGI, PHOSHORUS NUTRITION, AND *PRATYLENCHUS VULNUS* INFECTION ON GROWTH OF OHF-333 PEAR ROOTSTOCK. A. Lopez, J. Pinochet, C. Fernández, C. Calvet, and A. Camprubí, Departamento de Patología Vegetal, Institut de Recerca i Tecnologia Agroalimentàries (IRTA), Crta. de Cabrils s/n, 08348 Cabrils, Barcelona, Spain.—The interaction between 2 arbuscular mycorrhizal fungi and the root-lesion nematode *Pratylenchus vulnus* was studied on micropropagated OHF-333 pear rootstock under field microplot conditions during a growing season. Inoculation with *Glomus intraradices* and *G. mosseae* significantly increased growth of plants in low phosphorus (P) soil and was more effective than P fertilization in promoting plant development. Mycorrhizal plants achieved higher values for all measured growth parameters. *P. vulnus* did not affect the percentage of root colonization by both endomycorrhizal fungi.

Root colonization by mycorrhizae suppressed nematode increase in host roots. Nitrogen (N) and P were deficient in *P. vulnus* inoculated plants without mycorrhizae. Mycorrhizal treatments with and without the nematode resulted in increased foliar levels of N, P, and Zn. Most elements were within sufficiency levels. Early inoculation with *G. intraradices* and *G. mosseae* favored pear rootstock growth and conferred protection against *P. vulnus* by inhibiting nematode reproduction and by enhancing plant nutrition.

SURVEY OF NEMATODES OF THE GENUS *MELOIDOGYNE* PARASITIC ON CROPS IN CONGO. P. M. Loubana, Laboratoire de Defénse des Cultures, ORSTOM/DGRST, B.P. 1286, Pointe Noire, Congo.—*Meloidogyne* spp. can be considered a threat to crops in Congo. In a survey of 293 samples, 79% of soil samples and 71% of root samples contained *Meloidogyne* juveniles. Crops sampled included vegetables (tomato, eggplant, etc.), banana, and cassava. The specific determination by acrylamide gel electrophoresis of esterases showed 5 species of *Meloidogyne* in Congo: *M. incognita* (62%), *M. javanica* (27%), *M. incognita acrita* (7.6%), *M. arenaria* (2.2%,) and an unidentified and atypical species, *M.*n.sp. (2.2%). Moreover, the observed populations were mainly high, on the order of 2 000 per L of soil or 1 000 per g of dry root. There seemed to be little correlation between the *Meloidogyne* species found and the plant species. In contrast, the geographical location was more obvious. For example, *M. incognita acrita* was frequently found in the forest area and *M. javanica* occurred mainly in the coastal region. *M. incognita*, however, occurred everywhere in Congo and with high frequency. In addition, it has been observed that a common cover crop in Congo, *Stizolobium aterrimum*, seems to be a promising control method since its use in crop rotation decreased *Meloidogyne* spp. populations. It was found to be resistant and even toxic against nematodes in pot experiments.

PLANT-PARASITIC NEMATODES FOUND IN THE SWEDISH *SALIX*-ENERGY FORESTS. C. Magnusson, and M. Insulander, The Norwegian Crop Research Institute, Plant Protection Centre, Department of Entomology and Nematology, Fellesbygget, N-1432 Ås, Norway, and Swedish University of Agricultural Sciences, Department of Plant Pathology, Box 7044, S-750 07 Uppsala, Sweden.—In Sweden, fast-rotation forestry for biofuel production is based on fast-growing clones of *Salix viminalis*. These "*Salix*-energy forests" are at present grown on 6 500 ha of agricultural soil. So far, the occurrence and importance of plant-parasitic nematodes in *Salix*-energy forests has not been studied. In October 1993 and in May 1994, samples were obtained from areas of poor and vigorous growth within 19 *Salix*-stands. Thirteen genera of plant-parasitic nematodes were found. Among obligate root feeders, *Tylenchorhynchus sensu lato, Trichodorus, Paratrichodorus*, and *Pratylenchus* were especially common. In October 1993, the mean number of obligate root feeders tended to be higher (P = 0.06) in areas of poor growth compared to areas of vigorous growth. *Macroposthonia* sp., *Trichodoridae* and *Helicotylenchus* spp. seemed more closely associated with poor growth (P = 0.10-0.16) than *Tylenchorhynchus s.l.* and *Pratylenchus* spp. (P = 0.34-0.48). In May 1994, no such stratification of the nematodes could be detected. In a pot experiment, *Pratylenchus penetrans* (pi = 2 000 ind. per plant) at 90 days caused a 28% reduction in shoot (P = 0.041) and a 34% reduction in root (P = 0.045) fresh weights of *S. viminalis* clone 78183. Extrapolated to the field situation, this would correspond to a loss of 2 tonnes of biomass per ha. This level is similar to current losses caused by frost and fungi. The results indicate the need for extended studies on the role of plant-parasitic nematodes in *Salix*-energy forests.

A STEINERNEMATID NEMATODE OCCURRING IN FOREST HABITATS IN EASTERN JAPAN. Y. Mamiya, Department of Agriculture, Tamagawa University, Machida, Tokyo 194, Japan.—Surveys were done in eastern Japan forests to determine the occurrence of *Steinernema* spp. and *Heterorhabditis* spp. and to elucidate the role of these nematodes along a trophic chain within a forest ecosystem. Soil samples from forest habitats were tested for the presence of nematodes by baiting with *Galleria mellonella* larvae. *Steinernema* spp. were found at 12 sites. No heterorhabditid nematodes were found in surveyed forests. *S. carpocapsae* occurred at 1 site. An undescribed *Steinernema* species occurred at 11

sites. The nematode closely resembled *S. feltiae*, but they did not interbreed. It was indicated that this nematode might be widely distributed and of common occurrence in eastern Japan forests. In the larch forest of Tamagawa University Forest in Hokkaido where an outbreak of larch sawfly, *Cephalcia* sp. occurred, sawfly larvae in soil were naturally infected with the nematode. This is the first known record of this genus in a sawfly in Japan. This nematode is active in cooler weather since they produced infective juveniles at 10°C. The horizontal spatial pattern of the nematode in its natural habitat was patchy or aggregated.

PATHOGENICITY OF BURROWING NEMATODE POPULATIONS ON BANANAS. D. H. Marin,[1] C. H. Opperman,[1] D. T. Kaplan,[2] and T. B. Sutton,[1] Department of Plant Pathology, North Carolina State University, Raleigh, North Carolina 27695-7616,[1] and USDA-ARS, Orlando, Florida, U.S.A.[2]—Monoxenic cultures of burrowing nematode were established on carrot discs from populations extracted from banana roots from Belize, Guatemala, Honduras, and Costa Rica. Cultures of *Radopholus* spp. also were obtained from Florida, Puerto Rico, Dominican Republic, and Ivory Coast. Selection of sampled sites was based on observed pathogenicity of nematode populations in the field. Pathogenicity and aggressiveness of these populations were evaluated by inoculating banana plants (*Musa* AAA, cv. Grande Naine) with 200 nematodes/plant, using a mixture of juveniles and adults. Banana plants produced by tissue culture were grown in 0.4-L styrofoam cups, containing a 1:1 mix of a coarse and a fine sand at ca. 25°C and 80% RH. Banana plants were held in acclimation for 3-4 wks prior to inoculation. Fresh root weights and nematode populations were determined 8 wk after inoculation. Additional experiments to determine the genetic variability of the populations involved the evaluation of the molecular diversity of the nematode populations, using Random Amplification of Polymorphic DNA (RAPD's). Presence and/or absence of DNA fragments was scored and used for grouping the different populations, based on the simple matching coefficient and cluster analysis.

ALTERNATIVES TO METHYL BROMIDE IN COLOMBIAN FLOWER PRODUCTION. N. Martínez-Ochoa, and R. Rodríguez-Kábana, Department of Plant Pathology, and Biocontrol Institute, Auburn University, Auburn, Alabama 36849-5409, U.S.A.—Floriculture represents Colombia's third major export after coffee and bananas, being among the largest exporter of carnations, roses, and chrysanthemums in the world. First attempts to use methyl bromide (MeBr) indicated that this type of fumigation was too expensive. Colombian floricultural soils are generally acidic and very high in organic matter content, conditions that result in long MeBr retention and consequent phytotoxicity. To date, the vast majority of the flower growers do not use MeBr and only a few remember using this fumigant some 20 years ago. Basically, all soil disinfestations are achieved by application of nematicides, fungicides, and steam. Jardines de los Andes, one of the largest cut flower producers in the country located in the Bogotá Savannah of the Colombian Western Andes, has pioneered composting as one of the most important components of its management for disease control, plant residue recycling, and fertilization. This compost-IPM system has been economically successful as well, eliminating the costs of the use of other fumigants such as metam sodium and dazomet. Nematode problems (i.e. root-knot in roses and cyst in mini-carnations) are treated on small localized areas using the nematicide phenamiphos only or steam when possible.

A COMPARISON OF TWO SPLIT-ROOT METHODS FOR THE STUDY OF INDUCED SYSTEMIC RESISTANCE AS A MECHANISM FOR CONTROL OF THE ROOT-KNOT NEMATODE IN CUCUMBER. N. Martínez-Ochoa, J. W. Kloepper, and R. Rodríguez-Kábana, Department of Plant Pathology, Auburn University, Biological Control Institute, Auburn, Alabama 36849-5409, U.S.A.—The study of acquired or induced systemic resistance (ISR) as a mechanism to control plant-parasitic nematodes using different inducing agents has been reported. In order to demonstrate fully ISR, physical separation between the pathogen and the inducing agent must be maintained to exclude the possible role of antagonism or competition. Recently, studies were conducted using plant growth-promoting

rhizobacteria (PGPR) as mediators of ISR against soilborne diseases in cucumber such as the root-knot nematode, *Meloidogyne incognita*, and *Fusarium oxysporum* f. sp. *cucumerinum*, using root halves attached to the same plant by the stem, and planted into separate pots. Another possible method is to use natural separation of the root system, thereby eliminating the need for physical injury to roots. The objective of this study was to evaluate the consistency of these two split-root methods for detecting PGPR-mediated ISR against *M. incognita*. The comparison of both methods to non-induced disease and healthy controls was based on parameters such as root weight, stem length, root-knot index, number of galls and number of egg masses per root system 4 weeks after inoculation. Both methods allowed detection of PGPR-mediated ISR against *M. incognita*.

ENTOMOPATHOGENIC NEMATODES AGAINST FOLIAGE FEEDING PESTS IN THE TROPICS. J. M. Mason, and D. J. Wright, Department of Biology, Imperial College of Science, Technology and Medicine, Silwood Park, Ascot, Berkshire, SL5 7PY, U.K.—Entomopathogenic nematodes (*Steinernema* spp. and *Heterorhabditis* spp.) were recovered from selected sites within Peninsular Malaysia. Use of entomopathogenic nematodes against foliage pests is commonly perceived to be limited by their tolerance to temperature, desiccation, and UV radiation. The effect of these abiotic factors on the infective juveniles was examined. Infectivity, for example, at different temperatures was found to differ both within and between species. Optimal numbers of *Steinernema* spp. (SSL85) were found to infect at 20-25°C, whereas *Steinernema* spp. (T87) optimal range was 25-30°C. Desiccation studies revealed more marked differences between the isolates. Infective juveniles of some isolates of *Steinernema* spp. (SSL85) survived longer than 60 min at 80% relative humidity whereas those of *Steinernema* spp. (T87) rarely survived longer than 40 min. Screening was conducted to test the isolates for their suitability as control agents against major pests in the crucifer-lepidopteran pest complex using a leaf-disc assay. The results from the above studies were discussed in relation to the selection of suitable nematode isolates for use in the field.

THE INFLUENCE OF RENIFORM NEMATODE ON PHOMOPSIS BOLL ROT OF COTTON. K. S. McLean, and G. W. Lawrence, Department of Agriculture, Northeast Louisiana University, Monroe, LA 71209, U.S.A., and Department of Entomology and Plant Pathology, Mississippi State University, Mississippi State, Mississippi 39762, U.S.A.—Tests were established in field microplots to examine the association between the reniform nematode (*Rotylenchulus reniformis*) and a cotton boll rot determined to be caused by a *Phomopsis* sp. Cotton cv. DES-119 was inoculated with initial population densities (Pi) of 0, 1 000, 2 500, 5 000, 7 500, and 10 000 reniform nematodes per 500 cm^3 of soil. *Phomopsis* sp. boll rot occurred naturally beginning at bloom and continued throughout the season. Disease incidence ratings of mummified dead bolls attached to the cotton stem characteristically hanging by the peduncle were made 16 August 1995. Natural disease incidence ranged from 15.1 to 44.4% diseased bolls in the Pi = 0 and 10 000 reniform nematodes, respectively. The number of diseased bolls increased significantly with increasing levels of the reniform nematode. The number of diseased bolls and aborted bolls was greater in the reniform treated plots compared to the control. An average 20.43 bolls were diseased and aborted at the initial population density of Pi = 5 000 compared to 12.47 bolls at Pi = 0. The number of healthy bolls were inversely related to the initial nematode population densities. Seed cotton yield ranged from a 2 644 kg per ha to 1 552 kg per ha in the initial inoculum levels of Pi = 0 and Pi = 5 000 reniform nematodes per 500 cm^3 of soil. Yield reductions ranged from 7 to 30% compared to the control.

MANAGEMENT OF LESION NEMATODE, *PRATYLENCHUS ZEAE*, IN SUGARCANE FIELDS OF INDIA. U. K. Mehta, and P. Sundararaj, Nematology Section, Sugarcane Breeding Institute, Coimbatore - 7, India.—The efficacy of organic amendments and plant products of naemin, neemark, farmyard manure, pressmud, and leaves of *Calotropis procera* L. for control of the lesion nematode, *Pratylenchus zeae*, was compared to the chemical nematicide carbofuran on sugarcane. All the treat-

ments increased plant growth significantly in comparison to the uninoculated control. Naemark proved best in increasing plant growth and reducing the nematode population in soil and roots. Application of ground nut, sesame, neem, cotton seed and coconut oilcakes, significantly increased the yield and quality of sugarcane. Population of *P. zeae* in soil and roots was significantly suppressed by all treatments. Control of *P. zeae* in sugarcane fields was demonstrated in different zones of south India. Pressmud, neem products and carbofuran on 2 sugarcane cultivars, Co Si 86071 and Co 6304, produced significant differences in sugarcane yield, NMC and qualitative characters. Pressmud proved to be highly effective followed by carbofuran, neemark and naemin. Differences in plant growth were more pronounced in the plant crop than in the ratoon crop. Influence of sunnhemp, pressmud and carbofuran in 9 different combinations on 3 sugarcane cultivars, Co 6304, CoC 671 and CoC 85061, were investigated. Yield and CCS/ha of all the 3 cultivars showed significant increases in all the treatments as compared to the control. Combinations of sunnhemp, pressmud, and carbofuran showed maximum increase of all the above characters as compared to control.

EFFECT OF FREQUENCY OF FERTILIZATION ON *GLYCINE MAX* RESPONSE TO NEMATODE INFECTION. H. Melakeberhan, Department of Entomology, Michigan State University, East Lansing, Michigan 48824, U.S.A.—The possibility of alleviating the impact of separate or combined inoculations of *Pratylenchus penetrans, Heterodera glycines*, and *Meloidogyne incognita* on *H. glycines* resistant, tolerant, and susceptible *Glycine max* cultivars by increased fertilization was tested in 2 greenhouse (26±2°C) experiments. Both experiments included nematode inoculum of 0 (control) or 6 000 eggs (*H. glycines* and *M. incognita*) or vermiform stages (*P. penetrans*) inoculated either separately or in combinations of 2 (50:50) or 3 (33.3 each) into 800 cm^3 sandy loam soil contained in clay pots. Experiment I was watered daily with tap water and fertilized twice weekly with Hoagland solution (HS); whereas, Experiment II was watered daily with HS only for 5 weeks. In Experiment I, separate inoculations of *H. glycines* and *M. incognita* on the susceptible cultivar and *M. incognita* on the tolerant cultivar resulted in significantly less shoot weight and nitrogen content compared with the controls. Combined inoculations had little effect on biomass accumulation. With the exception of concomitant inoculations of *H. glycines* and *M. incognita*, all other combinations treatments resulted in a significantly lower shoot nitrogen in the susceptible cultivar. In Experiment II, none of the nematode treatments had any effect on plant weight or nutrient content on all 3 soybean cultivars. Daily fertilization had little effect on *P. penetrans* on all 3 cultivars, and only some effect on *H. glycines* in the susceptible and *M. incognita* in the tolerant cultivar. The results indicate that increased fertilization may help a susceptible cultivar to compensate for nematode damage.

COMPARISON OF SOYBEAN CULTIVARS FOR *IN VITRO* CULTURE OF SOYBEAN CYST NEMATODE. S. L. F. Meyer, and D. J. Chitwood, USDA, ARS, Nematology Laboratory, Beltsville, Maryland 20705-2350, U.S.A.—Nematodes free of contaminating microorganisms can be produced in root explant culture and used for research purposes. While it has been observed that greater numbers of soybean cyst nematodes (*Heterodera glycines* Ichinohe) are produced on some susceptible soybean cultivars than on others, there has been no quantitative enumeration of nematode populations on different cultivars. Consequently, 24 soybean (*Glycine max* (L.) Merr.) cultivars representing maturity groups 0-8 were tested with 1 isolate of *H. glycines* race 3 to determine which cultivar would produce the highest nematode populations. Excised soybean root tips were grown on Gamborg's B-5 medium (two root tips per petri dish). When the root tips were two-days-old, 10 females (each with an attached egg mass) were inoculated onto each petri dish and incubated at 28.8°C. Thirty-five days later, the total female and cyst population (excluding inoculum) was counted from each of 10 petri dishes. The experiment was repeated once for each cultivar. The highest numbers of females and cysts were produced on cultivar Bass (mean of 148 females and cysts per petri dish) and the lowest numbers on cultivar Chesapeake (mean of 32 per petri dish). *Heterodera glycines* population numbers were not affected by maturity group. For example in maturity group 3, Bass differed significantly from Pioneer

9392 (mean of 39 per petri dish). Due to the labor-intensive nature of rearing uncontaminated nematodes, this research demonstrates that it is worthwhile to test efficacy of various cultivars, since populations can be more than quadrupled by soybean cultivar selection.

NATURAL DISTRIBUTION OF ENTOMOPATHOGENIC NEMATODES (RHABDITIDAE: HETERORHABDITIDAE AND STEINERNEMATIDAE) IN BELGIAN SOILS. J. S. Miduturi, L. Waeyenberge, and M. Moens, Centrum voor Landbouwkundig Onderzoek, 9820 Merelbeke, Belgium.—To study the natural distribution of entomopathogenic nematodes (EPN) in Belgian soils, 378 soil samples were taken from 85 sites of agronomically and ecologically diverse habitats. Using the *Galleria* larva bait technique, 35 soil samples were found positive for entomopathogenic nematodes. Species identification was based on morphometric characters and confirmed by biochemical characterization. Thirty-four samples were found to contain *Steinernema* spp. (23 *S. feltiae*, 10 *S. affinis*, one *Steinernema* species B3). The remaining positive sample contained *Heterorhabditis* (North West European strain). The EPN's were isolated from 50%, 14.3%, 11.1%, 9%, and 7.3% of the samples, respectively, from sand dunes, grassland, cultivated land, roadside verges, and woodlands. *S. feltiae* was isolated in these 5 habitats while *S. affinis* was found in all except in cultivated land. *Heterorhabditis* was found in a grassland; *S. feltiae* was prevalent in sandy soils with a wide range of organic matter content; and *S. affinis*, *Steinernema* species B3, and *Heterorhabditis* were isolated in sandy loam soils. All the positive sample sites were in the pH range of 3.6-8.1.

LIFE CYCLE OF *PRATYLENCHUS PENETRANS* ON TRANSFORMED LADINO CLOVER ROOTS. T. Mizukubo, and H. Adachi, Kyushu National Agricultural Experiment Station, Kumamoto, 861-11 JAPAN and Biological Science Research Center, Lion Co., Kanagawa, 256 JAPAN.—Reproduction of *Pratylenchus penetrans* was studied using genetically transformed ladino clover roots at constant temperatures of 17°C, 20°C, 25°C, 27°C, and 30°C. Solitary females inoculated on transformed roots in nutrient gellan gum medium (pH 5.5) deposited 1.23, 1.53, 1.62, 1.78, and 1.97 eggs per day, respectively, over the range of 17-30°C. The number of eggs deposited was significantly ($r = 0.968$; $P \leq 0.001$) correlated with temperature ($y = 0.3404 + 0.0537\ x$). Reduction in egg-laying rates at the start of egg-hatching was commonly observed at all temperatures, which could largely be attributed to mortality of juveniles inside roots. Mortality of juveniles was higher at 17°C (50.4%), 20°C (50.3%), and 30°C (58.4%) than at 25°C (34.6%) and 27°C (37.6%), suggesting optimal reproduction at temperatures between 25-27°C on ladino clover. Life cycle (egg deposition to egg deposition) was completed in 46, 38, 28, 26, and 22 days, following approximately 3 days after the appearance of females at the respective temperatures. No development of *P. penetrans* was estimated to occur at 5.09°C. The effective cumulative temperature required for hatching, female emergence, and onsets of oviposition (completion of one generation) were calculated to be 177, 513, and 564 day-degrees, respectively. Results showed that *P. penetrans* can adapt and reproduce in a wide range of temperatures.

TOXICITY OF ISOTHIOCYANATE DERIVATIVES TO *MELOIDOGYNE CHITWOODI* AND *PRATYLENCHUS PENETRANS* IN SOIL ENVIRONMENT. H. Mojtahedi, and G. S. Santo, Washington State University, IAREC, Prosser, Washington 99350, U.S.A.—Various concentrations (0.1-40 µg/g of soil) of commercially purified methyl, ethyl, benzyl, propyl, G-phenyl, allyl, phenyl, and butyl isothiocyanates and one naturally-occurring 3-butenyl isothiocyanate were applied to a loamy sand soil infested with a mixture of eggs and second-stage juveniles of *Meloidogyne chitwoodi*. Treated soil was incubated in Mason jars for 1 wk before determining the number of infective nematodes on a tomato seedling. Methyl isothiocyanate (the fumigant released from metham sodium) was the most effective with an $ED_{50} \leq 1$ µg/g of soil while butyl isothiocyanate was the least effective with an $ED_{50} \leq 10$ µ/g of soil. The 3-butenyl isothiocyanate, a form released by decomposing rapeseed tissue, had intermediate effectiveness with an $ED_{50} \leq 3$ µg/g of soil. In another test, *Pratylenchus penetrans* were introduced into loamy sand soil and treated with the concentrations of isothiocyanate derivatives that affected 95% of *M. chit-*

woodi in the previous test, and the treated nematodes were bioassayed with wheat seedlings. *P. penetrans* proved to be less sensitive to some of the isothiocyanate derivatives than *M. chitwoodi.* This may explain the higher rate of survival of lesion compared to root-knot nematodes in field soil samples collected after metham sodium application.

EQUIPMENT FOR DECONTAMINATION OF RESIDUAL WATER AND SLUDGE AFTER EXTRACTION OF NEMATODES CYSTS: CASE OF *GLOBODERA ROSTOCHIENSIS* (WOLL.) AND *GLOBODERA PALLIDA* (STONE) IN THE POTATO. V. Molinero-Demilly,[1] D. Mugniery,[2] G. Giroult,[1] N. Valette,[1] A. Gaste[3] and J. Léchappé.[1] SNES, rue Georges Morel, B.P. 24, 49071 Beaucouzé Cedex, France,[1] INRA Pathologie, Domaine de la Motte, B.P. 29, 35650 Le Rheu, France,[2] and TCA, 2 rue de la Claie, 49070 Beaucouzé Cedex, France.[3]—The SNES (National Seed Testing Station) laboratory at the GEVES (Varieties and seeds Study and Testing Group) has developed a nematode unit with the objective of testing resistance to cyst nematodes *Globodera rostochiensis* and *G. pallida* in varieties of potato. The tests are conducted in a greenhouse in accordance with OEPP recommendations and require extraction of newly formed cysts from the soil. In order to satisfy regulatory requirements for environmental protection necessary for parasites in quarantine, special equipment had to be developed for processing residual water and sludge for *Globodera* cysts extraction from the soil. After elimination of chemical treatment and filtration for reasons related to cost, unreliability or failure to respect the environment, a system was developed using pasteurization principles developed in the food processing industry. It is capable of destroying cysts and free forms of nematodes and treating up to 20-25 m^3 per day of washing water. This installation consists of decanting the heavy parts and keeping them at a temperature of 90°C for 8 hrs, while water and light particles pass through coils in which steam is injected. Lightweight parts are then decanted in a second container and only sterilized water is thrown into rain water. Heavy and light solid wastes are recovered in sealed bags and incinerated in the plant. This equipment forms a reliable treatment system and satisfies constraints for manipulating these organisms and is done with a general quality assurance procedure.

A SURVEY OF PLANT-PARASITIC NEMATODES IN SENEGAL. D. Mounport, and P. Cadet, Department de Biologie Animale, Faculté des Sciences, UCAD, Dakar, Senegal, and Laboratoire de Nematologie, Centre ORSTOM, B.P. 1386, Dakar, Senegal.[2]—A survey of plant-parasitic nematodes was conducted in Senegal during 1995. The main objectives of the project were to inventory plant-parasitic nematodes present in Senegal in cultivated and natural areas and in fallows. For this first year, sampling was made in the central regions of Senegal from East to West. New records of plant-parasitic nematodes were registered as follows: *Tylenchorhynchus avarious* and *T. labiatus* on millet (*Pennisetum typhoides*), *Hemicriconemoides mangiferae* on sweet potato (*Ipomoea batatas*) and 4 species of *Xiphinema* belonging to the "*Xiphinema americanum*" group. *Scutellonema cavenessi* and *Helicotylenchus dihystera* were the major plant-parasitic nematodes in fields and fallows in the Central and East regions. In the Capvert region, *Rotylenchulus reniformis, Meloidogyne* spp., and species of *Xiphinema americanum* were the dominant nematodes.

PRE-PLANT DRIP IRRIGATION APPLICATION OF 1,3-DICHLOROPROPENE FOR NEMATODE CONTROL IN ANNUAL CROPS. J. P. Mueller, J. M. Richardson, and M. W. Melichar, Dow-Elanco, Indianapolis, Indiana 46268, U.S.A.—Twenty-one field studies were conducted in 1994 and 1995 on vegetable crops, nursery crops, and pineapples, comparing drip application of 1,3-dichloropropene (1,3-D) and other products to the standard soil injection methods. Efficacy data are available for several root-knot nematodes (*Meloidogyne* spp.), cyst nematodes (*Heterodera* spp.), and reniform nematode (*Rotylenchulus reniformis*). In these studies, drip-applied 1,3-D generally was as effective as the standard soil injection application at comparable rates, and sometimes at much lower rates of active ingredient per unit area. In 12 of the studies, the minimum effective drip-applied 1,3-D rates resulted in 76% to 295% of the yields in the 1,3-D injection treatments and 123% to 1 458% of the yields

in untreated areas. These 1,3-D drip treatment rates were 33% to 66% of the soil injected rates. The best drip-applied 1,3-D treatments resulted in 96% to 309% of the yields in the 1,3-D injection treatments, and 129% to 1 526% of the yields in untreated areas. Drip-applied 1,3-D was not effective in very sandy soils in Florida, due to the lack of lateral water movement from the drip tape. More research is needed to improve lateral movement in these sandy soils. Delivery systems research has identified several hardware configurations that will safely and conveniently deliver 1,3-D through drip irrigation systems. Preliminary air monitoring data indicate that, with a properly installed drip irrigation system, drip application may result in less 1,3-D loss to the atmosphere than standard soil injection application. Studies also are in progress to evaluate the effectiveness of drip-applied 1,3-D for post-plant applications in tree, vine, and nursery crops, and drip-applied 1,3-D/chloropicrin combinations for soilborne disease control. Drip irrigation application of 1,3-D currently is registered for melons in Arizona and for several other crops in Europe. Due to the fact that few, if any, new nematicides will be developed in the near future, it is critical that we adapt currently available nematicides to alternative application methods.

THE EFFECT OF PHOTOPERIOD ON HATCHING OF *GLOBODERA* SPECIES. D. Mugniéry, D. Fouville, and M. Oger, INRA, Laboratoire de Zoologie, BP 29, 35650 France.—Influence of photoperiod acting on the plant on which populations of different species of *Globodera* developed was studied on the emergence of the juveniles of newly developed cysts. Photoperiods of 8, 12, and 16 hrs were applied on tomato, a common host of *G. pallida, G. rostochiensis, G. tabacum sensu lato,* and *G. mexicana.* The potato cyst nematodes and two *G. tabacum* populations originated from Europe. All the others were from the U.S.A. and Mexico. Hatching factors were applied to the newly developed cysts immediately after extraction. Hatch was checked once per week during 8 weeks. As expected, hatching of *G. rostochiensis* (maximum 21%) and *G. pallida* (maximum 19%) was small. For *G. rostochiensis,* the shorter the photoperiod, the more important is the hatching. It is exactly the reverse for *G. pallida.* For all the other populations-species, hatching was fairly large, from 30 to 100%. Statistically, hatching was greater for 8hr photoperiod than for 12 hr, and for 12 hr photoperiod than for 16 hr. The hatching of the 2 French *G. tabacum* populations was optimum for 8 hr, followed by the 16 hr treatment. Diapause of the North American tobacco cyst nematodes and *G. mexicana* is weak, if it exists. Evidence for photoperiodic adaptation was clear for the 2 French populations. The strong diapause of potato cyst nematodes may be explained by the fact they developed on the potato, which has a short growth duration. The consequence of this is the inability of the old roots to stimulate the hatching, to attract the juveniles and to permit them penetration and development. Conversely, with tobacco, the quasi permanent development of new roots limit the need to establish a strong diapause for *G. tabacum* and *G. mexicana.*

A PRELIMINARY INVENTORY OF MARINE NEMATODES FROM THE SEA OF CORTEZ, MEXICO. M. Mundo-Ocampo, and James G. Baldwin, Department of Nematology, University of California, Riverside, CA 92521, U.S.A.—The Sea of Cortez in northwest Mexico is ecologically unique with extensive tidal size ranges resulting in inter-tidal areas about 5 km wide which vary in water depth, temperature and salinity. The region is rich in fauna and endemic species, but since little is known about nematode diversity, preliminary surveys of tide pools during low tide were conducted in winter/spring of 1995-96. Extraction from substrate and plant fragments was by sieving and specimens were heat relaxed, fixed in TAF/FA (4:1), and infiltrated with glycerin for light microscopy (LM) or critical point dried for scanning electron microscopy (SEM). Images were enhanced by computer image analysis and reproduced with high resolution printing. Although identification is continuing, findings demonstrate diversity representing: Chomodoridae: *Paradraconema* spp., *Epsilonema* spp., *Cyatholaimus* spp.; Desmoscolecida: *Desmoscolex* sp., *Greeffiella* sp., *Quadricoma* sp.; Enoplida: *Polygastrophora* sp., *Enoploides* sp., *Oncholaimus* sp., *Phanoderma* sp., *Prooncholaimus* sp., *Tobrilus* sp. as well as several Monhys-

terida and Araeolaimida. Surveys reveal new taxa and characters which provide insight into unique ecological systems and biogeography as well as phylogenetic relationships and classification.

GENETIC COMPARISON OF *HELIGMOSOMOIDES POLYGYRUS POLYGYRUS* AND *HELIGMOSOMOIDES POLYGYRUS BAKERI* PARASITES OF MURIDS. G. N'Zobadila,[1] J.-F. Humbert,[2] M. C. Durette-Desset,[1] and J. Cabaret,[2] Laboratoire de Biologie Parasitaire-Protistologie-Helminthologie, 61 rue Buffon, M.N.H.N., F-75231 Paris Cedex 05, France,[1] and Laboratoire d'Ecologie des Parasites, Pathologie Avlaire-Parasitologie, INRA, F-37380 Nouzilly, France.[2]—Two putative sub-species of the nematode, *Heligmosomoides polygyrus*, were genetically compared. The first one, *H. p. polygyrus*, is a parasite of the field mouse *Apodemus* sp. and of domestic mice. The second one, *H. p. bakeri*, might originate from the European *H. p. polygyrus* from domestic mouse, that were introduced 5 centuries ago at the onset of colonization of North America. In the U.S.A, *H. p. bakeri* infects the domestic mouse and several cricetids. Due to the limited amount of morphological variation, a genetic study was undertaken. For the present study, we isolated *H. p. polygyrus* from the wild murid *A. flavicollis* in France and utilized a laboratory strain of *H. p. bakeri* maintained in the CD1 laboratory mouse for 20 years. The genetic variability was assessed using 2 techniques: first, isoenzyme electrophoresis using 5 enzymes (PGM, MDH, LDH, GPI and G6Pd), of which PGM only was not polymorphic. The distance of Nei (0.03) and the average heterozygosity (0.12) were low, which indicated that the 2 putative subspecies were much alike and that within variability also was limited. Secondly, the genomic DNA study was carried out using the RAPD (Random Amplified Polymorphic DNA) using 9 primers. Variability was again found to be limited (within sub-species similarity indices ranged from 0.50 to 0.80). Nevertheless, it was shown that the nematodes belonging to the same sub-species had a monophyletic origin as derived from UPGMAs with the 9 primers, indicating that taxa were initiating differentiation. These first findings should be corroborated using natural populations of *H. p. bakeri* and not on a laboratory strain that probably underwent through several bottlenecks.

A METHOD FOR IDENTIFYING *MELOIDOGYNE* SPP. USING *PASTEURIA PENETRANS* ATTACHMENT SPECIFICITY. T. Narabu, and H. Adachi, Department of Plant Protection, National Agriculture Research Center, Tsukuba, Ibaraki 305, Japan and Lion co. ltd., 100 Tajima, Odawara, Kanagawa 256, Japan.—A new method was established to identify second-stage juveniles (J2) of *Meloidogyne* spp. easily by using 3 Japanese strains of *Pasteuria penetrans* which differ in attachment to 4 species. The strain PPMI-1 attached only to *M. incognita* and *M. javanica*, strain PPMA attached only to *M. arenaria*, and strain PPMH attached only to *M. hapla* populations tested. Attachment of *P. penetrans* spores caused strong aggregation or clumping of 100-200 J2 in 0.35 ml culture dish wells containing suspensions of 10 000 spores in 10 mM phosphate buffer (pH 7.0) and Triton X-100 (0.3%) solution after 1 or 2 hr incubation. On the other hand, there was no clumping if none or few spores attached to J2. Preincubation treatment of spores with a protease enhanced the attachment of spores and clumping of J2. Air-dried spores in solution could be used for more than 3 months without loss of activity. Populations of *M. arenaria* and *M. hapla* could be identified to species based on clumping in wells containing 3 strains of spores. However, *M. incognita* and *M. javanica* could not be separated using this method. There were also a few populations of *M. incognita* which failed to clump in the presence of any of the 3 strains.

FIELD WHEAT YIELD REDUCTIONS CAUSED BY THE ROOT LESION NEMATODE *PRATYLENCHUS THORNEI* IN SOUTH AUSTRALIA. J. M. Nicol, J. M. Fisher, T. Hancock, and K. A. Davies, University of Adelaide, Waite Inst. PMB 1, Glen Osmond 5064, South Australia.—A permanent two-year field trial with 130 plots was grown in Tanunda, South Australia. Thirteen cereals and non-leguminous hosts including a fallow treatment were selected to manipulate nematode numbers on the basis of previous laboratory tests which indicated a wide range of susceptibilities. The initial and final nematode densities in each plot were measured establishing a range of densities between 0 to 9 400

Pratylenchus thornei/200 g soil for the second year of the trial. In the second year the highly susceptible wheat, Warigal and 2 potentially resistant wheat cultivars, GS50A and AUS4930, were planted. At high initial densities, extensive lesioning and severe cortical degradation was more evident with Warigal than either GS50A or AUS4930. Regression analysis revealed a significant linear relationship between initial density of *P. thornei*/200 g soil and Warigal grain yield number (t/ha) with the estimated regression equation Y= 1.86 - 5.57exp(5x). Low initial densities (1 000 *P. thornei*/200g soil) caused a limited yield loss of 3%, and high initial densities (9 000 *P. thornei*/200 g soil) yield losses were up to 27%. In contrast, the two suggested resistant varieties showed no significant relationship between the initial density of *P. thornei*/200g soil and yield (t/ha).

DISTRIBUTION OF STRAINS OF TOBACCO RATTLE TOBRAVIRUS AND THEIR VECTOR NEMATODES (*TRICHODORIDAE*) IN GERMANY. C. Nieser,[1] U. Zunke,[1] D. Heinicke,[2] and D. J. F. Brown,[3] University of Hamburg, Institute of Applied Botany, 20355 Hamburg, Germany,[1] Department of Plant Protection, 30453 Hanover, Germany,[2] and Scottish Crop Research Institute, Dundee DD2 5DA, Scotland, U.K.[3]—Tobacco rattle tobravirus (TRV) causes "spraing" disease, a necrotic arcing in the tuber flesh of potatoes. A consequence of reduced tuber quality caused by the disease is that affected crops are sold for animal feed, rather than as a human food commodity, which incurs substantial financial loss for the producer. In Germany, application of nematicides/nematastats to control the vector nematodes has been disallowed due to the negative impact of these highly toxic chemicals on the environment. A consequence of this decision is that "spraing" disease is rapidly becoming a major problem, especially in the main potato growing areas. Alternative control measures are urgently required and will necessitate the use of resistant/tolerant potato cultivars and the inclusion of antagonistic plant species in the crop rotation. Studies have revealed that the development of "spraing" symptoms in potatoes depends on the TRV strain and the potato cultivar, and that different TRV strains are each specifically transmitted by a particular trichodorid species. The occurrence and geographical distribution of TRV strains and their associated vector species in Germany is being determined. Also, the reactions of potato cultivars to different TRV strains is being investigated. Several TRV strains occurring in association with particular trichodorid species have been determined. The results from these studies will be used when recommendations are formulated as to where particular potato cultivars can be grown with minimum risk from the disease.

FOLIAR NEMATODE DISEASE OF ALFALFA: AN EMERGING ISSUE IN COLORADO AGRICULTURE. R. K. Niles and D. W. Freckman, Natural Resource Ecology Laboratory, Colorado State University, Fort Collins, Colorado 80523, U.S.A.—Alfalfa's value to Colorado has increased from $157 million in 1983 to $255 million in 1993, ranking alfalfa as the state's third most important crop, after wheat and corn. Acreage grown to alfalfa is expanding and increased 11% over the past 10 years. Stem nematode, *Ditylenchus dipsaci*, has been found in Colorado's alfalfa fields since the 1940's, and plants expressing the symptoms of foliar nematode disease are common throughout the state's alfalfa production regions. A second nematode species, *Aphelenchoides ritzemabosi*, infects alfalfa in north-central counties, and in 1995, we detected this species in the Arkansas River Valley and western slope regions of the state. Alfalfa producers and CSU scientists suspect foliar nematodes are causing an increasing amount of damage to alfalfa, and producers in the state require better information about foliar nematode disease. We are investigating plant resistance and environmental modification as tactics for managing the disease. Two alfalfa variety trials in their third year of growth were located in eastern and western production regions and sampled for foliar nematodes during the 1995 growing season. Varieties expressed a range of host suitabilities to foliar nematodes, but 'Lahontan', a standard resistant variety, supported low nematode populations. *D. dipsaci* dominated the concomitant populations of *D. dipsaci* and *A. ritzemabosi* inhabiting plant stems.

EFFECTS OF TILLAGE ON YIELD OF SOYBEAN AND POPULATION DEVELOPMENT OF *HETERODERA GLYCINES*. G. R. Noel and L. M. Wax, USDA ARS, University of Illinois, Urbana, Illinois 61801, U.S.A.—A long-term experiment was established in 1994 to investigate population development of *Heterodera glycines* and yield of resistant ('Fayette') and susceptible ('Williams 82') soybeans grown in rotation with maize under conventional and no-till production systems. The experimental design was a split-plot with tillage as main plots and cultivars as subplots. Nematode populations were determined at planting and at harvest. In 1994 initial numbers of eggs/250 cm^3 of soil averaged 1 700 (range = 75-24 000). Ratios of final populations of eggs to initial populations (Pf/Pi) were determined. Under conventional tillage, Pf/Pi for 'Fayette' and 'Williams 82' were 2.5 and 14.9, respectively. Under no-till, the ratios were 4.9 and 28.6 for 'Fayette' and 'Williams 82', respectively. Nematode reproduction was affected significantly ($P \leq 0.003$) by planting 'Fayette'. Effects of tillage on Pf/Pi were significant at $P \leq 0.16$. A significant tillage × cultivar interaction for Pf/Pi was not observed. Yield of 'Fayette' (3 200 kg/ha) was greater ($P \leq 0.0001$) than 'Williams 82' (2 150 kg/ha), but yield was not affected by tillage. In 1995 maize was planted, and numbers of nematodes declined and were lower ($P \leq 0.05$) in plots planted to resistant 'Fayette' in 1994. In contrast to 1994, numbers of *H. glycines* were lower ($P \leq 0.02$) in no-till plots in 1995.

CHARACTERIZATION OF THE SURFACE PROPERTIES OF DESICCATION TOLERANT MUTANTS OF *HETERORHABDITIS*. S. A. O'Leary, A. M. Burnell, and J. R. Kusel, Department of Biology, Maynooth College, Co. Kildare, Ireland, and Department of Biochemistry, University of Glasgow, Glasgow G12 8QQ, U.K.—Entomopathogenic nematodes have become increasingly important for the control of insect pests, and there is much interest in their genetic improvement. Nematode strains with increased desiccation tolerance would be beneficial in currently used storage procedures and may also be useful for field applications. Mutant lines isolated from a rapid desiccation mutant screen are described which have a slower rate of water loss than the parental strain at 0% rh. Heterorhabditid IJs retain the sheath of the previous larval stage. The mutant lines possess alterations in the surface properties of the sheath. Differences were observed in fluorescent lipid analog insertion. Furthermore, cationized ferritin binding studies demonstrated that the mutant lines possessed an increase in net negative surface charge. Removal of the surface layer of the sheath resulted in the loss of the mutant phenotype and a reduction in the desiccation tolerance of the parental strain. Therefore, the negatively charged "surface coat" appears to play an important role in the desiccation tolerance of *Heterorhabditis* species.

A COMPARATIVE NEMATOLOGICAL STUDY OF LAKES IN THE MALAGA AND GRANADA PROVINCES (SPAIN). A. Ocaña and R. Monterrubio, University of Granada, Departamento Biologia Animal y Ecologia and Instituto del Agua, Granada 18071, Spain.—Nine lakes located in the Antequera area (province of Málaga) and 6 in the Loja area (province of Granada) were sampled over a four-year period. The lakes in the province of Malaga are salty, highly mineralized with Cl^- values ranging between 0.526 g/l and 3.450 g/l and $SO_4^=$ values between 2 118 and 3 411 mg/l, whereas the lakes in Loja showed considerably lower anion and cation levels (Cl^- values less than 0.1 g/l and $SO_4^=$ values between 10 and 55 mg/l). The striking differences in mineralization and in nutrient values as a consequence of variations in organic pollution (mainly phosphorus and nitrogen) found between the 2 groups of lakes explains the significant differences in the type of nematode fauna found. The more representative species found in the Málaga province lakes are: *Etmolaimus multipapillatus* Paramonov, *Monhystrella iranica* Schiemer and *M. lepidura* (Andrássy) Andrássy. The more abundant species found in the Loja area Lakes are: *Monhystera paludicola* de Man, *M. stagnalis* Bastian, *Eumonhystera andrássy* (Biró) Andrássy and *Tobrilus gracilis* Bastian. Moreover, in both groups of lakes during periods of drought, typically continental-water species with varied feeding habits were substituted by other amphibians with saprobacteriophagous and even phytophagous feeding habits.

A PROTEIN INDUCED BY JASMONIC ACID AND RECOGNIZED BY WHEAT GERM AGGLUTININ ANTIBODY IS ALSO INDUCED BY INVASION OF *HERERODERA AVENAE* TO OAT ROOTS. **Y. Oka,[1] I. Chet,[2] and Y. Spiegel,[1] Department of Nematology, Agricultural Research Organization, Bet-Dagan 50250, Israel,[1] and Department of Plant Pathology and Microbiology, The Hebrew University of Jerusalem, Rehovot 76000, Israel.[2]**—Wheat germ agglutinin (WGA) has not been detected in oat (*Avenae sativa*) seed nor in the plant. However, a protein that cross-reacted with WGA-antibody was induced by the cereal cyst nematode, *Heterodera avenae* after its invasion of oat seedlings. The protein was detected 2 days after inoculation and lasted at least 8 days longer. The protein was also induced by jasmonic acid (JA) in non-infected roots, but not by elicitors of pathogenesis-related proteins or by abscisic acid (an enhancer of WGA). Immunohistological studies revealed the presence of this protein mainly in the vascular cylinder in both nematode-infected and non-infected JA-treated roots. Inhibition of lipoxygenase activity, an enzyme involved in JA synthesis, reduced the protein level in nematode-infected roots. Moreover, lipoxygenase activity was higher in nematode-infected and JA-treated non-infected roots as compared with non-infected root. The biological role of this protein in *H. avenae*-infected plants is not clear, but it is suggested that JA serves as a signal for pathogen invasion in the plant.

EVALUATION OF ALTERNATIVE FUMIGANTS FOR TOMATO PRODUCTION IN NORTH FLORIDA, U.S.A. **S. M. Olson,[1] J. R. Rich,[1] and J. W. Noling,[2] University of Florida, IFAS, NFREC, Route 2, Box 4370, Quincy, Florida 32351, U.S.A.,[1] and University of Florida, IFAS CREC-Lake Alfred, Florida 33850, U.S.A.[2]**—Methyl bromide (MBr) is the standard broad spectrum fumigant used for weed, disease and nematode control on about 20 000 ha of tomatoes in Florida each year. Since future availability of MBr is in question, alternative fumigates must be evaluated for potential replacement. Results of trials conducted from 1993-1995 were presented. Materials compared to MBr included metham sodium, dazomet, 1,3-dichloropropene (1,3-D) + chloropicrin, chloropicrin and an untreated check. Only the 1,3-D + chloropicrin came close to equaling MBr for root-knot nematode (*Meloidogyne* spp.) control, but none of the treatments equaled MBr for nutsedge control. In most cases, yields were not affected by treatments, even though root gall ratings and populations were high, due to optimum water and fertilizer management. At present the most viable alternative is use of 1,3-D + chloropicrin, with pebulate, a herbicide added for nutsedge control.

EFFECT OF THE PREVIOUS CROP ON POPULATION DENSITIES OF *MELOIDOGYNE JAVANICA* AND YIELD OF CUCUMBER. **C. Ornat, S. Verdejo-Lucas, and F. J. Sorribas, IRTA. Crta. de Cabrils s/n. 08348, Cabrils, Barcelona, Spain, and ESAB. Comte d'Urgell 187. 08036 Barcelona. Spain.**—The effect of planting a Mi-resistant or a susceptible tomato cultivar on yield of the following crop, cucumber Dager II, was determined in a commercial plastic polytunnel greenhouse infested by *M. javanica*. Cucumber was transplanted 9July 1995, and the Pi at planting were 3 and 132 juveniles/250 cm^3 soil in tunnels where a resistant or a susceptible tomato cultivars had been cultivated, respectively. As fruits reached maturity, they were harvested, weighted, and counted. Fourteen weeks after planting, the gall index and Pf were determined. Cucumber grew well in tunnels that had been planted with a resistant tomato cultivar as the previous crop. They showed a gall index of 2.3, and yielded 41 kg/m^2. In contrast, those plants in tunnels where a susceptible tomato had been cultivated showed a gall index of 7.9, and produced 25 kg/m^2. Population densities at harvest were 248 and 846 juveniles/250 cm^3 in tunnels that had been planted with resistant or susceptible tomato cultivars, respectively. The low Pf left by a resistant tomato cultivar allowed an increase in production of 62% in these tunnels with respect to those planted with a susceptible cultivar.

NONHOST CULTIVARS FOR A CYST-FORMING NEMATODE PATHOGEN OF TOMATO, EGGPLANT, AND TOBACCO AND COMPARISON WITH CERTAIN OTHER CYST SPECIES. W. W. Osborne, IAI Inc., South Boston, VA 24592 U.S.A.—*Globodera tabacum* (TCN), the tobacco cyst nematode, was found in Connecticut by Lownsbery on tobacco (*Nicotiana tabacum*) in 1951. He reported a 22% loss on the tobacco cultivar Connecticut 49 and stated that TCN is of "no consequence on tomato (*Lycopersicon esculentum*) or eggplant (*Solanum melongena*)". *Globodera solanacearum*, the Osborne's cyst nematode (OCN), was found in 1961 on severely stunted tobacco in Amelia County, Virginia. High OCN populations cause losses of 30% to 50% in tobacco, tomato, and eggplant in Virginia. The OCN also occurs on stunted tobacco in North Carolina. *Globodera virginiae*, (HCN) the horsenettle cyst nematode, was found in a soil sample from Isle of Wight County in Virginia in 1958. The HCN occurs only on weed hosts and is of no economic importance. The above mentioned nematode species differ in geographic distribution, pathogenicity, morphology, and host range. Sweet pepper (*Capsicum frutescens*) is a host to TCN but a nonhost to OCN. Three additional pepper cultivars-Better Belle, Bonnie Sweet Bell Pepper, and California Wonder- are reported here as nonhosts for OCN. Information was presented to suggest that species differences do not allow TCN, HCN, or OCN to be members of a "species complex".

STRUCTURE OF NEMATODE COMMUNITIES DURING A FALLOW PERIOD IN SENEGAL. E. Pate,[1] P. Cadet,[1] and D. Debouzie,[2] Laboratoire de Nématologie, BP 1386 Orstom, Dakar, Sénégal,[1] and Laboratoire de Biométrie, Université Lyon 1, 69622 Villeurbanne Cedex, France.[2]—Fallow is traditionally used to restore soil fertility. In developing countries, demographic pressure leads farmers to reduce the duration of fallow. Phytoparasitic nematodes are a component of the biological soil fertility. Understanding the dynamics of nematodes during fallow periods is of great importance in seeking better management of sustainable farming systems. Soil was sampled, at 7 dates (June 94-June 95), along 11 transects under fallows of different ages in Senegal. Correspondence Analysis (CA) was used to analyze the structure of nematode communities. Inertia of CA between-sampling date represented only 15%, despite sharp climatic variations within a year. Yearly change was greatest for the increase of relative proportions of *Xiphinema* spp. and *Gracilacus parvula* during the rainy season. Inertia of CA between transects represented 63% of total inertia. Within the first year of fallow, communities were close to that in cultivated fields, with high proportions of *Tylenchorhynchus gladiolatus* and *Scutellonema cavenessi*. *T. gladiolatus* in the 8-year fallow was less abundant that in the 1-year fallow. By contrast, relative proportion of *T. mashoodi* and *Pratylenchus sefaensis* were highest in the 8-year fallow. The proportion of *S. cavenessi* dropped in the 18-year fallow, while relative proportions of *Trichotylenchus falciformis* and *G. parvula* increased. These results confirm that nematodes could be used as bioindicators of ecological processes of agro-ecosystems.

DESICCATION SURVIVAL OF ENTOMOPATHOGENIC NEMATODES (RHABDITIDA: STEINERNEMATIDAE). M. N. Patel,[1] R. N. Perry,[2] and D. J. Wright,[1] Department of Biology, Imperial College of Science, Technology and Medicine, Silwood Park, Ascot, Berkshire, SL5 7PY, U.K.,[1] and Entomology and Nematology Department, IACR-Rothamsted, Harpenden, Hertfordshire, AL5 2JQ, U.K.[2]—Exsheathed infective juveniles (IJs) of 4 *Steinernema* species (*S. glaseri* (NC), *S. feltiae* (UK76), *S. carpocapsae* (All) and *S. riobravis* (Biosys 355)) were desiccated on glass slides, after removal of superficial water at 0, 20, 40, 60 and 80% relative humidity (RH). Survival was assessed after rehydration with water, and movement was used as the criterion for survival. At all RHs tested, *S. carpocapsae* showed the greatest survival and had the slowest rate of water loss (measured by interference microscopy); for example, at 80% RH, the survival time for 50% (S_{50}) of *S. carpocapsae* IJs was ca. 45 min compared with 5 to 20 min for the other species. The survival of aged IJs (75 days old) was markedly reduced in all cases. The presence of the 2nd stage juvenile cuticle (sheath) was not a significant factor in aiding survival. Drying IJs slowly on artificial substrates (0.5% agar or 1% agarose) greatly improved the survival of all 4 species. On agarose, *S. glaseri* and *S. feltiae* survived proportionally better;

at 80% RH, 30 to 40% of IJs were alive after 7 days desiccation compared with 0% for *S. riobravis* and ca. 20% for *S. carpocapsae.* The work is discussed in relation to possible mechanisms for survival of IJs during fast and slow drying conditions.

THE PRODUCTION OF *PASTEURIA PENETRANS* WITH REGARD TO THE EFFECTS OF TEMPERATURE AND WATERING REGIMES. B. Pembroke, University of Reading, Department of Agriculture, Earley Gate, Reading, RG6 2AT, U.K.—Consistent mass production of *Pasteuria penetrans* on root-knot nematodes is difficult to guarantee. Temperature is known to be a critical factor for the development of *P. penetrans* but even when the temperature is calculated in degree days for nematode development, the yield of mature endospores cannot be predicted. Temperature fluctuation could possibly explain unsatisfactory results. The degree day system may not be adequate when temperature is not constant or controlled. A secondary factor could be that of watering. It has been suggested in the literature that the amount of water given to *Pasteuria*-treated plants is crucial not only at the time of nematode inoculation/invasion but throughout the duration of the experiment. This hypothesis may be unfounded. Soil temperature fluctuation created by watering may affect the number of accumulated degree days calculated.

A TABULAR KEY TO SPECIES OF THE GENUS *TYLENCHOLAIMELLUS* COBB IN M. V. COBB, 1915 (NEMATODA: DORYLAIMIDA). R. Peña Santiago, and M. Peralta, Departamento de Biología Animal, Vegetal y Ecología, Universidad de Jaén, Virgen de la Cabeza nº 4, 23008-Jaén, Spain.—The species hitherto classified under *Tylencholaimellus* form a rather homogeneous group whose separation is far from easy. A tabular (polytomous) key to their identification has been prepared as a result of a revision of the available literature and is presented here. Several morphological and morphometric features are especially useful to distinguish the species: perioral region shape (disc-like, not disc-like, liplets), morphology of the lip region (continuous, set off by constriction), stylet (odontostyle plus odontophore) length, prevulvar uterine sac length (compared to the corresponding body width), "V" ratio, "c" ratio, and body length. The intrageneric taxonomy of the group is briefly discussed.

GUS GENE ACTIVITY AS A TOOL TO QUANTIFY NEMATOPHAGUS FUNGI IN SOIL. L. Persmark, Y. Persson, and H-B. Jansson, Department of Microbial Ecology, Lund University, Ecology Building, S-223 62 Lund, Sweden.—*Arthrobotrys oligospora* is one of the most commonly occurring nematode-trapping fungi, and it captures nematodes by means of adhesive three-dimensional network traps formed on the hyphae. Apart from its nematode-trapping ability, it is regarded as a fairly good saprophyte. Detection and quantification of nematophagous fungi in soil are quite laborious tasks. Therefore, new techniques have to be developed for studies of these (and other) fungi in soil. The need for specific markers is acute. β-Glucuronidase (GUS) is a commonly used reporter enzyme for plant and fungal genetic research. One of the advantages of using GUS as a reporter gene is that most plant and fungal systems lack appreciable GUS-activity, and that GUS assay substrates are available for histochemical, spectrophotometric and spectrofluorometric analyses. A GUS-producing strain of *A. oligospora* was created by cotransformation of protoplasts by 2 different plasmids. The transformant was stable through several single spore isolations, and also after re-isolation from non-sterile soil, where it had been grown for 1 month. The transformant did not differ from the wild type strain in growth rate, spore production or production of adhesive traps. The detection limit for spores from the transformed *A. oligospora* added to non-sterilized agricultural soil was 100 000 spores/g soil and for hyphae 3 mg wet weight mycelia/g soil. Further development of the GUS method is in progress and might improve the sensitivity of the method. The transformant will be important in basic studies of the ecology of *A. oligospora* in the soil and the rhizosphere, and in applied research focused on using this fungus in biological control of plant-parasitic nematodes.

DETECTION OF ENDOPARASITIC FUNGI IN NEMATODES. Y. Persson, A. Roumegoux, S. Erland, and H-B. Jansson, Department of Microbial Ecology, Lund University, Ecology Building, S-223 62 Lund, Sweden.—Many nematode species are parasites of plants and animals, including economically important crops and livestock. Nematodes, in their turn, can be parasitized by several parasitic fungi, the so-called nematophagous fungi. These fungi are used for development of control methods for nematode pests in plants and animals. Endoparasitic, nematophagous fungi develop their hyphal system within their host where they spend their entire vegetative lives; only spore-bearing structures will be produced outside the body of the nematodes. The purpose of this work was to investigate the possibility of using molecular techniques to detect and identify endoparasitic fungi inside single infected nematodes. PCR was used to amplify the ITS (internal transcribed spacer) region of the rDNA. The amplification product was cut with different restriction enzymes to produce RFLP patterns. Six different species of endoparasitic fungi, identifiable in axenic culture by the PCR/RFLP method, were used to infect nematodes. The amplification products from infected nematodes were represented by 2 bands: one band resulted from the amplification of the nematode ITS-region and the other band from the ITS of the fungi. Using restriction analysis, it was possible to identify 3 out of 6 fungal species within the infected hosts.

DISTINGUISHING NEMATODE RACES USING SEQUENCE VARIABILITY IN THE NON-TRANSCRIBED RIBOSOMAL SPACER. D. J. Petersen, and T. C. Vrain, Pacific Agriculture Research Centre, Agriculture and Agri-Food Canada, 6660 NW Marine Drive, Vancouver, B.C. V6T 1X2, Canada.—Sequence variability in the internal transcribed ribosomal spacer (ITS) is used to separate species of many genera of plant parasitic nematodes, but the ITS nucleotide sequences of many species are too conserved to be useful in distinguishing between races. Variability in the nucleotide sequence of the non-transcribed ribosomal spacer (NTS) may provide a diagnostic tool to separate races of several species of *Meloidogyne* and other nematode genera. Primers for PCR amplification of *M. chitwoodi* NTS were synthesized based on conserved regions of the 26S and 18S ribosomal genes. One population of race 1 and one population of race 2 of *M. chitwoodi* each yielded an amplified fragment of approximately 4.5 Kb, containing the 3' end of the 26S gene, the NTS, and the 5' end of the 18S gene. RFLP patterns were analyzed to detect differences between the 2 races. Subclones of the amplified fragments were constructed to facilitate DNA sequencing of the NTS region.

AN ANALYSIS OF POTATO CYST NEMATODE GENETIC VARIATION USING RAPDS. M. S. Phillips,[1] V. C. Blok,[1] R. T. Folkertsma,[2] and J. N. A. M. Rouppe van der Voort,[2] Scottish Crop Reasearch Institue, Invergowrie, Dundee DD2 5DA, Scotland,[1] and Wageningen Agricultural University, PO Box 8123, 6700 ES Wageningen, The Netherlands.[2]—RAPD analysis on European populations of potato cyst nematodes (*Globodera rostochiensis* and *G. pallida*) have been conducted to determine (i) the intra- and interspecific variation of these species within Europe and (ii) to determine the number and molecular constitution of introductions of both species into Europe. These data were subjected to a number of forms of analysis in order to identify and display the groupings. These methods include the use of dendrograms, UPGMA, principle co-ordinate analysis and phylogenetic approaches. The results are displayed and discussed in relation to both the molecular technique, employed in displaying the genetic variation, as well as the biological interpretation that can be placed on the groupings identified in the analyses.

EVALUATION OF AGRONOMICALLY IMPROVED *PRUNUS* ROOTSTOCK TO ROOT-KNOT AND LESION NEMATODES IN SPAIN. J. Pinochet, M. Anglès, E. Dalmau, C. Fernández, A. Felipe, and D. Esmenjaud, Departamento de Patología Vegetal, Institut de Recerca i Tecnologia Agroalimentaries (IRTA), Crta. de Cabrils s/n, 08348 Cabrils, Barcelona, Spain, and Institut Nationale de la Recherche Agronomique, Lab. de Biologie des Invertebres, 06606 Antibes, France.—Two screening and one resistance verification trial involving 20 *Prunus* rootstocks were conducted under greenhouse

conditions against root-knot nematodes and *Pratylenchus vulnus*. Most of the rootstocks were experimental genotypes or new commercial peaches and plums of Spanish and French origin. Nearly all were interspecific hybrid rootstocks. In the first trial, the rootstocks Bruce, Cadaman, Mirac, G× N No 15, Cachirulo × (G × N No 9) and *P. myra* × peach were immune or resistant to a mixture of 7 isolates of *M. incognita*. In the second screening trial, the hybrid plum P 2588 was a poor host to a mixture of 4 isolates of *P. vulnus*. The remaining 7 rootstocks were good hosts to the root-lesion nematode. In a resistance verification trial, the rootstocks GF-31, G× N No 15, Torinel, AD-101, Monpol, Nemaguard, Cadaman maintained their high level of resistance when tested against a mixture of 17 isolates comprising 5 root-knot nematode species (*M. incognita, M. javanica, M. arenaria, M. hapla* and *M. hispanica*). Barrier peach suffered a partial loss of resistance not detected in previous tests.

THE OCCURRENCE THE OF TRICHODORID NEMATODES AND TOBACCO RATTLE VIRUS IN RELATION TO TOBACCO RATTLE DISEASE IN TULIPS. A. T. Ploeg,[1] F. C. Zoon,[2] J. de Bree,[2] C. J. Asjes,[3] and P. W. Th. Maas,[2] University of California Riverside, California 92521, U.S.A.,[1] Institute for Plant Protection Research IPO-DLO, Wageningen, The Netherlands,[2] and Bulb Research Center, Lisse, The Netherlands.[3]—The Netherlands are the largest producer of tulip bulbs with approximately 8 000 ha and an economic value of 700 million Dutch guilders. Growers are faced with increasing restrictions on the use of soil fumigants. Among the organisms controlled by soil fumigation are virus-transmitting trichodorid nematodes. Both the nematodes and tobacco rattle virus, transmitted by these nematodes, commonly occur in sandy soils in tulip growing areas. It was earlier demonstrated that serological variants of the virus are associated with different vector nematode species. In a recent study, a field with a history of tobacco rattle virus problems was sampled for nematodes and tobacco rattle virus. Subsequent to sampling, tulips were grown in this field to determine whether a correlation exists between the occurrence of virus-transmitting nematodes and/or TRV in soil and outbreaks of TRV in tulip. Three trichodorid species were found in the field: *Paratrichodorus nanus, P. pachydermus* and *P. teres*. Tobacco rattle virus was isolated from soil by bait-tests and serologically characterized. From serology it was concluded that *P. teres* was the most likely vector, which was later confirmed in laboratory tests using individual *P. teres*. No correlation between total numbers of trichodorid nematodes in soil samples and the presence/absence of TRV was observed. The occurrence of TRV over the field was clustered and disease incidence in tulips was correlated with the presence/absence of TRV in soil but not with numbers of trichodorid nematodes. It is concluded that a bioassay of soil for TRV offers possibilities for predicting outbreaks of TRV in tulip. More research under different field conditions is needed to evaluate these findings.

ISOLATION OF ROOT-KNOT NEMATODE INDUCED/REPRESSED PLANT GENES BY DIFFERENTIAL SCREENING OF A cDNA LIBRARY FROM ALFALFA ROOTS. C. Potenza,[1] S. Thomas,[2] and C. Sengupta-Gopalan,[1] Department of Agronomy and Horticulture[1] and Department of Entomology, Plant Pathology and Weed Science,[2] New Mexico State University, Las Cruces, New Mexico 88003, U.S.A.—Using the *Meloidogyne incognita* (RKN) susceptible alfalfa cultivar 'Lahonotan', mRNA populations from non-infected roots and roots infected with RKN were analyzed by translating the RNA *in vitro* and subjecting the translation products to 2D gel electrophoresis. Substantial differences in the RNA populations were observed between non-infected and infected roots 72 hrs postinoculation. A cDNA library to the poly(A) RNA from infected roots (48 hr to 96 hr postinoculation) was constructed in Mgt11. The library was subjected to differential hybridization with radio labeled cDNA synthesized from 2 magnetically anchored (DYNAL-Dynabeads) cDNA libraries to non-infected and infected roots. The 2 radiolabeled populations of cDNA synthesized from the Dynabead libraries were hybridized to duplicate nitrocellulose lifts of the Mgt11 cDNA library. Preliminary screening of 10^4 plaques showed approximately 5% of the defined plaques differentially hybridized. Primary selected plaques were subjected to further rounds of purification and re-selected based on differential screening. Experiments are in progress to use the inserts of the selected cDNAs as probes in northern anal-

ysis of RNA from infected and non-infected roots of susceptible and resistant cultivars of alfalfa. We presented data on the characterization of cDNAs representing genes that are either induced or repressed due to RKN infection.

HOST RANGE OF *PRATYLENCHUS PENETRANS* AMONG NATIVE SAND PRAIRIE PLANT SPECIES FROM ONTARIO, CANADA. J. W. Potter, and A. W. McKeown, Agriculture and Agri-Food Canada, Vineland Stn, ON L0R 2E0, and Horticultural Research Institute of Ontario, Simcoe, ON N3Y 4N5, Canada.—Sand prairie is an unusual habitat in Ontario, existing mainly just north of Lake Erie where tobacco is the principal cash crop. The native plant species adapted to this hot, dry sandy area include several grasses and forbs uncommon elsewhere in the Province. Since *Pratylenchus penetrans* is a major pest of tobacco in this region, we considered that native plant species might show resistance to this nematode. Over 20 species of indigenous plants were grown from seed in pots, inoculated with lesion nematodes, and sampled after 98-140 days. Two species, *Rudbeckia hirta* and *Houstonia longifolia*, were immune and had no lesion nematodes present in either roots or surrounding soil. Three other species, *Asclepias tuberosa*, *R. serotina* and *Helenium purpurescens*, showed only slight root infestation and few nematodes in the soil. Three native grasses, *Andropogon gerardii*, *Sporobolus cryptandrus* and *A. scoparius*, failed to support the original inoculum; over half of the test species did likewise. These results confirm findings from soil sampling in the sand prairie area which also indicated that many plant species native to the habitat are poor hosts of the lesion nematode, *P. penetrans*.

NEMATODE INTERACTIONS WITH ENDOPHYTES II: EFFECT OF NEMATODE DENSITY ON COLONIZATION OF ENDOPHYTIC BACTERIA. A. Quadt-Hallmann, J. Hallmann, R. Rodríguez-Kábana, and J. W. Kloepper, Department of Plant Pathology, Auburn University, Auburn, Alabama 36849, U.S.A.—Plant-parasitic nematodes are considered to function as an entrance gate for plant pathogens. The effect of plant-parasitic nematodes on non-pathogenic endophytes has not yet been studied in detail. We have chosen the cotton - *Meloidogyne incognita* system to evaluate the effect of different juvenile concentrations (0, 300, 2 000, and 5 000 J2/plant) on colonization density of 2 selected endophytic bacteria. Strains being tested were *Pseudomonas fluorescens* (strain 89B-61), which has shown biocontrol potential against *M. incognita* on cotton and cucumber, and *Enterobacter asburiae* (strain JM-22), an excellent colonizer of several plant species. Both bacteria were applied as a seed treatment. The experiments were conducted under greenhouse conditions with heat-treated sand as planting substrate, and samples were taken 2 and 4 weeks after juvenile inoculation. The endophytic bacteria colonized the host tissue even in the absence of *M. incognita* reaching population densities of 1.0×10^7 cfu/g surface-disinfested root tissue. However, the presence of *M. incognita* increased endophyte populations significantly. Maximum endophyte densities of 1.0×10^7 cfu/g were obtained after inoculation of 2 000 J2/plant. Further increasing number of *M. incognita* inoculum did not result in higher endophyte populations. Cytological studies of nematode-infested cotton roots showed high numbers of bacteria in association with wounded tissue. Both endophytic bacteria colonized the root cortex, with 89B-61 predominantly staying in the intercellular spaces close to the root surface, while JM-22 also colonized the intercellular spaces close to the conducting elements. JM-22 also was found within individual cells of the root epidermis. The results indicate that plant-parasitic nematodes are not necessary for endophyte colonization, but their presence increases endophyte densities significantly, which for biocontrol agents might be correlated with higher control potential.

POPULATION DECLINE OF *MELOIDOGYNE INCOGNITA* AND *ROTYLENCHULUS RENIFORMIS* DURING SHORT-TERM ROTATION FROM VEGETABLES TO *MUCUNA PRURIENS* IN MARTINIQUE. P. Quénéhervé, B. Martiny, P. Topart, and S. Marie-Luce, Laboratoire de Nématologie ORSTOM-INRA, Centre ORSTOM, BP 8006, 97259 Fort-de-France Cedex, Martinique (F.W.I.).—The volcanic type of soil found in Martinique and in the Lesser Antilles is highly favorable for infection and reproduction by both the root-knot nematode, *Meloidogyne incognita*, and the reniform nematode,

Rotylenchulus reniformis, on cultivated crops. Therefore, there is a need to find nonhosts which can be grown easily as rotation crops in infested greenhouses and fields planted in vegetables. The influence of intercropping with *Mucuna pruriens* (L.) DC, var. utilis (Wallich ex Wight) Bak. ex Burck. [velvetbean, syn. *Stizolobius aterrimum* Piper and Tracy, *Mucuna aterrima* (Piper and Tracy) Holland, *Mucuna deeringiana* (Bort.) Morr., *Stizolobium deeringianum* Bort.] was examined on field populations of *M. incognita* and *R. reniformis* in vegetable crops cultivated in Martinique. In field experiments, velvetbean was always a poor host for both *M. incognita* and *R. reniformis* and significantly reduced nematode numbers compared to fallow or susceptible crops after only 3 months. The use of *Mucuna pruriens* var. utilis as a rotation crop may provide growers a practical and environmentally safe means to reduce population densities of both *M. incognita* and *R. reniformis* on a short term basis prior to cultivation of susceptible vegetable crops while also improving soil fertility.

ANHYDROBIOSIS IN THE SECOND-STAGE JUVENILES OF *HETERODERA SACCHARI*. **G. Reversat, C. Sannier, and A. Pando-Bahuon, Laboratoire d'Ecologie des Sols Tropicaux, ORSTOM, 32 avenue Henri Varagnat, 93143 Bondy Cedex, France.**—From previous studies published by other authors, juveniles of *Heterodera sacchari* were considered unable to withstand desiccation. We succeeded, however, in inducing anhydrobiosis in juveniles of 3 strains of this species (from Congo, Chad and Senegal). The desiccation method involved an inert support of filter paper and the use of classical solutions of increasing concentrations of glycerol. The desiccation stress was increased very slowly (the experiment lasted 2 weeks), and until pF 5, the rate of survival was high (about 60%). This value of pF 5 was among the highest values observed currently during the dry season in soils of subsaharian Africa. The mineral composition of the inoculation solution influenced the rate of survival in relation to the nature of the support. On the other hand, the mineral composition of the recovery medium (during rehydration) affected the rate of survival to a lesser extent. Differences from results obtained previously by other authors are discussed in relation to genetics (strains from different geographic origins), physiology (our strains may have been selected in connection with the storage of the cysts in hyperosmotic medium between 2 multiplication periods) or methodology (the use of filter paper as support and the rather slow increase in pF during desiccation).

PERFORMANCE OF 1,3-DICHLOROPROPENE (1,3-D) AND FENAMIPHOS IN TWELVE TOBACCO FIELD TRIALS CONTAINING *MELOIDOGYNE JAVANICA*. **J. R. Rich, and D. J. Zimet, Route 3, Box 4370, University of Florida, Quincy, FL 32351, U.S.A.**—Twelve tobacco (*Nicotiana tabacum*) nematicide trials were conducted over 12 years in north-central Florida, U.S.A. In each of these trials, treatments of 1,3-D, fenamiphos 3SC and a control were utilized. Trials were conducted on fine sand soils (93% sand, 4% silt, and 3% clay, < 1% o.m.). The 1,3-D treatments were applied with a single chisel in the row at rates between 68 and 102 kg a.i./ha. Fenamiphos was applied broadcast at 6.7 kg a.i. in 185 L/ha water and disc-incorporated. Plots were 2 rows wide (1.12 m wide/row) and 6.1 m long. Tests were arranged in a randomized complete block design with 6 replicates. Tobacco leaves were harvested 3-4 times upon maturity and cured weights recorded. Root-gall index ratings were made at or near final harvest from 4 plants in each plot and rated on a 0-4 scale where 0 = 0 and 4 > 75% root galling. Average cured yield increase of tobacco in the 1,3-D treatments was 781 kg/ha providing added value of $2 754 (U.S.)/ha at current prices. Average yield increase of tobacco in the fenamiphos treatments was 585 kg/ha providing added value of $2 062/ha. Average root-galling index was 3.2 for the control and 1.0 and 1.1 for the 1,3-D and fenamiphos, respectively. Fenamiphos and 1,3-D have performed consistently over the years in the deep sands where tobacco is grown in Florida.

IMPORTANCE OF ROOT-KNOT NEMATODES ON MAIZE PRODUCTION IN SOUTH AFRICA. **H. F. Riekert, Grain Crops Institute, Agricultural Research Council, Private Bag X1251, Potchefstroom, 2520, South Africa.**—Root-knot nematodes are known plant parasites of maize in South Africa but are generally regarded as being of little importance. Since the adaptation of extraction

techniques specifically for root-knot nematodes on maize a few years ago, the true distribution and impact of these nematodes on maize is being realized. Greenhouse and microplot trials indicated yield losses of up to 50 g per plant. Possible plant compensation due to nematode damage on root systems also was observed. Application of registered granular nematicides to maize fields did not result in decreased numbers of root-knot nematodes. Soil fumigation with EDB, however, resulted in population decreases. The low yield potential and cash value of dryland cultivated maize limits the economical feasibility of chemical control. Irrigated maize with higher yield potentials could benefit economically by application of granular nematicides.

USE OF ELECTROPHYSIOLOGICAL TECHNIQUES TO DETERMINE THE RESPONSE OF *GLOBODERA ROSTOCHIENSIS* MALES TO FRACTIONS OF THEIR HOMOSPECIFIC SEX PHEROMONE. E. Riga, R. N. Perry, and D. R. Holdsworth, Entomology and Nematology Department, IACR-Rothamsted, Harpenden, Herts, AL5 1LX, U.K.—Electrophysiology has considerable potential for evaluating the responses of nematodes to semiochemicals. The reaction of individual adult males of the potato cyst nematode, *Globodera rostochiensis*, to sex pheromones from adult females has been investigated in Rothamsted using electrophysiological techniques with detailed computer analysis of the responses. Each male nematode was pierced with an electrode near the cephalic region and then exposed to crude pheromones from virgin females. The spike frequency produced by *G. rostochiensis* males increased significantly after the application of the homospecific pheromone. Using reverse phase HPLC techniques, the crude sex pheromone was fractionated and the response of males of *G. rostochiensis* was tested when exposed to each of 4 fractions presented sequentially to individual nematodes; the nematode responses enabled the most active fraction to be defined. Nematode sensory responses to known concentrations of semiochemicals and their fractions can be quantified, spike activity before, during and after stimulation can be compared and the occurrence of aspects such as sensory adaptation can be demonstrated.

DEVELOPMENT OF IMPROVED AND APPROPRIATE PEST MANAGEMENT STRATEGIES FOR POTATO-CYST NEMATODES (PNC) IN BOLIVIA. E. Riga, and H. J. Atkinson, Centre for Plant Biochemistry and Biotechnology, University of Leeds, Leeds, LS2 9JT, U.K.—Historically, frequent cropping of potato as a staple food has resulted in a prevalence of high soil populations of PCN in the Altiplano. Our contribution to an international collaboration seeks to quantify decline rates of the nematode in this agricultural ecosystem. Seasonal decline of egg populations in soil can be monitored with reduced variation when cyst size is considered using image analysis. Physiological indicators of dormancy, readiness to hatch and relative infectivity are being developed. Lipid reserves of unhatched juveniles decline with progress of a rotation course, but variation at a locality is also influenced by other agronomic parameters. The importance of nonhost crops on dormant populations is one factor under study. We aim to develop an expert system to optimize cropping frequency and potato yield in the prevailing low input production system.

EFFECT OF ORGANIC AMENDMENTS ON *MELOIDOGYNE ARENARIA* POPULATION IN POTTED SOIL. C. H. S. P. Ritzinger, and R. McSorley, Department of Entomology and Nematology, University of Florida, Gainesville, Florida 32611-0620, U.S.A.—Organic amendments (OA) were evaluated for their effectiveness in suppressing *Meloidogyne arenaria* populations in 2 greenhouse experiments in naturally infested soil. Vegetative shoots from castor (*Ricinus communis*), collard (*Brassica oleracea*), sesame (*Sesamum indicum*), sorghum (*Sorghum bicolor*), velvetbean (*Mucuna deeringiana*), and zinnia (*Zinnia elegans*) were chopped into small pieces, and placed on the soil surface in plastic pots. Control pots received no OA. Nematode numbers and yield were measured on okra (*Hibiscus esculentus*) planted into each pot. In the spring experiment, 4 g of the fresh OA or 4 g of the dried OA were used as separate treatments. The main effect of fresh vs. dry OA on nematode population and its interaction with OA type were significant ($P \leq 0.05$), with greater efficacy from dried OA than fresh OA.

Reduction of juveniles (J2) in the root system was obtained with dry OA from zinnia, castor, velvetbean, and collard treatments. The lowest gall indices were noted when castor, velvetbean, sorghum, and zinnia were used. There were no differences among OA for egg masses ($P \leq 0.05$). In the summer trial, 4 g of dry OA and the fresh weight of each OA corresponding to 4 g dry weight did not differ in their ability to reduce J2 ($P \leq 0.05$). Castor and velvetbean gave best suppression of J2 in soil, followed by collard and zinnia. Reduction of J2 in the root systems was higher from velvetbean, followed by collard, castor, and sorghum. For both seasons, the best growth responses of okra were obtained with OA of castor, velvetbean, collard, and zinnia. In general, castor and velvetbean were the most effective OA source, and sesame and sorghum the least effective.

A SPECIMEN OF *LONGIDORUS ELONGATUS* WITH TWO VULVAS FROM RUSSIA. R. T. Robbins, and T. V. Rubtsova, University of Arkansas, Department of Plant Pathology, Fayetteville, Arkansas 72701, U.S.A., and Agronomic Faculty, Moscow Agriculture Academy, Timirjazevskaja St. 49, Moscow, 127550, Russia.—A single *Longidorus elongatus* female with 2 vulvas was found in a collection from about the roots of *Artemisia campestris* in the town of Starocherkassk on the bank of the Don River in the Rostov region of European Russia by V. Chizov and T. Rubtsova in the summer of 1995. The vaginas appear to be normal in length and structure, except that they are connected by a inner-vaginal chamber (tube) of the otherwise normal appearing uteri. The morphometrics (in μm) of this individual are as follows: L = 6 604; a = 155; b = 17; c = 171; c' = 1.2; V-anterior = 49.4; lip width = 15.2; anterior end to guide ring = 33.5; odontostyle length = 91.4; odontophore length = 46.7; body width = 42.6; tail length = 38.6; hyaline length of tail = 10; anterior genital tract length = 542; posterior genital tract length = 573; distance between vulvas = 22.5. Both vulvas protrude slightly. The lip region is slightly expanded. The tail is conical with a broadly rounded tip. There are also 3 normal *L. elongatus* females and a single male on the same slide and 14 females, 10 males on accompanying slides. The morphometrics of the 3 normal females agree closely with those of the abnormal female.

1,3-DICHLOROPROPENE PRODUCT SAFETY. D. M. Roby, B. A. Houtman, M. W. Melichar, and W. T. Stott, DowElanco, Indianapolis, Indiana 46268-1054, U.S.A., and The Dow Chemical Company, Midland, Michigan 48674, U.S.A.—Over the past several years, use of 1,3-dichloropropene (1,3-D) has come under increased regulatory scrutiny. The U.S. Environmental Protection Agency initiated the reregistration and special review processes and other regulatory bodies have conducted extensive assessments on the effects of 1,3-D on human health and the quality of the environment. Resolution of product safety issues has required a comprehensive approach that includes definition of benefits and potential risk associated with 1, 3-D use, development of state-of-the-art risk refinement and management technologies, research to define effectiveness of potential elements of risk mitigation, and exposure reduction measures through labeling modifications and enhanced product stewardship efforts.

DIRECT DAMAGE AND SYNERGISTIC INTERACTIONS OF NEPOVIRUSES AND VIRUS VECTOR NEMATODES ON SMALL FRUITS. N. D. Romanenko, and A. A. Tomilin, Institute of Parasitology of the Russian Academy of Sciences, Leninskii Prospect 33, Moscow, 117071, Russia, and Settl. Kopili, Kievskaya, 17, Poltava, 315011, Ukraine.—Direct damage and synergistic interactions of virus-vector nematodes and their associated nepoviruses on strawberry and raspberry were investigated during 1989-1995 in laboratory and field experiments. The yield and height of strawberry plants infected with arabis mosaic virus transmitted by *Xiphinema diversicaudatum* were reduced 4.0 and 2.0-fold, respectively, compared with healthy, nematode-free control plants. Raspberry ringspot virus (RRSV) transmitted by *Longidorus elongatus* reduced the fruit yield and vegetative production of strawberry plants by 2.0 and 1.8-fold, respectively, as compared with the control plants. With raspberry plants, RRSV transmitted by *L. elongatus* reduced the fruit yield, the number of shoots and the height of the raspberry plants by 2.9, 3.1 and 1.3-fold, respectively. The yield and vegetative production of strawber-

ry and raspberry plants were reduced 1.5-2.0-fold more when the virus and its associated vector nematodes were present together than when either the virus or the vector nematodes were present separately. Raspberry and strawberry plants infected together with the 2 viruses and their associated vectors were only a third as vigorous as the control plants.

DISTRIBUTION OF LONGIDORID NEMATODES IN DIFFERENT SOIL TYPES IN THE FSU. N. D. Romanenko, and E. N. Romanenko, Institute of Parasitology of the Russian Academy of Sciences, Leninskii Prospect 33, Moscow, 117071, Russia, and Department of Soil Sciences, M.V. Lomonosov University, V-234, Moscow, 119899, Russia.—Ten soil types were investigated in 65 regions of the European and 19 regions of the Asian zone of the FSU. Longidorids were found in the soil types: podzol, sod podzol, grey forest, chernozem, chestnut, red, brown earth, alluvial and peat-bog. Longidorids were not found in tundra-gley soil. The largest diversity of longidorid species were found in chernozem (16 species), sod-podzol, red and brown earth soils (7-10 species) and the least in podzol, grey-forest, alluvial and peat-bog soils (2-3 species). *Longidorus elongatus* and *Xiphinema diversicaudatum* were associated with hydromorphic soils occurring in natural biotopes, and their presence was independent of climatic conditions. Also, *L. elongatus* was recovered mainly on fluviogenic soils both temperate and in semi-arid climatic zones. In automorphic soils, longidorid nematodes were found mainly in agricultural soils in which suitable host plants were growing. Longidorids were most numerous in river flood-plains, and occurred most frequently at sites adjacent to the rivers.

***AEGILOPS TRIUNCIALIS*, A SOURCE OF RESISTANCE TO *HETERODERA AVENAE* (CCN). D. Romero,[1] M. F. Andrés,[1] J. M. Balagué,[2] A. Delibes,[3] A. Duce,[1] A. Lara,[3] I. López Braña,[3] J. A. Martín Sánchez,[2] C. Martínez,[2] A. Michelena,[2] and E. Sin,[2] Centro de Ciencias Medioambientales, CSIC, Serrano 115 dupl. 28006 Madrid, Spain,[1] Centro UDL-IRTA, Rovira Roure 177, 25198 LLeida Spain,[2] and Departamento Biotecnología, ETSI Agrónomos, Ciudad Universitaria, 28040 Madrid, Spain.[3]**—*Aegilops triuncialis* (sin. *Triticum triuncialis*), with a genomic constitution CCUU, has been employed to transfer *H. avenae* resistance to *T. aestivum* (AABBDD) using *T. turgidum* (AABB) as a bridge species. One resistant line was used as a donor in crossing and further backcrossing with commercial wheat varieties. Wheat lines segregating for resistance have been obtained. The selection for resistance to CCN were carried out in greenhouse and field experiments with nematode infested soils. The results obtained are compatible with a monogenic inheritance, the gene for resistance being dominant. The presence of markers of *Ae. triuncialis* (proteins, enzymes and DNA), which are absent in wheat, and its possible linkage with the gene for resistance were studied. The relationship of this gene with resistance factors (Cre1 and Cre2), and the interactions between the nematode and other resistant/susceptible plants.

CHARACTERIZATION OF SINGLE-CHAIN ANTIBODIES DIRECTED TO SALIVARY SECRETIONS OF ROOT-KNOT AND POTATO CYST NEMATODES. M. N. Rosso,[1] A. Schouten,[2] J. Roosien,[2] T. Borst-Vrenssen,[2] R. S. Hussey,[3] F. J. Gommers,[2] J. Bakker,[2] A. Schots,[2] and P. Abad,[1] Laboratoire de Biologie des Invertébrés, INRA, BP 2078, 06606 Antibes Cedex, France,[1] Wageningen Agricultural University, Department of Nematology - L.M.A., P.O. Box 8123, 6700 ES Wageningen, The Netherlands,[2] and Department of Plant Pathology, University of Georgia, Athens, Georgia 30602, U.S.A.[3]—Expression in plants of antibodies directed to salivary secretions, and capable of inhibiting their activities by simple binding, will help understanding the molecular events taking place during the plant/nematode interaction. Furthermore, such antibodies constitute major tools for creating new forms of resistance to sedentary phytoparasitic nematodes. This strategy implies the synthesis of small size antibodies, such as single-chain antibodies (scFv) which can be expressed in different cellular locations of the plant cell. From several monoclonal antibodies selected for their activity on nematode secretions at different parasitic stages, 3 scFvs directed against salivary secretions of *Globodera rostochiensis* and 2 scFvs directed against salivary secretions of *Meloidogyne incognita* have been con-

structed *via* polymerase chain reaction (PCR). The produced scFvs have first been expressed in *Escherichia coli*. All of them proved to be active on larvae homogenates in ELISA tests. Among these, the 6D4 scFv, directed to salivary secretions of *Meloidogyne*, is highly specific and presents an affinity for its antigen at least identical to the affinity of the monoclonal. To go further in its characterization before plant transformation, the stability of the 6D4 scFv has been shown in transient expression assays by immunofluorescence and after blotting of the intra-and extracellular fractions on membranes. The observed expression yields gave promising results regarding its use in plant transformation experiments. Their expression *in planta* and their ability to block the development of the parasite are being analyzed.

EFFICACY OF SOME NIGERIAN HERBS IN THE CONTROL OF *MELOIDOGYNE INCOGNITA*. M. O. Rotimi,[1] M. Moens,[2] and R. Moermans,[2] Department of Crop Production, Federal University of Technology, Akure, P.M.B. 704, Ondo State, Nigeria,[1] and Centrum voor Landbouwkindig Onderzoek, 9820 Merelbeke, Belgium.[2]—The *in vitro* nematicidal properties of leaves of 5 Nigerian herbs, *Azadirachta indica, Cymbopogon citratus, Acacia alata, Ocimum gratissimum* and *Acalypha ciliata*, were tested on *Meloidogyne incognita*. Comparative assessment of water, methanol and n-hexane extracts showed that extract activities were influenced by the extraction procedures employed. The standard concentrations (SC) of water extracts were superior to either of the other two, while n-hexane extracts exhibited the poorest activity. In the comparative assessment of *A. ciliata* and *A. indica* extracts for their inhibition of egg hatch, the former showed superiority over the latter. Except for the *A. ciliata* extracts, SC of the water and methanol extracts of all the herbs investigated hampered nematode movement after 1 and 2 hr exposure to them. One hr exposure resulted in reversible paralysis while there was less reversible paralysis after a 2 hr exposure. SC of water and methanol extracts hindered migration of freshly hatched second-stage juveniles in sand columns up till 3 cm away from point of extract introduction. Dilution of SC of extracts reduced the activities of the extracts.

SYNERGISTIC INTERACTION OF *PRATYLENCHUS PENETRANS* AND *VERTICILLIUM DAHLIAE* FOR PHOTOSYNTHESIS AND TRANSPIRATION OF POTATO. I. A. Saeed, A. E. MacGuidwin, and D. I. Rouse, University of Wisconsin, Department of Plant Pathology, Madison, Wisconsin 53706, U.S.A.—The effects of solitary and joint infection of Russet Burbank potato by 3 inoculum densities of *Pratylenchus penetrans* and 1 inoculum dose of *Verticillium dahliae* were determined in growth chamber experiments. Nematode and fungus inoculum levels were insufficient to reduce photosynthesis or transpiration in solitary infections. The oldest leaves of plants jointly infected with both organisms exhibited significantly reduced net photosynthesis, light use efficiency, stomatal conductance, transpiration, and increased water use efficiency. Levels of intercellular CO_2 were not reduced, indicating "non-stomatal" limitations to CO_2 diffusion. Joint infection similarly affected younger leaves but to a lesser extent. The onset of reductions in photosynthesis and transpiration due to co-infection occurred prior to visual symptoms on leaves. The sensitivity of both photosynthesis and transpiration to co-infection was similar; reduction in both parameters occurred at about the same time in each of 4 experiments. However, co-infection caused more drastic reduction in photosynthesis than in transpiration. The extent of the reductions in photosynthesis and transpiration was invariant to nematode inoculum densities ranging from 0.8 to 8 *P. penetrans* per cm^3 soil.

ECOLOGICAL STUDIES ON *MELOIDOGYNE JAVANICA* AND *TYLENCHULUS SEMIPENETRANS* INFECTING GRAPES. F. M. Salem, M. E. Sweelam, G. I. Zohdy, and A. M. Atia, Economic Entomology and Agricultural Zoology Department, Faculty of Agriculture, Menoufia University, Shebin El-Kom, Egypt.—Field experiments were conducted in different grape vineyards at El-Bagour district, Menoufia Governorate, Egypt. The purpose of this research was to investigate the effect of some ecological aspects, i.e. grape varieties (Thompson seedless and Bez Elanza), training method (on wires and on arches) and vineyard ages (5 and 25 years) on the dynamics of *Meloidogyne javanica* and *Tylen-*

chulus semipenetrans populations in the soil and roots of grape. Bi-weekly soil and root samples were taken during the vegetative period of vineyards (March-October, 1991). No significant differences between *M. javanica* populations in the soil of the two ages of Bez Elanza were found, while differences in *T. semipenetrans* and opposite relations for nematodes in the roots. There were differences between *M. javanica* populations in the soil of Thompson seedless under the two training methods, while no differences were observed between *T. semipenetrans* in the soil, but significant differences between both nematode populations in the roots. There were significant differences in *M. javanica* in the soil of both grape varieties while, there were not in *T. semipenetrans*, but there were differences in both of the nematodes extracted from the roots of the grape varieties.

REVIEW OF RECENT RESEARCH CONDUCTED IN THE NEMATOLOGY LABORATORY OF NATIONAL CENTER OF PLANT AND ANIMAL HEALTH (CENSA) CUBA. L. Sánchez, M. G. Rodríguez, I. Rodríguez, L. Hildalgo, A. Iglesia, L. Gómez, and L. Castellano, CENSA, Apdo. 10, La Habana 32700, Cuba.—The research in our laboratory is divided into three areas: the diagnosis of phytonematode, (mainly root-knot nematodes), biological control of plant-parasitic nematodes and entomopathogenic nematodes in pest control. The diagnosis of root-knot nematodes parasitizing coffee crops in the central and eastern regions in Cuba was made by morphology, host test and chromosome numbers. *Meloidogyne arenaria, M. javanica, M. mayaguensis and M.* sp. were detected. In these soils two nematophagous fungi, *Verticillium chlamydosporium* var. *chlamydosporium* and *V. chlamydosporium* var. *catenulatum*, and trapping fungi, *Arthrobotrys conoides* and *A.* spp., were also detected. Through a survey, four isolates of *Heterohabditis bacteriophora* and one of *Steinernema* sp. were found. The strain HC1 of *H. bacteriophora* showed higher pathogenicity and reproductive rate in *Galleria mellonella* than others. This strain was applied successfully against coffee floury bedbug complex (*Homoptera: Pseudococcidae*) in field conditions.

EFFECT OF MOTILITY AND LOCATION OF JUVENILES IN SOIL AGGREGATES ON *PASTEURIA PENETRANS* ENDOSPORE ATTACHMENT TO *MELOIDOGYNE INCOGNITA*. Z. Sano, and J. T. Gaspard, Kyushu National Agricultural Experiment Station, Nishigoshi, Kumamoto 861-11, Japan, and Nematec Company, LTD., Miyukigaoka, Tukuba 305, Japan.—The nature of attachment of *Pratylenchus penetrans* endospores to *Meloidogyne incognita* juveniles (J2) was examined in field plots. Soil samples were collected from *M. incognita*-infested field plots where the bacteria was inoculated and susceptible crops were grown for 2 or 4 years prior to sampling. The soil samples were collected at depths of 10-15 cm and 25-30 cm in April and July, and J2 were extracted separately from inside and outside of 0.25-4 mm diam soil aggregates by a combination of sieving and centrifugal flotation technique. The extracted J2 were placed on modified Baermann funnels consisting of cotton wool filters placed in glass vials. Juveniles collected at 3 hrs were designated highly motile, and those collected at 72 hrs were designated as low motility. Attached endospores were observed and counted after staining with BBG. More endospores were attached to J2 extracted from outside soil aggregates than those extracted from inside soil aggregates regardless of soil depth or date of sampling. Also, endospore attachment was greater for high motility J2 than low motility J2. These results suggest that motility of J2 is a major factor affecting attachment of endospores to *M. incognita* in soil.

PLANT-PARASITIC NEMATODES OF RAIN-FED AND TREE CROPS IN NIGER. E. Sarr, DFPV, CENTRE REGIONAL AGRHYMET/CILSS, BP 12625, NIAMEY, NIGER.—Surveys conducted on principal rain-fed and tree crops in 23 localities of the agricultural zone of Niger revealed 25 genera of plant-parasitic nematodes. These are distributed into 12 families of which the most important are Heteroderidae, Pratylenchidae and Hoplolaimidae. The predominant genera in these families are *Meloidogyne, Pratylenchus* and *Helicotylenchus*, respectively. They were collected from more than 50% of the localities and plants sampled and generally at high population densities. This frequency and high degree of polyphagy make them primary pests in many zones and on different crops surveyed. The

principal species recorded in these genera are *Meloidogyne* sp., *M. javanica, M. incognita, Pratylenchus* sp., *P. loosi, P. brachyurus, P. scribneri, Helicotylenchus* sp.1, *Helicotylenchus* sp. 2, *H. dihystera, H. africanus, H. digonicus* and *H. abunaamai.* Other phytonematodes species such as *Tylenchorhynchus* sp., *Xiphinema savanicolla* and *Criconemella curvata* also seem to constitute a problem respectively on millet, sorghum and maize on which they were found in high population densities in some localities. *Hirschmanniella spinicaudata* and *H. oryzae* were found only on rice in high populations. The genus *Scutellonema* was found on many crops but generally at low population densities. Other recorded genera of plant parasitic nematodes seem to be less important both by population density and locality. As far as nematofauna diversity, the plant species most infested are banana, mango, cotton, millet, sorghum and maize.

PHYLOGENETIC RELATIONSHIPS OF SOME FREE-LIVING AND ZOOPARASITIC NEMATODES AS DEDUCED FROM 18S rRNA SEQUENCE ANALYSIS. P. Scheldeman, M. Blaxter, P. DeLey, and A. Reid, Instituut voor Dierkunde, Universiteit Gent, Ledeganckstraat 35, B-9000 Gent, Belgium, Institute of Cell, Animal and Population Biology, Edinburgh University, West Mains Road, Edinburgh EH9 3JT, U.K, and International Institute of Parasitology, 395a Hatfield Road, St Albans AL4 0XU, U.K.—Nearly complete sequences of the small subunit ribosomal RNA gene have been determined for *Brumptaemilius* sp. (Rhigonematida), *Heterorhabditis bacteriophora* and *Steinernema carpocapsae* (Rhabditida), *Syngamus trachea* and *Ostertagia ostertagi* (Strongylida), *Toxocara canis* (Ascaridida) and *Brugia malayi* (Spirurida). Neighbor-joining and parsimony analysis of the resultant data strongly supports 2 separate origins from rhabditid ancestry for the major zooparasitic lineages in Secernentea. Both methods suggest that a strongylid clade arose independently from anascarid/rhigonematid/spirurid clade. The two entomopathogenic genera of Rhabditida, *Heterorhabditis* and *Steinernema*, are only distantly related to each other. They actually display stronger affinities with, respectively, the strongylid clade and the ascarid/rhigonematid/spirurid clade. Relationships within free-living Rhabditida are less clearly resolved, but most of the previously analyzed species of the family Rhabdtidae are grouped in a sister clade of *Heterorhabditis* and Strongylida. Paraphyly of the order Rhabditida was not previously proposed in the literature despite the fact that it can be deduced from several morphological characters as well as our molecular data. We predict that several other orders of free-living nematodes will be shown paraphyletic, and that the main lines of current classification of nematodes can only be conserved if numerous paraphyletic taxa are allowed for.

CHARACTERIZATION OF POPULATIONS OF CYST-FORMING NEMATODES BY A POPULATION STRUCTURE-INDEX. J. Schlang, Federal Biological Research Centre for Agriculture and Forestry (BBA), Institut für Nematologie und Wirbeltierkunde, Außenstelle 50189 Elsdorf, Germany.—The rate of population decline of *Heterodera schachtii* in set aside fields planted to resistant catch crops was significantly slower when the catch crop was grown in the spring after sugar beet than after winter barley. The results indicated that hatching may be influenced by the age of the individuals in a population. Therefore, a population structure-index (psi) was developed to describe the age structure and condition of a population. The index is based on the (J) juvenility, (V) vitality and (A) age structure of an individual field population of *H. schachtii* or on another cyst-forming nematode population. Calculation of the components were as follows: (J) based on the number of newly formed females and cysts; (V) on the number of viable eggs and larvae per cyst; and (A) on the ratio of newly formed females and cysts to older cysts. These factors are then used to calculate the psi. The psi-values can be arranged in 14 log-divided classes. With assistance of the psi, it is possible to characterize field populations of cyst nematodes and to identify homologous populations. In addition, the population dynamics and population models of cyst-forming nematodes can be described more exactly.

RESISTANCE AND TOLERANCE TO STEM NEMATODE IN OATS, PEAS, AND FABA BEANS IN SOUTH AUSTRALIA. M. Scurrah, and D. Szot, South Australian Research And Development Institute, Field Crops Pathology Unit, Plant Research Centre-Waite, GPO Box SA 397, Adelaide 5001, Australia.—The oat race of the stem nematode, *Ditylenchus dipsaci*, occurs in the mid and lower north regions of South Australia, affecting 150 000 ha of cropping land. It attacks oats, faba beans and field peas. The preferred method of control is to develop resistant and tolerant cultivars. Oats are especially susceptible and intolerant. Genes for resistance have been identified and utilized. Two resistant varieties have been released to date, Echidna and Bettong. Field pea seedlings are very intolerant and susceptible: up to 50% of the plants may be killed or stunted. After about 10 weeks, plants become resistant and nematode numbers decline, and at harvest, hardly any nematodes are recovered. As a result, work has focused on trying to identify variation in seedling tolerance. Lines (800) from the South Australian germplasm collection have been screened for seedling tolerance and 12 lines are currently being tested in infested and uninfested plots. Faba beans, by contrast, exhibit seedling tolerance but adults are susceptible and will increase stem nematodes to very high numbers by the end of the season. Seed are easily infested. No symptoms are correlated with nematode multiplication and resistance is rated solely by recovering nematodes at the end of the season. Four lines selected for lower multiplication rates in the field were not significantly different in pot trials, due to large plant variation. Progeny of these lines are currently being assessed to test if lower multiplication lines can be identified. Of 28 new lines selected as promising in a first pot test, only 6 lines maintained their resistance rating. These promising lines will be reassessed both in the field and pots. So far no major genes for resistance have been detected.

MULTIPLICATION OF PLANT-PARASITIC NEMATODES ON SAHELIAN FOREST TREES AND ANTAGONISTIC EFFECTS OF ECTOMYCORRHIZAE. K. Senghor, and R. Duponnois, Laboratoire de Nématologie, ORSTOM, B.P. 1386, Dakar, Sénégal.—Plant-parasitic nematodes and mycorrhizal fungi can be commonly present in the roots and the rhizosphere of the same plants, but they have opposite effects on plant vigor. The multiplication of the root-knot nematode, *Meloidogyne javanica*, was evaluated on 7 Australian *Acacia* species in the greenhouse: *A. holosericea, A. mangium, A. hilliana, A. lysipholia, A. sclerosperma, A. trachycarpa* and *A. timuda* were all susceptible to *M. javanica*. Nematode development was greater on *A. sclerosperma, A. hilliana, A. holosericea* and *A. mangium* than on *A. trachycarpa, A. timuda* and *A. lysiphloia*. The nematode reduced the growth of *A. holosericea*, stimulated the growth of *A. timuda* and had no effect on the other species. The antagonistic effects of 31 strains of ectomycorrhizae (genus *Pisolithus*) on the viability of *M. mayaguensis* eggs were studied. Exudates of 12 *Pisolithus* spp. strains produced *in vitro* killed eggs or reduced their eclosion. The phenol concentration in the exudates was up to 0.60 mg/ml. The use of *Pisolithus* spp. to control other tylenchids that also affect the growth of some forest trees (e.g. *Eucalyptus camaldulensis*), such as *Helicotylenchus dihystera, Pratylenchus loosi, Rotylenchulus reniformis, Scutellonema cavenessi* and *Tylenchorhynchus gladiolatus*, was discussed.

SEASONAL CHANGES IN THE FAUNA OF FREE-LIVING NEMATODES OF *PHYLLOPHORA BIOCENOSIS* (*PHYLLOPHORA NERVOSA* GREV.) IN THE BLACK SEA. N. G. Sergeeva, Institute of Biology of Southern Seas, National Ac. Sci. of Ukraine, Sevastopol, Ukraine.—Nematodes of 108 species, 63 genera and 24 families are found near Sevastopol in the growths of the attached phyllofora at depths of 15 and 20 cm. The maximum quantitative development of the nematodes was observed in spring-summer, (56 000-78 000/kg of phyllofora), the minimal development was in autumn-winter. The Desmoscolecida, Chromadorida and Enoplida orders prevail in the nematode association. The structure of this association changed noticeably during the research period. Number of juveniles relative to adults varied slightly. The male-female ratio varied within a wide range (from 1:2 to 1:58). *Desmoscolex minutus, D. tenuiseta* and *Chromadorina obtusa* were among the dominant species. In the

association of the nematodes on phyllofora, all feeding groups are represented. The deposit-feeding species and epistrate-feeding forms play a leading role during the year.

THE EFFECT OF TEMPERATURE ON THE DEVELOPMENT OF *PASTEURIA PENETRANS* IN *MELOIDOGYNE ARENARIA*. **M. Serracin,[1] D. W. Dickson,[1] A. C. Schuerger,[2] and D. P. Weingartner,[3] Department of Entomology and Nematology, Gainesville, Florida 32611, U.S.A,[1] The Land, EPCOT P.O. Box 10,000, Lake Buena Vista, Florida 32830, U.S.A,[2] and University of Florida, IFAS AREC-Hastings, Florida 32145, U.S.A.[3]**—The development of *P. penetrans* isolate P-100 infecting *M. arenaria* race 2 was followed by tracking accumulated degree-days at 3 different temperatures (21, 28, and 35°C) with plants maintained in hydroponic conditions. *Pasteuria penetrans*-infected nematodes were extracted from galled roots at 100 accumulated degree-day intervals. Nematodes were placed into a drop of lactophenol containing 1% methyl blue stain, and the developmental stages of *P. penetrans* were determined with a compound microscope under oil immersion. Scanning electron microscopy was used to confirm specific life stages observed with light microscopy at selected sampling times. Five predominant developmental stages could be identified with light microscopy: germination, vegetative growth, differentiation, sporulation, and maturity. Temperature affected development and duration of the life cycle of *P. penetrans*. Mature endospores were detected at 28 and 35 cumulative days at 35 and 28°C, respectively. However, mature endospores required more than 90 cumulative days to become the predominant stage at 21°C. Number of degree-days required for *P. penetrans* to reach a specific developmental stage were different at each temperature. The stage of development against degree-days was estimated with regression. Significant differences were observed in the development of *P. penetrans* at 21, 28, and 35°C based on regression values fitted for data from 100 to 600 accumulated degree-days. A linear response was observed between 100 to 600 accumulated degree-days; however, after 600 accumulated degree-days, the rate of development leveled off. Results indicate that accumulated degree-days may only be useful in predicting early development of *P. penetrans*.

EFFECT OF SEED TREATMENT WITH DELTA-ENDOTOXINS OF *BACILLUS* SPP. ON THE MULTIPLICATION OF *HETERODERA GLYCINES* ON SOYBEAN AND CORN. R. D. Sharma, and A. C. Gomes, EMBRAPA/CPAC, Caixa Postal 08223, CEP 73301-970, Planaltina, DF, Brazil.—In two experiments, the effect of delta-endotoxins of *Bacillus sphaericus* (Bs 2362), *B. thuringiensis* var. *israelensis* (Bt-H-1), and *B. thuringiensis* var. *kurstaki* (Btk-HD-1) applied to seed of *Glycine max* cv. Cristalina (susceptible to *Heterodera glycines*) and *Zea mays* cv. Pioneer 3041 (nonhost to *H. glycines*) were evaluated on the multiplication of *H. glycines* cultured under greenhouse conditions for 26 days after sowing. Forty plastic pots were filled with 500 g of soil infested with *H. glycines* (99 cysts + 3 juveniles + 3 eggs/50 g of soil) of which half were used for soybean and the rest for corn. In soybeans, the treatments used were: 1) untreated control, 2) Bs 2362 1 g, 3) Btk-HD-1 10 g, and 4) Btk-HD-1 20 g per kg of soybean seed. In corn, the treatments used were: 1) untreated control, 2) Bti-H-14 1 g, 3) Bs 2362 10 g, and 4)Bs 2362 20 g per kg of corn seed. The percentage increase in the first generation females per plant over the untreated control in soybean roots with treatments 2, 3, and 4 were 63.2, 138.8 and 145.9, respectively. The percentage decrease of females in soil in relation to the control with treatments 2, 3, and 4 were 56.4, 53.3 and 16.7, respectively. No females were found in the corn roots and the populations in the soil were slightly reduced but remained equal regardless of the treatment. In general, seed treatment with toxins stimulated corn plant growth but a significant ($P \leq 0.05$) increase (42%) over the untreated control was observed only in treatment Bs 2362 with the 20 g dose. The stimulatory effect of delta-endotoxins in increasing the female population in soybean roots can be used to increase the efficacy of soybeans as a trap crop for the control of *H. glycines*.

BIOCONTROL EFFICIENCY OF *PASTEURIA PENETRANS* AGAINST *MELOIDOGYNE JAVANICA*. **R. D. Sharma, and L. J. Vivaldi, EMBRAPA/CPAC, Caixa Postal 08223, CEP 73301-970,Planaltina, DF, Brazil.**—In a greenhouse experiment, the efficiency of *Pasteuria penetrans* in soil obtained from a

greenhouse culture was evaluated against *Meloidogyne javanica* on soybean cv. Cristalina. Four doses of 0, 10, 50 and 100 g of infested soil with 1.10^5 endospores/g of culture soil (i.e. 0, 1×10^5, 5×10^5 and 100×10^5 endospores/pot)) were thoroughly mixed in 1 kg of autoclaved soil (w:w) per PVC pot. Immediately after mixing the *P. penetrans* culture soil, 1 000 second-stage juveniles were inoculated into each pot. Two days later, a 3-day-old soybean seedling was transplanted into each pot. Eighty- nine days after transplanting the seedlings, the maximum increase in fresh pod weight, the number of egg masses, and the final nematode population density per plant were evaluated. The fresh pod weight for treatment with 100 g culture soil/kg of autoclaved soil differed significantly ($P \leq 0.05$) from the other treatments. The increases in pod weight for 50 and 100 g treatments were respectively 18.7 and 53.7% higher than the untreated control. The number of egg masses per plant for treatments 10, 50 and 100 g were significantly ($P \leq 0.05$) lower than the untreated control.The final nematode density in soil for treatments 50 and 100 g was significantly ($P \leq 0.05$) lower than the untreated control. The final nematode population in soil for treatments 10, 50 and 100 g were 50.9, 89.1 and 81.8% lower than the untreated control.

TOLERANCE TO *ROTYLENCHULUS RENIFORMIS* IN PIGEONPEA (*CAJANUS CAJAN*). S. B. Sharma, and K. C. Jain, International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) Asia Center, Patancheru, Andhra Pradesh 502 324, India.—Pigeonpea is one of the major grain legumes of subsistence agriculture in the semi-arid tropics and the reniform nematode, *Rotylenchulus reniformis*, is an important parasite of this crop. Fifty-five short duration (maturity in 100-150 days) and 46 medium-duration (151-200 days) pigeonpea lines were evaluated between 1987 and 1994 for resistance and tolerance to the nematode in greenhouse and field trials. Shoot and root masses of each genotype in nematode-infested and nematode-free soils were compared and roots were screened for number of nematode egg sacs on a 1 (no egg sacs = highly resistant) to 9 (>50 egg sacs = highly susceptible) scale. Plant biomass production in carbofuran and dibromochloropropane treated plots was compared with that in nontreated plots in a field naturally infested with *R. reniformis*. Three pigeonpea genotypes, ICPL 87, ICPL 227, and ICPL 270, were used as nematode susceptible checks. Genotypes with good growth both in nematicide as well as in control treatments were identified and evaluated for plant growth and yield for 3 years. All short- and medium-duration lines were susceptible; however, two short-duration lines, ICPL 83024 and ICPL 85045, seven medium-duration lines, ICPL 8357, 85068, 85073, 87911, 89050, 89051, and 90097 showed tolerance and their growth and yield was much better than other lines. Single plant selections in medium-duration genotypes were made. Progenies from individual plants were evaluated for plant biomass and seed yield and promising lines were selected based on their uniformly good growth in the nematode-infested soil.

THE REPLACEMENT OF METHYL BROMIDE IN TOBACCO SEEDBEDS IN ZIMBABWE. J. A. Shepherd, Tobacco Research Board, P.O. Box 1909, Harare, Zimbabwe.—The most important nematode pest of tobacco in Zimbabwe is the root-knot nematode, *Meloidogyne javanica*. Anything less than complete control of this nematode in the seedbeds, as is currently possible with methyl bromide fumigation, can lead to severe losses of yield in the field and to the break down of the field control program. Alternatives to methyl bromide are being tested since it may not be available after 2001 A.D. Dazomet at rates up to 75 g/m^2 was found to provide inadequate nematode control. The combination of ethylene dibromide (41% m/m) at 35 ml/m^2 and dazomet at 30 g/m^2 gave complete nematode and adequate weed control.

APPLICATION OF A PLANT-INDICATOR METHOD FOR REVEALING ELEMENTS OF EPIPHYTOTIC FOCI OF *GLOBODERA ROSTOCHIENSIS* (GLOBODEROSIS). A. A. Shesteperov, All-Russian K.I. Skrjabin Institute of Helminthology, Bloshaya Cheremushkinskaya Str., 28. Moscow, 117259, Russia.—The first discovery of *Globodera rostochiensis* (*G.r.*) infection on individual land in the village of Uljahino, Vladimir region was observed in 1982. After 13 years (1995) all individual and some in-

dustrial land lots were infested with *G. rostocheinsis* in this village. Cysts were discovered in gardens, orchards, hothouses, potato storehouses, pigsties and dung. Females of *G. rostochiensis* were detected on roots of indicator plants transplanted near houses and roads (dust-holes, heaps of rubbish and others) and near the river, reservoir, brook and ravines. Live *G. rostochiensis* juveniles were discovered near a cattle-breeding complex. Additionally, specimens of *G. rostochiensis* were found on garage territories, a park of agricultural techniques, meliorative ditches and other places. The duration of existence of *G. rostochiensis* epiphytotic foci depends on long vitality of juveniles in cysts (more than 6-7 years) in the absence of susceptible host plants.

ANALYSIS AND PURIFICATION OF SUBVENTRAL ESOPHAGEAL GLAND SECRETORY PROTEINS FROM THE POTATO CYST NEMATODE *GLOBODERA ROSTOCHIENSIS*. **G. Smant, A. Goverse, J. W. P. G. Stokkermans, J. M. de Boer, H. A. Overmars, J. F. Zilverentant, F. J. Gommers, J. Helder, A. Schots, and J. Bakker, Wageningen Agricultural University, Department of Nematology and Laboratory for Monoclonal Antibodies, P.O. Box 8123, 6700 ES Wageningen, The Netherlands.**—Antigens localized in the stylet secretions of the potato cyst nematode, *Globodera rostochiensis*, were characterized by two-dimensional gel electrophoresis and Western blotting. Six protein species on a Western blot of a protein extract of preparasitic juveniles were recognized using a monoclonal antibody directed to the contents of secretory granules in the subventral esophageal glands. The proteins focused in the pH gradient between 6.8 and 8.6, with estimated molecular masses between 30 and 49 kDa. At least one protein species is N-glycosylated. Secretion of the antigens is influenced by the hatching environment of the juvenile. No antigens emanate from the stylet tip when juveniles are hatched in tapwater, while juveniles hatched in root exudates of potato (*Solanum tuberosum*) produce large quantities of secretions containing the antigens. We have devised a protocol for purification of the antigens from a homogenate of juveniles using preparative continuous flow gel electrophoresis and affinity chromatography.

THE STRUCTURE OF SYNCYTIA IN *ARABIDOPSIS THALIANA* INDUCED BY *HETERODERA SCHACHTII* AND ITS RELEVANCE TO THE SEX OF THE NEMATODE. **M. Sobczak,[1] F. M. W. Grundler,[2] and W. Golinowski,[1] Katedra Botaniki, SGGW, Warsaw, Poland,[1] and Institut fuer Phytopathologie, Universitaet Kiel, Germany.[2]**—Light and electron microscopic studies were performed to clarify the relation between the structure of the syncytia in *Arabidopsis thaliana* and the sex of *Heterodera schachtii*. Conditions could be established which induced a proportion of more than 90% males of all developing juveniles, compared to others that promoted female development. Females developed on syncytia induced in procambial cells. These were preferentially selected for induction. Under male inducing conditions, initial syncytial cells in the procambium mostly degenerated, whereas in the pericycle functional syncytia differentiated. Female-related syncytia consisted of few strongly hypertrophied cells, whereas syncytia related to males differentiated from many weakly hypertrophied cells. A number of ultrastructural features were found in both types of syncytia, e.g. a condensed cytoplasm, an increased amount of organelles and the loss of a central vacuole replaced by numerous cytoplasmic vacuoles. These were more frequent in syncytia related to male juveniles. Remarkably, cell wall protuberances and wall dissolutions were only found in syncytia of females.

NEMATODE BIODIVERSITY: A POSSIBLE INDICATOR OF SOIL ENVIRONMENT HEALTH. **N. Somasekhar, and U. K. Mehta, Nematology Section, Sugarcane Breeding Institute, Coimbatore-7, India.**—The relative biomass and biodiversity of nematodes in 3 different ecosystems representing relatively undisturbed, less-disturbed and frequently disturbed ecosystems has been studied as a reference base for interpreting their relationship with soil health. Free-living and plant-parasitic nematodes were extracted from 3 composite soil samples (each consisting of 10 random samples/acre) collected from each of the 3 representative ecosystems. The undisturbed ecosystem represented by soils of evergreen forests of Nilgiris consisted of 81% free-living nematodes, 19% plant-parasitic

nematodes. Significantly high population of free-living nematodes in this ecosystem reflects its richness in organic matter as well as good soil condition that supports large populations of microflora upon which these nematodes feed. The less-disturbed ecosystem represented by soils of open grassland contained 58% free-living nematodes and 42% plant-parasitic nematodes, and the frequently-disturbed ecosystem represented by a cultivated field in which sugarcane was grown for more than 5 years had 24% free-living nematodes and 76% plant parasitic nematodes (of this 80% belongs to a single species, *Pratylenchus zeae*). Significantly high populations of plant-parasitic nematodes in this ecosystem reflected poor organic matter status of soil due to intensive cropping patterns, elimination of natural enemies because of the extensive use of agrochemicals and significant reduction in nematode biodiversity which act as stabilizing factors in natural ecosystems. These preliminary observations indicate that nematode biodiversity is highly influenced by changes in soil environment and can be used as bioindicators of soil environment health.

EVALUATION OF *MUSA* HOST PLANT RESPONSE TO *RADOPHOLUS SIMILIS* AND *HELICOTYLENCHUS MULTICINCTUS* USING NEMATODE DENSITIES AND DAMAGE INDEX. P. R. Speijer, and F. Ssango, International Institute of Tropical Agriculture, East and Southern Africa Regional Center, P.O. Box 7878, Kampala, Uganda.—Plant-parasitic nematodes are a major constraint to *Musa* production. Host plant resistance to nematodes could contribute to sustainable production of the crop. Nematode reaction in *Musa* germplasm can be assessed using nematode densities in a standard subsample of the root system and a damage index, which is a combination of root and rhizome health indices. This method, used at the International Institute of Tropical Agriculture, revealed increasing nematode susceptibility of Pisang Awak (*Musa* AAB), Sukali Ndizi (*Musa* AB) and Gros Michel (*Musa* AAA), the Highland cultivars Nabusa and Mbwazirume (*Musa* AAA), and Valery (*Musa* AAA) ad Obino l'Ewai (*Musa* AAB). Nematode densities per 100 g fresh root weight for *R. similis* ranged from 390 for Gros Michel to 33 650 for Valery and for *H. multicinctus* from 1 020 for Pisang Awak to 17 920 for Obino l'Ewai. Damage index ranged from 32.9 for Gros Michel to 83.3 for Valery.

ACCUMULATION OF WHEAT GERM AGGLUTININ AND BARLEY LECTIN IN WHEAT AND BARLEY ROOTS INFECTED WITH *HETERODERA AVENAE*. Y. Spiegel,[1] I. Chet,[2] and Y. Oka,[1] Department of Nematology, Agricultural Research Organization, Bet-Dagan 50250, Israel,[1] and Department of Plant Pathology and Microbiology, The Hebrew University of Jerusalem, Rehovot 76000, Israel.[2]—Wheat (*Triticum aestivum*) lectin, wheat germ agglutinin (WGA), and WGA-like lectins have been found in several cereals, mainly in seed embryos, but also in root tips of young seedlings. When wheat and barley (*Hordeum vulgare*) roots were inoculated with the cereal cyst nematode (CCN), *Heterodera avenae*, WGA and the barley lectin accumulated in resistant and susceptible cultivar roots. In non-infected roots, amount of the WGA and barley lectin declined rapidly 4 days after seed germination, whereas in nematode-infected roots, the amount of the lectins declined slowly. In nematode-infected wheat roots, a WGA precursor was observed 4 days after inoculation, whereas in barley roots, this precursor was not detected. Immunohistological studies revealed the presence of WGA in root tip epidermal cells and the nematode feeding site (syncytium) region. Commercial WGA bound to the surface of *H. avenae*. In wheat roots inoculated by another CCN, *H. latipons*, which invades mature roots, WGA did not accumulate, and the commercial WGA did not bind to the surface of *H. latipons*. It is suggested that the lectins may play a role in *H. avenae*-plant interaction, and to our knowledge, this is the first report of direct involvement of a lectin in a plant-nematode interaction.

ATTACHMENT OF *PASTEURIA PENETRANS* SPORES TO THE SURFACE OF *MELOIDOGYNE JAVANICA* SECOND-STAGE JUVENILES. Y. Spiegel, M. Mor, and E. Sharon, Department of Nematology, Agricultural Research Organization, Bet Dagan 50250, Israel.—*Pasteuria penetrans* spore adhesion to *Meloidogyne javanica* second-stage juveniles (J2) was examined following several pretreatments of the latter. The detergents sodium dodecyl sulfate and Triton X-100, the carbohydrates fucose and

methyl-D- mannoside and the lectins con-canavalin A and wheat germ agglutinin reduced spore attachment. Spores exposed to *M. javanica* surface coat (SC) extract exhibited decreased adherence to the J2 surface. J2 which had been treated with antibodies recognizing a 250-kDa antigen of J2 SC extract had many less spores attached to their surfaces, as compared to non-treated J2, except for the head region. This inhibition pattern was similar to that of antibody-labelling on *M. javanica* J2 as observed by electron microscopy. It is suggested that several SC components, such as carbohydrate residues, carbohydrate-recognition domains and the 250-kDa antigen, are involved in *P. penetrans* spore attachment to the surface of *M. javanica*.

ASSESSMENT OF INTRASPECIFIC VARIATION WITHIN *GLOBODERA* SPP. USING MICROSATELLITE REPEATS. P. R. Stancombe, M. J. McPherson, and H. J. Atkinson, Centre for Plant Biochemistry & Biotechnology, University of Leeds LS2 9JT, U.K.—We have investigated intraspecific variation in U.K. populations of *Globodera* spp. Study of microsatellite DNA proved to be of particular value. Oligonucleotide primers complementary to these repeats were used in polymerase chain reactions (PCR). Some of the primers amplified discrete products from genomic DNA. Sequencing of a number of PCR products revealed clusters of non-complementary, microsatellite repeats in the genome. Hybridization of these PCR products to oligonucleotide probes of different microsatellites highlighted a sub-set of products. Profiles proved to be reproducible and to identify intraspecific polymorphisms within *Globodera* spp. This will form a basis for assessing genotypic variation in U.K. field populations.

EVALUATION OF WEEDS AND COVER CROPS AS HOSTS FOR BANANA NEMATODES IN HONDURAS. R. Stoffelen, J. Kestemont, M. T. Castro, and D. De Waele, Laboratory of Tropical Crop Improvement, Katholieke Universiteit Leuven, Belgium, and Standard Fruit De Honduras, La Ceiba, Honduras.—The host suitability of the following weeds and cover crops to banana nematodes was tested: *Geophila repens, Arachis pintoi, Syngonium podophylum* (conde), *Sorghum bicolor* (forage sorghum), *Sorghum sudanense* (black sorghum), *Tagetes erecta* (french marigold), *Medicago sativa* (alfalfa). In a first experiment, these plants and Ecuador Dwarf banana tissue plants were planted in plastic bags containing field soil infested with nematodes. After 4 weeks, *Radopholus similis, Helicotylenchus multicinctus, Meloidogyne* spp. and *Rotylenchulus reniformis* were extracted from the roots. Based on the number of nematodes per root unit, the weeds and cover crops were moderate hosts with only *Tagetes erecta* being a poor host. In a second experiment, the crop residue effects were evaluated. Only the residues of *Medicago sativa* suppressed the nematode infection of subsequently grown banana tissue plants. In a third experiment, the weeds and cover crops were grown together with banana tissue culture plants in field soil infested with nematodes. The presence of the weeds and cover crops did not affect the nematode densities per banana root unit.

THE USE OF *TAGETES ERECTA* AND *PASTEURIA PENETRANS* FOR THE CONTROL OF *MELOIDOGYNE* SPECIES IN ZIMBABWE. V. Stubbs, and S. R. Gowen, Department of Agriculture, University of Reading, Reading, RG6 2AT, U.K.—*Meloidogyne* species are serious pests of many crops in Zimbabwe. As the use of chemical control measures is increasingly restricted, the need for effective alternative management strategies becomes ever more urgent. *Pasteuria penetrans*, which is found in many parts of the world including Zimbabwe, is a naturally occurring bacterial parasite of *Meloidogyne* spp. *P. penetrans* has been used alone and in conjunction with a commercially important cultivar of *T. erecta* in a series of crop cycles in microplots. Tomato (*Lycopersicon esculentum*), pea (*Pisum sativum*), and maize (*Zea mays*) were included in the rotations. Populations of *Meloidogyne* spp. were not substantially reduced where *P. penetrans* was applied. *Tagetes erecta* roots did not support reproduction of *Meloidogyne* spp. Although the number of juvenile *Meloidogyne* in the soil was considerably reduced after *T. erecta*, the nematode population increased rapidly in the subsequent tomato crop. In spite of this,

tomato yield was greater after *T. erecta* than after peas. *T. erecta* may be effective in the suppression of *Meloidogyne* populations when it is integrated with other control measures.

EFFECT OF *MELOIDOGYNE* SPP., *MACROPHOMINA PHASEOLINA* AND *FUSARIUM OXYSPORIUM* ON *PSIDIUM GUAJAVA* L. AND THE INDUCED HISTOPATHOLOGICAL CHANGES. Z. Suárez H., L. C. Rosales, A. Rondón, and M. S. González, Dentro Nacional de Investigaciones Agropecuarias, Departamento Protección Vegetal, Apdo. 4653, Maracay 2101, Aragua, Venezuela.—Guava plantations in Zulia State, Venezuela, are frequently infested with *Meloidogyne* spp.(Me), *Macrophomina phaseolina* (Ma) and *Fusarium oxysporum* (Fu). The objective of this study was to determine the effect of these pathogens on the development of guava plants. Treatments were arranged in a split-plot design with soil moisture as the main factor and pathogen inoculations as the subunits. Treatments were inoculated with *Meloidogyne* (5 070 juveniles and/or eggs/plant), *Macrophomina* (3.9×10^6 conidia/cm^3/plant) and *Fusarium* (1.1×10^6 propagules/cm^3/plant) alone or in combination nematode-fungus, (Me + Ma) and (Me + Fu), and a control treatment (uninoculated). Nematodes were inoculated one month prior to the fungi. Plants were harvested 17 weeks after inoculation. Plant height (cm) and fresh weight and dry weight of the shoots and roots were measured. Data were analyzed using ANOVA, and means were separated by Duncan's multiple range test. Histopathological studies were done fixing roots in Craff III. Roots were then dehydrated in a terbutanol series, embedded in paraffin, cut in 15µm sections and dyed with Quadruple Triarch's Stain modified by Suárez. Statistical differences were significant among subunits but not significant between main plots for all variables tested. There were differences in the height of the plants only in the first 4 weeks after nematode infection. Me + Ma and Me + Fu resulted in the lowest fresh weight of the shoot, while all treatments with nematodes had the greatest root fresh weight. Histological sections showed abundant starch grains in the xylem of Fu-inoculated plants. In plants inoculated with Me + Fu, fungal mycelia invaded giant cells, and cortical parenchyma was disintegrated. The plants with only Me showed normal giant cells. Me + Ma and Me + Fu caused a higher detrimental effect than that observed for Me, Ma or Fu alone.

BIOECOLOGY OF *PRATYLENCHUS ZEAE* IN SUGARCANE. P. Sundararaj, and U. K. Mehta, Nematology Section, Sugarcane Breeding Institute, Coimbatore - 7, India.—Lesion nematodes, *Pratylenchus* spp., are the most important pathogens of sugarcane in India. Investigations were carried out on different aspects of bio-ecology of this nematode in the sugarcane ecosystem. Survey results (5 000 samples) showed that *Pratylenchus spp.* are the predominant nematode pests of sugarcane in all 8 sugarcane growing states of India, with percent occurrence being maximum in Tamil Nadu and minimum in Maharashtra. Seven species of *Pratylenchus* (*P. brachyurus, P. coffeae, P. dellaterei, P. neglectus, P. pratensis, P. scribneri* and *P. zeae*) were identified from 21 sugar factory zones of South India. In the majority of zones, *P. zeae* showed a negative association with *Hoplolaimus indicus* and *Helicotylenchus dihystera* and a positive association with *Tylenchorynchus annulatus*. Studies on the population dynamics of *P. zeae* over 5 years (20 520 samples) at 2 depths, (20 cm and 40 cm), and 3 distances (0, 25, and 45 cm) from base of the sugarcane clump indicated the occurrence of peak populations at the eighth and tenth month of crop age. Further, the 20 cm depth from the base of the clump was found to be the appropriate zone for sampling. A progressive increase in the total nematode population was discernible due to monoculturing of sugarcane. Yield losses due to *P. zeae* was up to 23% in cv. Co 6304 and 30% in cv. CoC 671. Interaction of *P. zeae* with *H. indicus* and *T. annulatus* on sugarcane crops indicated that when these 3 nematodes were inoculated together, the damage to the plant was more than when any were inoculated alone. Root penetration studies indicated that all stages of *P. zeae* from egg to adult can be located inside the roots, suggesting the ability of the nematode to complete its life cycle within sugarcane roots. Maximum damage was observed in the cortical cells. Effect of temperature, soil type and cultivars on penetration of *P. zeae* into roots indicated that the maximum penetration occurred between 23-27°C in clay soil. Rate of penetration was maximum in cv. CoC 671 followed by cvs. Co 6304, CoC 8501 and Co 8021, respectively.

EFFICACY OF ABG-9008 ON THE BURROWING NEMATODE OF BANANAS. T. B. Sutton, D. H. Marin, and K. R. Barker, Department of Plant Pathology, North Carolina State University, Raleigh, North Carolina 27695-7616, U.S.A.—ABG-9008 is a biological nematicide of microbial origin with potential for control of several plant-parasitic nematodes in different horticultural crops. Efficacy of ABG-9008 to control the burrowing nematode of bananas (*Radopholus similis*) was evaluated using direct contact assays, root-penetration assays, and greenhouse tests. Nematodes were incubated for 48 hrs at 25°C in suspensions of ABG-9008 corresponding to 56, 112 and 224 kg/ha on a broadcast basis. Suspensions of ABG-9008 at 1.5 and7.5% w/v also were included. Root-penetration assays were performed at the same rates of ABG-9008 with banana-root segments in microtiter plates. Efficacy of ABG-9008 was evaluated under greenhouse conditions by applying it at day 0 and 5 after inoculation. Water and fenamiphos (4.5 kg a.i./ha) were included in all experiments as standard controls. Percentages of mortality in suspensions, which ranged from 16.8 to 24.6%, were not statistically significant between those for 56, 112 and 224 kg/ha and 1.5% of ABG-9008. Percentages mortality for fenamiphos (76.4) and 7.5% ABG-9008 (69.8) did not differ statistically. Nematode recovery was greatly suppressed after 48 hrs of incubation of infected banana roots exposed to 7.5% ABG-9008 or fenamiphos and was significantly greater than the other ABG-9008 rates used.

***PAECILOMYCES LILACINUS* AS A POTENTIAL CONTROL AGENT FOR *ROTYLENCHULUS RENIFORMIS*. M. E. Sweelam, and M. A. Mostafa, Economic Entomology and Agricultural Zoology Department, Faculty of Agriculture, Menoufia University, Shebin El-Kom, Egypt, and Plant Pathology Institute, Agricultural Research Center, Giza, Egypt.**—The use of the nematophagous fungus, *Paecilomyces lilacinus*, as a biological control agent against reniform nematode, *Rotylenchulus reniformis*, on 6 cotton cultivars (Giza 45, 75, 76, 80, 81 and 84) was evaluated under glasshouse conditions. There were different rates of susceptibility of the cotton cultivars to reniform nematode. The fungus effectively controlled the reniform nematode in cotton cultivars. Results showed that the fungus allowed a significant decrease in nematode population counts of more than 60 percent compared with the non-treated pots in both soil and roots of all cotton cultivars ($P \leq 0.05$). Data also showed that there were significant differences ($P \leq 0.05$) in the numbers of fungal colony-forming units per g of soil between cotton cultivars with better results obtained from Giza 45 and 75.

POPULATON STRUCTURE AND SYSTEMATICS OF POTATO CYST NEMATODES USING rDNA SEQUENCE POLYMORPHISMS. A. L. Szalanski,[1] C. C. Fleming[2] and T. O. Powers,[1] Department of Plant Pathology, University of Nebraska, Lincoln, Nebraska 68583-0722, U.S.A.,[1] and Nematology Section, Applied Plant Science, Food and Agriculture Centre, Newforge Lane, Belfast BT9 5PX, North Ireland.[2]—An 824 bp region of the nuclear ribosomal DNA repeating unit, including ITS1 and a portion of the flanking 18 S and 5.8 S genes, was sequenced to determine the variability and genetic population structure of *Globodera pallida* and *G. rostochiensis*. Sequence analysis of 9 *G. pallida* and 10 *G. rostochiensis* populations from Europe, South America and North America revealed approximately equivalent levels of genetic variability in both species. *G. pallida* intraspecific variation averaged 1.8% (range 0.3-3.4%) and 2.2% (range 0.4-3.9%) for *G. rostochiensis*. Interspecific variation in PCN averaged 2.8% (range 1.6-5.8%). Thirteen polymorphic nucleotide sites were found to be fixed within species, and 2 of these species-specific changes could be detected using PCR-RFLP analysis. No correlation was detected between geographic location and level of genetic similarity. Phylogenetic analysis supports the view of multiple long distance dispersal events for PCN. *G. tabacum* and several Mexican cyst isolates formed a distinct lineage closely related to both PCN species. Average genetic distances for the Mexican isolates were 2.8% and 2.6% for *G. pallida* and *G. rostochiensis*, respectively.

EFFECT OF NEMATICIDES ON THE DEVELOPMENTAL BIOLOGY OF FREE-LIVING NEMATODES. Q. Tahseen, M. S. Jairajpuri, and I. Ahmad, Section of Nematology, Department of Zoology, Aligarh Muslim University, Aligarh 202002, India.—The study was undertaken mainly to analyze the

impact of commonly used nematicides on non-target organisms which constitute beneficial soil meiofauna. Nematicides, (carbofuran, phorate, aldicarb, posse and endosulfan) were tested at 50 ppm, 100 ppm, 150 ppm and 200 ppm concentrations to determine their impact on the developmental biology of 2 species of soil nematodes, *Cephalobus persegnis* and *Mesorhabditis cranganorensis*. At 200 ppm, all nematicides inhibited all the reproductive activities of the nematodes. Eggs treated with this concentration developed for a certain period of time but later became non-viable. The normal life span of 20-22 days of *M. cranganorensis* was reduced to a minimum of 7-8 days in 50 ppm endosulfan. The 150 ppm concentrations of all nematicides except carbofuran inhibited the egg laying process. The maximum number of eggs laid by the females treated with 50 ppm concentration of nematicides was less than half of that laid normally. A 2½ times increased embryonation period was observed in *C. persegnis* eggs treated with 50 ppm of the nematicides. Single-cell eggs placed in 100 and 150 ppm concentrations could not develop beyond gastrulation. However, the eggs treated with the same concentrations of nematicides at gastrulation, hatched successfully. In *M. cranganorensis*, the life cycle was prolonged for 10-12 days from the normal when treated with 50 ppm of the nematicides.

DEPTH DISTRIBUTION AND SOIL SAMPLING TECHNIQUES FOR *PRATYLENCHUS NEGLECTUS* IN CALCAREOUS SANDS AND CLAY VERTISOLS OF SOUTH AUSTRALIA. S. P. Taylor, and M. L. Evans, South Australian Research and Development Institute, GPO Box 397, Adelaide 5001 & Mallee Research Centre, Walpeup, Victoria 3507, Australia.—The root lesion nematode, *Pratylenchus neglectus*, is a migratory endoparasite which infects the root cortex of many cereal, legume and oilseed crops in the semi-arid dryland cropping regions of southern Australia. The nematode survives anhydrobiotically in dry soil and appears susceptible to mechanical damage caused by soil disturbance while in this state. The effect of soil sampling on recovery of nematodes was assessed by using an auger, undisturbed cores and a commercial soil sampler ("Arborline corer") in wet and dry sands, sandy loams and clay vertisols. In dry soil of all types, significantly higher numbers of nematodes were recovered using the undisturbed core wet to field capacity than the auger. While higher numbers of nematodes were recovered using the undisturbed core plus water than the commercial soil sampler, differences were not significant. In soil naturally wetted to field capacity, there was no significant difference in nematode numbers recovered using any sampling technique. The distribution of nematodes in the soil profile was also determined in wet and dry soil. Nematodes were sampled to a depth of 60 cm in sands and to 90 cm in clay vertisols with 73% and 42% of the population recovered in the top 10 cm of the profile from the sand and clay types, respectively. Significantly higher numbers of nematodes were recovered after soil was wet to field capacity although percentages in each sampling depth were the same. The procedure used for sampling *P. neglectus* will depend on soil moisture and soil type in the area to be sampled.

PUTATIVE MICROCINE PRODUCED BY BOTH PHASE ONE AND PHASE TWO OF *XENORHABDUS NEMATOPHILUS* F1. J.-O. Thaler, and N. E. Boemare, Laboratoire de Pathologie Comparée, INRA-CNRS URA 1184, Université Montpellier II, 34095 Montpellier Cedex 5, France.—Bacteria symbiotically associated with insect pathogenic nematodes are known to produce several antibiotics. *Xenorhabdus* and *Photorhabdus* strains spontaneously occur in two colony form variants known as phase variants. Phase 1 variants absorb dyes on agar plates, produce exo-enzymes and antibiotics, while these properties are absent in phase 2. This report shows that in contrast with other bacterial species associated with nematodes, both phases of *X. nematophilus* strains could inhibit a wide range of microorganisms. The antibiotic activity of *X. nematophilus* F1/1 and F1/2 bacterial cultures was tested by spotting aliquots from the culture supernatant onto lawns of a bacterial indicator. A clear zone on the bacterial lawn at the drop location indicated an inhibition activity. For further investigations, samples of the culture supernatant were treated by heating or with proteases. In all strains of *X. nematophilus*, controlled-phase two form variants were always able to inhibit *M. luteus*. Phase two antibiosis was always less important than that of phase one and was heat-stable (30 min at 100°C) and protease-sensi-

tive (pronase E). These properties indicated that inhibitory activity of the phase two was probably due to a peptidic antibiotic. Moreover, chloramphenicol-treated culture of *X. nematophilus* F1/2 failed to inhibit *M. luteus*. Therefore, we think that *X. nematophilus* strains could produce a putative microcine (ribosomally-synthetised peptide antibiotic). *X. nematophilus* F1/1 produces two kind of antibiosis against *M. luteus*. One was similar to phase 2 activity, while the other was protease-resistant and was lost after extended dialysis. This second compound probably corresponds to the previously characterized antibiotics xenocoumacins which are only produced by the phase one of *X. nematophilus* All. Experiments are in progress to understand the occurrence of an antibiotic production by phase two form variants on the symbiotic relation between *Xenorhabdus nematophilus* and *Steinernema carpocapsae*.

PHYLOGENY OF *GLOBODERA* SPECIES FROM SOLANACEAE. M. Thiéry, D. Mugniéry, and M. Bossis. INRA, Laboratoire de Zoologie, BP 29, 35650 France.—Relationships between species of *Globodera* were studied by their host range, cross experiments, 2-D PAGE of females, RFLP of rDNA and RAPD. Tobacco species and cultivars discriminate 3 groups in *G. tabacum sensu lato*. On PB D6, only 2 French populations develop. Coker 254 is a very good host only for *G. tabacum solanacearum*. *G. tabacum virginiae* have the most variable populations. Results of *in vitro* cross experiments show 4 clear genetic groups: *G. rostochiensis*, *G. pallida*, *G. tabacum sensu lato* and *G. mexicana*. This last species is able, as males, to hybridize with *G. pallida* and *G. tabacum solanacearum*, giving viable and fecund hybrids. This cytoplasmic incompatibility is more or less strong, according the populations used. With the *G. pallida* Duddingston population Pa1, this incompatibility is nearly absent. 2-D PAGE confirm these 4 groups, with *G. pallida* and *G. mexicana* being close. The variability of *G. pallida* was studied from 15 European populations with quantitative analysis of proteins (present-absent) and a cluster was built. Quantitative variations of proteins were detected in some populations. Clusters from data of RFLP on rDNA-ITS and the 5.8S gene confirm the existence of the four groups, *G. mexicana* being close to *G. pallida*, but no difference between populations was detected. Clusters from RAPD data also confirm this phylogeny and show clear differences between populations. The differences detected between populations are in good agreement with data from hybridizations and host range: the Duddingston population is far away in the *G. pallida* group, the 2 French populations of *G. tabacum* and *G. tabacum solanacearum* are clearly separate from each other.

SUSCEPTIBILITY OF OKRA ACCESSIONS PREVIOUSLY REPORTED RESISTANT TO *MELOIDOGYNE INCOGNITA*. J. A. Thies, U.S. Vegetable Laboratory, USDA ARS, Charleston, South Carolina, U.S.A.—Forty-five okra accessions previously reported as resistant to *M. incognita* race 3, or to unidentified species or races, were evaluated in replicated greenhouse tests for resistance to *M. incognita* race 3. Seed coats of most accessions were hard and germination was erratic. Therefore, the seed was pregerminated to ensure even seedling emergence. Seed was soaked in 1% NaOCl for 20 min, rinsed in running tap water for 10 min, and the seed coat was pierced with a pin. Seed of each accession were placed in the bottom of a petri dish that had been lined with filter paper and moistened with 3 ml sterile water. Each petri dish was covered and incubated for 2 days at 28°C. On 14 October 1994, 3 pregerminated seeds per entry were planted on 12-cm-centers in greenhouse benches containing steam-sterilized sand and soil mixture. Each seed was inoculated with 1 500 eggs of *M. incognita* race 3. The experimental design was a randomized complete block design with 6 replicates. Greenhouse temperatures were maintained at 27+/-7°C with natural daylight. On 15 November, plants were lifted from soil, roots were washed, and scored for gall severity and egg mass production using a 1-5 scale (1=0 to 3% of root system galled or covered with egg masses; 5=81 to 100% of root system affected), and eggs were extracted from roots. Gall and egg mass indices ranged from 4.4 to 5.0 and 3.6 to 4.3, respectively. Numbers of eggs/g fresh root ranged from 76 000 to 119 000. The experiment was repeated with similar results. We concluded that all okra accessions tested were susceptible to our isolate of *M. incognita* race 3. Differences in our results and those previously reported may be attrib-

uted to: (1) differences in nematode isolates; (2) delayed seed germination that may have allowed okra roots to escape nematode infection.

SCREENING ISOLATES OF THE FUNGUS ARF 18 FOR EFFICACY AGAINST THE SOYBEAN CYST NEMATODE. P. Timper, and R. D. Riggs, Department of Plant Pathology, University of Arkansas, Fayetteville, Arkansas 72701, U.S.A.—Twelve fungi isolated from eggs of soybean cyst nematode (SCN) and superficially resembling ARF 18 (non sporulating, white mycelium) were compared for their ability to suppress number of SCN eggs. The fungi were grown for 7 days on a shaker in liquid pea juice medium, harvested on a filter, homogenized for 30 seconds, and added to heat-treated soil (1 g fungus/500 cm^3 soil). Heat-treated soil without fungus served as a control. The fungus-infested and uninfested soil was packed into 45-cm^3 conical pots (16.5-cm-high × 2.5-cm-diam) and planted with a soybean seedling cv. Lee. One week later, a suspension of 250 SCN second-stage juveniles was added to the soil surface. Juveniles and cysts were extracted from roots and soil 30 days after inoculation. Cysts were crushed in a tissue grinder, and the number of eggs and second-stage juveniles determined. Most of the fungal isolates suppressed SCN numbers; however, one isolate, designated TN 14 from Tennessee, was significantly more effective than were the other isolates.

THE INCIDENCE OF *PASTEURIA PENETRANS* IN ECUADOR AND SOME RESULTS OF IT'S FIELD DEPLOYMENT AGAINST ROOT-KNOT NEMATODES. C. Trivino, and S. R. Gowen, Department of Agriculture, The University of Reading, Earley Gate, PO Box 236, Reading, RG6 6AT, Berkshire, U.K.—An extensive survey of *Meloidogyne* spp. and *Pasteuria penetrans* in all regions of Ecuador has been completed. Of the 207 samples of root-knot nematodes taken, *P. penetrans* was found to be present at approximately 30% of the sites. In a microplot experiment in which *P. penetrans* was applied initially to some plots, 90% of the root-knot nematodes recovered from soil samples at the completion of 5 crop cycles had between 1-9 spores attached to their cuticles. Galling indices on host plant roots and numbers of J2 in soil were significantly less in plots in which *P. penetrans* had been applied.

THE PRODUCTION, SELECTION, IDENTIFICATION AND MANAGEMENT OF VIRULENT POPULATIONS OF POTATO CYST NEMATODES. S. J. Turner, and C. C. Fleming, Department of Agriculture for Northern Ireland, Applied Plant Science Division, Newforge Lane, Belfast BT9 5PX, N. Ireland, U.K.—The use of PCN resistant potato varieties has obvious advantages over other methods of PCN control such as nematicides, long rotations and legislative control. However, past research predicted the potential of European PCN populations to change genetically and overcome the resistance genes in potatoes. These problems have now been realized, with field observations revealing a reduction in the effectiveness of *Solanum vernei* based resistant potato varieties in controlling PCN. An obvious solution to this problem would be to utilize varieties containing alternative resistance genes, however, the problem has been accentuated by the narrow genetic base from which current resistant potato varieties have been derived. The DANI PCN Programme has screened a wide range of the *Solanum* germplasm (Commonwealth Potato Collection) and identified new sources of high levels of PCN resistance. Resistance/virulence and sequential selection experiments have demonstrated that 'resistance mechanisms' differ between *Solanum* species, offering a potential management tool against further selection of virulent PCN field populations. Additional studies on *Solanum*/PCN interactions further clarify these differences and will be discussed.

ROLES OF HOST-DERIVED HATCHING INHIBITORS IN CONTROLLING HATCH OF *GLOBODERA ROSTOCHIENSIS*. U. Twomey,* K. Devine, J. Byrne, and P. Jones, Department of Plant Science, University College, Cork, Ireland. (*Present address: Department of Entomology, IACR-Rothamsted, Harpenden, U.K.).—In addition to hatching factors (HFs), which stimulated the hatch of juveniles of the golden potato cyst nematode, *G. rostochiensis*, fractionation of potato root leachate

revealed the presence of hatching inhibitors (HIs), which inhibited HF-induced hatch. The level of PCN hatch induced in soil under host and non-host solanaceous and non-solanaceous species was positively correlated with the HF:HI ratio in the corresponding root leachate, with non-hosts (including *Petunia hybrida, Triticum aestivum, Vicia faba* and *Helianthus annuus*) producing in-soil PCN hatch levels below that exhibited in the absence of a plant (i.e. spontaneous hatch). HI activity was detectable at similar levels in all the species tested, the differences in PCN hatch (*in vitro* and in-soil) being related most closely to differences in HF levels. HFs were detected, however, in non-solanaceous species such as bean. Studies of the time course of hatching chemical production in potato root leachate showed that HIs appeared first, followed by the sequential production of individual HFs. The in-soil hatch time course closely paralleled these *in vitro* studies; for the first 2 weeks after planting unsprouted but non-dormant potato tubers, in-soil hatch was less than the spontaneous hatch (in soil in the absence of potato plants) as a result of the very low HF: HI content of the root leachate.

EFFECT OF TOMATO PLANT TREATMENT WITH AMARANTINE ON *MELOIDOGYNE INCOGNITA*. Z. V. Udalova, Institute of Parasitology of Russian Academy of Sciences, Leninskii Prospect, 33, Moscow, 117071, Russia.—It is known that some secondary metabolites of plants, in particular alkaloids, are one of the major factors determining plant resistance to pests. Preparation of amarantine (alkaloid) produced by of *Amaranthus cruentus* was tested against the plant-parasitic nematode, *Meloidogyne incognita*. Tomato plants susceptible to *M. incognita* were sprinkled with 50 and 100 ppm concentrations of amarantine hydrate solution before infestation. Analysis of nematodes isolated from treated and untreated plants showed a significant decrease in number of nematodes per g of root (2.1 times at 50 ppm, and 1.3 times at 100 ppm in comparison with control). Size of females from treated tomato was significantly less, and female fertility was reduced (15-20% in comparison to the control). This may indicate increased time of ontogenesis. Average weight of treated plants was 30% greater than that of control plants.

HISTOCHEMISTRY AND CYTOCHEMISTRY OF PHENOLIC COMPOUNDS IN BANANA ROOTS FOLLOWING INFECTION WITH THE NEMATODE *RADOPHOLUS SIMILIS*. C. Valette,[1] C. Andary,[2] L. Mondolot-Cosson,[2] M. Boisseau,[1] J. P. Geiger,[3] J. L. Sarah,[1] and M. Nicole,[3] CIRAD-FLHOR BP 5035, 34032 Montpellier, France,[1] Faculté de Pharmacie, 34060 Montpellier, France,[2] and ORSTOM BP 5045, 34032 Montpellier, France.[3]—Susceptible (Poyo and Fougamou) and partially resistant (Yangambi) banana cultivars infected with the burrowing nematode, *Radopholus similis*, were histochemically investigated for phenolic compounds during the infection process. In healthy plants, flavonoids, dopamine, caffeic esters, and lignin were present at higher levels in roots of resistant plants as compared to susceptible ones. In infected plants, the production of flavonoids was enhanced in both cultivars. These compounds were localized only in the vascular tissues of susceptible plants while they were detected at a higher intensity in all infected tissues of the resistant cultivar. Flavonoids were mainly found in cells close to the nematode. Ultrastructural cytochemistry demonstrated that phenolics accumulated in pectin-rich material associated with and in plant cell walls close to the parasite. Counting revealed the occurrence of numerous nematodes in all tissues of susceptible plants; the few detected in the resistant plants were observed in the cortical parenchyma only. These data suggest that resistance of banana to *R. similis* is likely based upon differences in constitutive phenolics between cultivars. After infection, the more extended accumulation of flavonoids in the resistant cultivar may contribute to the plant defense strategy, limiting nematode ingress within root tissues.

POPULATION DYNAMICS OF *MELOIDOGYNE JAVANICA* ON REGROWTH TOBACCO. E. R. van Biljon, and M. S. Botha, Tobacco and Cotton Research Institute, P/B X82075, Rustenburg 0300, Republic of South Africa.—The population dynamics of the root-knot nematode, *Meloidogyne javanica*, was studied on tobacco plants which had been cut off and allowed to regrow from suckers. Five tobacco cultivars (TL 33, OD 469, OD 272, LK 3/46 and LK 10/46) planted in EDB-fumigated

soil were cut off at different times (3, 6 and 9 wks) after planting and compared to tobacco that had not been cut off which served as a control treatment. The *M. javanica* numbers were determined every 3 wks from 3 wks before planting to 18 wks after planting. For the effect of time, polynomial contrasts were determined. The best fitting polynomial was determined where the treatment× time or cultivar × time interaction was significant and these polynomials were tested pairwise for differences between trends. It was found that the population density of all the stages of *M. javanica* was significantly higher where the tobacco was cut off, regardless of cut off time. Although none of the cultivars were resistant to *M. javanica* the cultivar OD 272 had significantly higher larval numbers over a longer period than the other cultivars.

CROSSING BARRIERS BETWEEN *MELOIDOGYNE CHITWOODI* AND A RELATED SPECIES. J. G. Van der Beek, and G. Karssen, Research Institute for Plant Protection, IPO-DLO, P.O. Box 9060, NL- 6700 GW Wageningen, The Netherlands, and Plant Protection Service, PD, P.O. Box 9102, NL- 6700 HC Wageningen, The Netherlands.—Crossing in *Meloidogyne* is hampered by the parthenogenetic nature of most species of this genus. Consequently, the species concept in root-knot nematodes has been based on criteria other than reproductive isolation. Crossings were attempted between *M. chitwoodi* and a related species found in the Netherlands, previously been referred to as *M. chitwoodi* type 'Baexem'. The maternal line was obtained by inoculating two-week-old seedlings of tomato with a limited number of juveniles of *M. chitwoodi*. Males of the related species were added several times to the maternal line, during a period of 2.5 wks. In a second experiment, mixtures of the 2 species were inoculated on tomato seedlings. In both experiments, hybrids were detected, using the isozyme malate dehydrogenase as a genetic marker. The progeny of these hybrids appeared to be inviable. The few inviable juveniles found in this progeny were abnormally shaped, and in most of the eggs, a disturbance was observed during various stages of their development. It is concluded that, between these two related *Meloidogyne* species, reproductive isolation is a valuable criterion for species distinction.

THE EARLY EMBRYONIC CELL LINEAGE OF THE MARINE NEMATODE *PELLIODITIS MARINA*. B. Vancoppenolle,[1] G. Borgonie,[2] R. Schnabel,[2] and A. Coomans,[1] Institute of Zoology U.G., Ledeganckstraat 35 B9000 Gent, Belgium[1] and MPI für Biochemie, Am Klopferspitz 18A D82152 Martinsried, Belgium.[2]—The early embryonic cell lineage of *Pellioditis marina*, a marine rhabditid with relatively short developing time (9 hrs at 25°C), was traced using a 4D-microscope. Although the general pattern of cell divisions is congruent with the lineage described for *Caenorhabditis elegans* by Sulston and co-workers, striking differences can be observed concerning migrations, timing of divisions and cell deaths. The AB, MS and C lineage of *P. marina* differ from those of *C. elegans* both in the occurrence of additional cell deaths as well as in the absence of certain cell deaths. Additionally, Caap does not divide in accordance with the characteristic period of the rest of the C lineage. In contrast with *C. elegans*, the E founder cell in *P. marina* undergoes a migration before gastrulation and divides into Ea and Ep only after E has entered the interior of the embryo. D and P4 divide in a similar way as in *C. elegans*. Additionally, the probable origin of observed variation in developmental time at 25°C was determined.

TOLERANCE AND YIELD LOSS IN CEREAL AND MEDIC VARIETIES CAUSED BY *PRATYLENCHUS NEGLECTUS* IN SOUTH AUSTRALIA. V. A. Vanstone, and S. P. Taylor, University of Adelaide, Waite Campus, Glen Osmond, 5064 South Australia and South Australian Research and Development Institute, GPO Box 397, Adelaide, 5001, South Australia.—The root lesion nematode (*Pratylenchus neglectus*) occurs throughout the cropping areas of South Australia, infecting all cereals as well as grain and pasture legumes and oilseeds. In the dryland cropping systems of southern Australia, wheat-medic rotations are common, along with a few other rotational options. Results from field trials demonstrate that variation in resistance and tolerance to the nematode exists in commercial cereal and medic varieties. Varietal tolerance and yield loss was established on the basis of response to nematicide applica-

tion (aldicarb at 2.5 kg/ha a.i.). Trials including 14 cereal varieties were conducted at a total of 9 sites in 1994 and 1995. Yield losses varied depending on seasonal conditions and the initial soil nematode density. Average yield loss recorded for the most intolerant varieties was 10-20%, with smaller losses (<8%) in the more tolerant varieties. Oat (*Avena sativa*) varieties were generally more intolerant than wheats (*Triticum aestivum*), with barley (*Hordeum vulgare*) and triticale (*Triticosecale*) among the most tolerant. Eight medic (*Medicago* spp.) varieties were assessed at 1 site in 1995. Large responses were observed in dry matter herbage production (up to 28%) for most varieties, indicating the intolerance of medics to *P. neglectus*. *P. neglectus* appears to cause considerable yield loss to cereals and medics commonly grown in rotational sequences in the dryland cropping areas of South Australia.

COMPONENTS OF NEMATODE-SUPPRESSIVE ACTIVITY OF VELVETBEAN *MUCUNA DEERINGIANA*. R. Vargas,[1] A. Rodríguez,[2] and N. Acosta,[1] University of Puerto Rico, Department of Crop Protection, Mayaguez, Puerto Rico, [2] and Department of Chemistry, Rio Piedras, Puerto Rico.—Roots of velvetbean, *Mucuna deeringiana* (MD), were chemically evaluated to identify and characterize the components involved in nematode suppressive activity. MD-roots were extracted in methanol and their components separated by a silica gel column. These components were fractionated with hexane, chloroform and water as solvents. The structure elucidation process was performed according to the fragmentation pattern by high-resolution electron impact mass spectra. We performed an extensive screening of MD-fractions for nematicidal activity. Our results showed that the organic phase of these extracts had the strongest nematotoxic effect on juveniles of *Meloidogyne incognita*. Bioassay of single compounds of root metabolites demonstrated a reduction of nematicidal activity, in relation to the crude chloroform fraction. Several phytoalexins of the pterocarpanes, isoflavanes and isoflavonones structural classes were isolated from the chloroform portion. The production of phytoalexins by MD-roots may be one of the mechanisms of antagonism to plant-parasitic nematodes.

IDENTIFICATION OF mRNA SPECIES EXPRESSED UPON NEMATODE INFECTION BY THE DIFFERENTIAL DISPLAY TECHNIQUE. I. Vercauteren, M. Van Montagu, and G. Gheysen, Laboratorium voor Genetica, Flanders Interuniversity Institute for Biotechnology, Universiteit Gent, Ledeganckstraat 35, B-9000 Gent, Belgium.—Root-knot nematodes induce specialized feeding sites in the plant root. Only few molecular data are available on the events in the early stages of the infection process. To obtain insight into the regulation of the host plant genes upon nematode attack, the differential display technique was chosen and optimized for *Arabidopsis thaliana* roots infected with the root-knot nematode, *Meloidogyne incognita*. This allowed us to isolate plant genes specifically expressed in infected *A. thaliana* roots but not in uninfected control roots. In short, mRNA is extracted from infected roots between 2 and 7 days after inoculation and from control roots. First-strand cDNA is made by a $T_{(12)}$MN primer and amplified by PCR using the same $T_{(12)}$MN primer and a random decamer. The PCR mixture is separated on a denaturing polyacrylamide gel. Bands only visible in the lanes displaying cDNA from infected plant roots and not in the lanes containing cDNA from control roots are of interest to us. These bands are then reamplified, cloned and sequenced. They can be used as a probe in Southern blots to determine whether they are from nematode or plant origin. Further, RNA gelblots have to be performed to confirm that the cDNA clones isolated are really induced upon nematode infection. The next step in the procedure is to check the cell specificity in the plant root of the cDNA clone by *in situ* hybridizations. So far, 14 differentially expressed cDNAs have been isolated, cloned, sequenced and confirmed on Southern blots. For the most interesting candidates, RNA gel blots and *in situ* hybridizations are being performed.

SCREENING CITRUS ROOTSTOCKS FOR RESISTANCE TO *TYLENCHULUS SEMIPENETRANS*. S. Verdejo-Lucas,[1] F. J. Sorribas,[1] J. B. Forner,[2] and A. Alcaide,[3] IRTA. Crta. de Cabrils s/n. 08348. Cabrils, Barcelona, Spain,[1] ESAB. Comte d'Urgell 187. 08036 Barcelona, Spain,[2] and IVIA. Apartado oficial 46113 Moncada Valencia, Spain.[3]—A breeding program was initiated at IVIA in 1974 to obtain

new citrus rootstocks tolerant to tristeza virus, *Phytophthora* root rot, and adapted to Spanish conditions, particularly calcareous soils and salinity. The reaction of 22 new citrus hybrid rootstocks to *Tylenchulus semipenetrans* in green-house and field tests is reported. The rootstocks tested included 11 hybrids of Cleopatra mandarin × *Poncirus trifoliata*, 2 hybrids of Cleopatra mandarin × Troyer citrange, 8 hybrids of Troyer citrange × Cleopatra mandarin, and 1 hybrid of Troyer citrange × common mandarin. The susceptible hybrid sour orange × Cleopatra mandarin was included as a reference. Five of the 11 rootstocks tested with *P. trifoliata* in their parentage reacted as resistant to the Mediterranean biotype of *T. semipenetrans* in both the greenhouse and in the field. Rootstocks with Troyer citrange in their parentage were susceptible to the nematode. The resistance to *T. semipenetrans* exhibited by 2 Cleopatra mandarin × *P. trifoliata* hybrids (03.01.05 and 03.01.18) is of great practical importance since these rootstocks have shown resistance or tolerance to tristeza virus, they produce many seeds, provide semi-dwarf trees and the fruit quality of the cultivars grafted on them is high.

IDENTIFICATION OF RESISTANCE TO *MELOIDOGYNE JAVANICA* IN THE *LYCOPERSICON PERUVIANUM* COMPLEX. J. C. Veremis, and P. A. Roberts, Department of Nematology, University of California, Riverside, California 92521, U.S.A.—Clones of *Lycopersicon peruvianum* PI 270435-2R2, PI 270435-3MH and PI 126443-1MH expressed novel resistance to 3 Mi-avirulent *M. javanica* isolates in greenhouse experiments. The 3 isolates were able to reproduce on 1 embryo rescue hybrid of PI 126443-1MH, but not on 3 *L. peruvianum-L. esculentum* bridge line hybrids of PI 126443-1MH when screened at 25°C (Mi expressed temperature). Clones of PI 270435-2R2 and all its hybrids with susceptible genotypes were resistant to the 3 *M. javanica* isolates at 25°C. The bridge line hybrid EPP-2 × PI 270435-2R2 was susceptible to *M. javanica* isolate 811 at 32°C, whereas PI 270435-2R2 and all other hybrids of PI 270435-2R2 were resistant at 32°C. At 32°C, 1 F2 progeny of PI 126443-1MH × EPP-1 and reciprocal test-cross progenies of [PI 270435-3MH × PI 270435-2R2] × PI 126440-9MH each segregated into resistant: susceptible (R:S) ratios close to 3:1. These results indicated that resistance to Mi-avirulent *M. javanica* is conferred by a single dominant gene on PI 126443-1MH and that different non-allelic dominant resistance genes are present in the clones PI 270435-2R2 and 270435-3MH. The expression of differential susceptibility and resistance to *M. javanica* and *M. incognita* in individual plants of the bridge line hybrid, embryo rescue hybrid, F2, and test-crosses indicated that at least some genes governing resistance to *M. javanica* differ from the genes conferring resistance to *M. incognita*. A new source of heat-stable resistance to *M. javanica* was identified in *Lycopersicon chilense*.

BIOLOGICAL STUDIES OF *MELOIDOGYNE INCOGNITA* ON POINTED GOURD IN EASTERN U.P. INDIA. A. C. Verma, and A. Anwar, Department of Nematology, N.D. University of Agriculture and Technology, Kumarganj, Faizabad, India.—A pot trial was conducted to determine the life cycle of root-knot nematode, *Meloidogyne incognita*, on pointed gourd (*Trichosanthes dioica* Roxb.). The experiment was based on inoculation of four-week-old plants with 2 000 freshly hatched second-stage juveniles (J2). Fifty-eight percent of J2 penetrated roots by 24 hrs after inoculation and 90% of these J2 moulted to various female stages. Moulting started after 3 days and development of young females from 18 days after inoculation. Two juvenile stages (J3 and J4) became sedentary. Maturation of females started 20-22 days after inoculation. Deposition of a gelatinous matrix and egg masses began after 20-24 days and emergence of J2 was initiated even before the egg masses turned brown. The fecundity of the nematodes was not affected by the host. The majority of the eggs were retained in the egg sac. During penetration, J2 incited necrosis and the formation of irregular-shaped syncytia. The infection also caused the formation of confluent round to spindle shaped galls laterally on roots and usually in a cancerous stage. We found that the root-knot nematode, *M. incognita*, is able to complete its life cycle from J2 to next generation J2 in 26 days at temperature ranges of 30-40°C.

RFLP MARKERS FOR LOCI CONFERRING BROAD-BASED RESISTANCE TO SOYBEAN CYST NEMATODE. R. A. Vierling,[1] J. Faghihi, V. R. Ferris,[2] and J. M. Ferris,[2] Indiana Crop Improvement Association and Department of Agronomy, Purdue University, West Lafayette, Indiana 47907-1150, U.S.A.,[1] and Department of Entomology, Purdue University, West Lafayette, Indiana 47907-1158, U.S.A.[2]—In ongoing studies to determine resistance to soybean cyst nematode (SCN), progeny from the soybean cross 'Williams 82' × Hartwig' have been screened with 2 inbred nematode lines. When 200 $F_{2:3}$ families were screened with either an SCN race 3 or race 4 inbred, the resistant phenotypes in each case indicated a two-gene system. Ward's minimum variance cluster analysis was used to separate data for plant families into cells of resistant, segregating and susceptible phenotypes. Progeny from the experiment with 1 inbred were then screened with 56 polymorphic markers, and stepwise regression used to analyze molecular marker allele states and phenotypes. The model selection was made at $P \leq 0.01$. Four unlinked RFLP markers (A006, A567, A487, A112) were associated with SCN resistance, with partial coefficients of determination (R^2) of 91%, 1%, 1% and 1%, respectively. A new major SCN resistance locus (A006) and 3 minor loci (A567, A587, A112) were mapped. This mapping will accelerate the transfer of broad-based resistance without linkage drag, and aid in the determination of relationships among various SCN resistant germplasm sources.

EVALUATION OF GRAFTING ON *COFFEA CANEPHORA* VAR. ROBUSTA AND CHEMICAL TREATMENT FOR CONTROL OF *PRATYLENCHUS* SP. IN *C. ARABICA* CROPPING SYSTEMS. L. Villain,[1] J. L. Sarah,[1] B. Decazy,[1] A. Molina,[2] and S. Sierra,[2] CIRAD-CP, BP 5035, 34032 Montpellier Cedex 1, France,[1] and ANACAFE, 5a. Calle 0-50 Zona 14, Guatemala, 01014 Central America.[2]—*Pratylenchus* sp., among other pests, has a significant impact on the economy of coffee plantations throughout Central America. A field experiment was undertaken in the southwest of Guatemala to assess damage due to the nematode and to evaluate 2 different methods of control: grafting on *C. canephora* var. Robusta, and chemical treatments (terbufos: 1 to 2 g/plant twice a year). This experiment included 4 treatments (1- no grafting without chemical; 2- no grafting plus chemical; 3- grafting without chemical; 4- grafting plus chemical) and was carried out for five years, including the first 3 commercial harvests. Observations of nematode numbers, total weight of harvested berries and number of dead plants demonstrated the high degree of pathogenicity of *Pratylenchus* sp. on *C. arabica* and the very good efficiency of grafting as a method of control (production of coffee berries multiplied by 3.6 on average). In contrast, chemical treatments resulted in a nonsignificant increase in production (×1.14).

INFLUENCE OF THE NATIVE SOIL MICROFLORA ON THE REPRODUCTION AND PATHOGENICITY OF *HELICOTYLENCHUS DIHYSTERA*. C. Villenave, and R. Duponnois, Laboratoire de Bio-Pédologie and Nématologie, ORSTOM, BP 1386, Dakar, Senegal.—Soils were sampled from 3 agro-systems: a field cultivated in millet, a 1-year-old fallow and a 17-year-old fallow in Senegal. Soils were autoclaved and inoculated with their own soil filtrates, either sterilized or not. *Helicotylenchus dihystera* were inoculated (2 rates of inoculation and uninoculated control) on transplanted millet seedlings in the different soils. The experiment lasted 2 months in the greenhouse. Irrespective of the microflora treatment, the reproductive index of *H. dihystera* was similar in the cultivated and the one-year-old fallow soils (Pf/Pi = 20) and higher than that in the old fallow soil (Pf = 6). The texture was sandy for the 2 first soils and clayey for the third one, which may explain the differences. The initial and final numbers of bacteria were lower in the millet and the one-year-old fallow soils than in the old fallow soil. The native microflora had no effect on *H. dihystera* reproductive index in the two sandy soils. No pathogen effects of the nematodes were measured on plant dry weight in these soils. In the clayey old fallow soil, native microflora doubled the reproductive index of the plant-parasitic nematode (8 versus 4). In this treatment, the dry weight of millet was lower than that in the uninoculated nematode control (15%). The pathogenic effect of *H. dihystera* increased in soil with high microbial status.

ON SOME NEW CEPHALOBIDS FROM SUBANTARCTIC AMERICA. M. T. Vinciguerra, and M. Clausi, Dipartimento di Biologia Animale, Università di Catania, Italy.—During a survey of soil nematodes in subantarctic Argentina and Chile, 2 new species and a new genus of Cephalobidae were found. The new genus is very similar to *Chiloplacus* Thorne 1937, but it differs from the latter in having very reduced cephalic probolae and the labial probolae apically located on the lips. The 2 new species, respectively, belong to the genus *Stegelletina* Andrassy 1984 and to the genus *Acrobelophis* Andrassy 1984. The details of the complex cephalic structures of specimens of the 3 taxa were shown by SEM photos. Some problems related to the identity and definition of the above mentioned genera of Cephalobidae were discussed, also in the light of the present research.

HOST SUITABILITY OF ROSE ROOTSTOCKS TO *MELOIDOGYNE HAPLA* USING A HIGH INOCULUM PRESSURE TEST. R. Voisin,[1] Y. Jacob,[2] J. C. Minot,[1] G. Pelloli,[2] S. Aloisi,[2] and D. Esmenjaud,[1] INRA-LBI, 06600 Antibes, France,[1] and INRA-SAPF, 83370 Fréjus, France.[2]—The root-knot nematode, *M. hapla*, is an important pest of greenhouse rose crops. Host suitability of 32 rose rootstocks from the INRA collection in Fréjus (France) of 14 *R. indica*, 8 *R. multiflora*, 2 *R. canina*, 2 *R. manetti* and 6 other rootstocks were evaluated under greenhouse conditions using an isolate of *M. hapla* from Canada. Cuttings rooted in 3-L containers were directly inoculated after 50 days by transferring into each rose container the whole root system and soil of one tomato plant grown in 0.25-L pot and previously inoculated at the 5-leaf stage with 1 000 juveniles of the nematode. Rose and galled tomato plants were then co-grown for 2 months before the removal of the top part of tomato. This method consequently provided a high and durable inoculum pressure. Rose plants were harvested 3 months after inoculation for gall index rating and root nematode extraction. *Rosa manetti* rootstocks were completely free of galls whereas *R. canina* exhibited a very light galling. *R. indica* genotypes ranged from moderately to heavily galled. *Rosa multiflora* genotypes ranged from lightly (clones K1 and K2) to moderately galled. Nematode numbers in the roots confirmed gall index ratings. In a complementary test, the 2 clones *R. multiflora* K1 and K2 were also resistant to 2 other isolates from England and Southern France, that confirmed their value as diploid sources of resistance to this RKN.

A COMPARISON OF THE MIGRATORY BEHAVIOUR IN *ARABIDOPSIS* ROOTS OF THREE *MELOIDOGYNE* SPECIES. N. von Mende, IACR Rothamsted, Harpenden, Herts. AL5 2JQ, U.K.—*Meloidogyne incognita, M. arenaria* and *M. javanica* are the most prevalent root-knot nematode species in world agriculture. The correlation between the aggressiveness of these 3 species and the degree of yield loss has been studied extensively by examining various factors such as root invasion, fecundity and gall size. In the present study the behavior of the second-stage juvenile after root invasion was monitored in order to correlate the physical ability of this stage during migration to the aggressiveness of each species. A comparison of the speed of the moving juvenile and the time interval between invasion and initiation of the feeding site was presented including a detailed analysis of the repeated cycles of stylet protrusion and medium bulb pumping during migration. Generally, the results indicate that *M. javanica* is the fastest amongst the 3 species tested which agrees with the previously determined rating of aggressiveness. Physical and physiological aspects of these observations were discussed.

INTERSPECIFIC VARIATION AMONG DIFFERENT SPECIES OF *PRATYLENCHUS* AND *RADOPHOLUS SIMILIS* USING rDNA RESTRICTION FRAGMENT LENGTH POLYMORPHISM. L. Waeyenberge,[1] M. Moens,[1] J. Pinochet,[2] and T. C. Vrain,[3] Centrum voor Landbouwkundig Onderzoek, 9820 Merelbeke, Belgium,[1] Institut de Recerca i Tecnologia Agroalimentàries, 08348 Cabrils, Spain,[2] and Pacific Agriculture Research Centre, 6660 Vancouver, British Columbia, Canada.[3]—Different parts of the tandem ribosomal repeats of rDNA evolve at different rates. This provides areas with varying degrees of sequence divergence for analysis. The Ploymerase Chain Reaction (PCR) was

used to amplify a rDNA-fragment of *R. similis* and 7 *Pratylenchus* species (*P. vulnus, P. goodeyi, P. scribneri, P. agilis, P. thornei, P. crenatus* and *P. penetrans*) with a total of 26 populations. The primers were conserved sequences of the 18S and 26S rRNA genes of *Caenorhabditis elegans*. They amplified a fragment ranging in size from 900 to 1200 bp. Amplified products were obtained from all species and populations except from *P. penetrans*. From this species the fragment was amplified from only one population. The rDNA fragments were cut with 8 restriction enzymes (AluI, HinfI, HpaII, HaeIII, CfoI, BamHI, HindIII and Sau3AI). The patterns of the restriction fragments showed clear differences between the *Pratylenchus* spp. and *R. similis*. With the exception of *P. agilis* and *P. scribneri*, all nematode species could be differentiated with all the restriction enzymes used. Only AluI and CfoI could also differentiate *P. agilis* from *P. scribneri*.

SURVEY OF *HETERODERA GLYCINES* RACES IN BRAZIL. A. L. Wain, and J. F. V. Silva, EMBRAPA-CNPSo, C.P. 231, 86001-970 Londrina, PR, Brazil.—During the 1994/95 and 1995/96 growing seasons, a total of 19 populations of the soybean cyst nematode, *Heterodera glycines*, were collected in infested fields in the states of Mato Grosso do Sul, Mato Grosso, Goiás, Minas Gerais, São Paulo, and Rio Grande do Sul. These nematode populations were cultured in the cultivar Cristalina under greenhouse conditions at the National Soybean Research Center, Londrina, state of Paraná. The races were identified using "Lee 68" as the susceptible host, and "Picket", "Peking", PI88.788, and PI90.763 as differential hosts. Plants were inoculated with eggs, and the number of white females that developed on each plant was determined approximately 35 days after inoculation. The female index (FI) was calculated for each differential soybean line, and a race was designated for each population. Races 1, 2, and 3 were identified in the samples collected in Mato Grosso. Races 3, 6, and 14 were identified in the samples from Mato Grosso do Sul. Races 3, 4, and 14 were identified in the populations from Goias. In the collections from Minas Gerais and São Paulo, only race 3 was identified. Race 6 was the only race identified in the collections from Rio Grande do Sul.

EVALUATING SOILLESS MEDIA SUITABLE FOR ANTHURIUM GROWTH AND UNFAVORABLE FOR NEMATODE INFECTIVITY. K. Wang,[1] B. S. Sipes,[2] and A. R. Kuehnle,[1] Departments of Horticulture[1] and Plant Pathology,[2] University of Hawaii, Honolulu, Hawaii 96822, U.S.A.—Alternatives to chemical control of *Radopholus* in anthurium and export restrictions of potted anthurium to Japan requires identifying media suitable for plant growth but unfavorable for nematode development. In this experiment, a 2:1 Big-R compost:perlite mix (V/V), volcanic cinder, a 1:1 cinder-peat mixture (V/V), and a 1:1:1 mixture of rockwool, cinder, and peat (V/V/V) were evaluated for their effects on growth of 'Alii' and 'Midori' anthurium cultivars and on the population increase of *Radopholus* in the greenhouse. Nine months after inoculation with 2 000 *Radopholus*, differences were observed among media. Media affected the growth of Alii more than Midori. Alii has a more compact root system with many fine lateral roots. Anthurium growth was better in the Big-R:perlite mixture than all other media in the absence of nematodes. Populations of *Radopholus* were lowest in plants grown in the cinder medium. *Radopholus* were recovered from the anthurium stem and petiole tissue as well as root tissue. Big-R compost or rockwool-cinder-peat mixture can be used in raised bench cultivation in conjunction with tissue cultured plantlets to produce nematode-free potted anthuriums for export to Japan.

THE EFFECT OF VELVETBEAN AS A ROTATION CROP FOR THE MANAGEMENT OF NEMATODE PROBLEMS IN COTTON. C. F. Weaver, R. Rodríguez-Kábana, and D. G. Roberston, Department of Plant Pathology, Auburn University, Alabama 36849, U.S.A.—A 3-year field experiment was conducted to determine the value of 'Florida' velvetbean (*Mucuna deeringiana*) as a rotation crop for the management of nematodes in cotton (*Gossypium hirsutum*). Principal nematode pathogens present in this field included *Meloidogyne incognita* and *Hoplolaimus galeatus* with other species (*Paratrichodorus minor* and *Pratylenchus* spp.) present in much lower numbers. Seven cotton cultivars were as-

sessed for yield and nematode populations (at-harvest) in the following rotation schemes: Cotton monoculture untreated [C-C-C(-)], cotton monoculture treated with aldicarb (2.8 kg a.i./ha at-plant) [C-C-C(+)], 2 years of velvetbean followed by 1 year of untreated cotton [V-V-C(-)] and 2 years of velvetbean followed by 1 year of treated cotton [V-V-C(+)]. In the first 2 years, cotton plots supported high levels of *M. incognita* (>300 juveniles/100 cm^3 soil) and lower levels of *H. galeatus* (≈30). First year velvetbean plots supported very low numbers of *M. incognita* (≈40 juveniles) while second-year velvetbean was a non-host for *M. incognita*. First and second-year velvetbean supported higher numbers of *H. galeatus* (≈50) than did cotton. In third-year cotton, effects of the rotation and nematicide were cultivar dependent, but on average across all cultivars aldicarb applications sharply reduced nematode populations while significantly increasing yield. Compared to [C-C-C(-)], the rotation scheme [V-V-C(-)] significantly increased yield and sharply reduced *M. incognita* but supported higher levels of *H. galeatus*. The rotation scheme [V-V-C(+)] resulted in the sharpest reduction of nematode populations and the most significant increases in yield.

ENTOMOPATHOGENIC NEMATODES - THE NEMATODES FOR ALL REASONS. J. M. Webster, G. Chen, K. Hu, J. Li, and K. Ng, Centre for Pest Management, Department of Biological Sciences, Simon Fraser University, Burnaby, Vancouver, Canada.—*Steinernema* and *Heterorhabditis* species are recognized biological control agents against insects. The bacteria mutualistically associated with these nematodes, *Xenorhabdus* spp. and *Photorhabdus luminescens*, respectively, are a primary cause of insect death and also of the death of other organisms within the insect cadaver or the surrounding soil. Controlled, mass culture of these bacteria releases the metabolites into the broth that are antifungal and antibacterial. In addition to the presence of previously isolated indole derivatives, stilbene derivatives, xenorhabdins and xenocoumacins in the bacterial culture broths, 2 new classes of compounds, namely xenorxides and nematophin, have been found to be bioactive. The minimum inhibitory concentration (MIC) of a xenorxide from *X. bovienii* is 6 µg/ml against *Bacillus subtilis*, and 0.3 µg/ml against *Stapthylococcus epidermidis*. Some of the nematophins have a MIC of 12 µg/ml against *B. subtilis*, 0.75 µg/ml against drug resistant isolates of *S. aureus*, and 0.75 µg/ml against *Aspergillus fumigatus* and *A. flavus*. When applied to *Phytophthora infestans* (late blight) infected potato plants, the mixed organic extract of the culture broth of *X. bovienii* completely inhibited fungal growth at 10.0 µg/ml. Ammonia and a stilbene derivative are nematicidal components in the culture broth of *P. luminescens*. The stilbene caused 94% mortality of *Bursaphelenchus xylophilus* (pinewood nematode) at 50 µg/ml but had no effect on *Meloidogyne incognita* or *Heterorhabditis megidis*.

***MELOIDOGYNE ARENARIA* AND *PASTEURIA PENETRANS* POPULATION DENSITY DEVELOPMENT AS AFFECTED BY AN INTERCROPPING SYSTEM. E. Weibelzahl-Fulton, and D. W. Dickson, University of Florida, Department of Entomology and Nematology, Gainesville, Florida 32611, U.S.A.**—A microplot study on the influence of a corn (*Zea mays* L.) and bean (*Phaseolus vulgaris* L.) intercropping system in rotation with peanut (*Arachis hypogaea* L.) on population density development of *M. arenaria* race 1 and *P. penetrans* was initiated in the spring of 1994. The initial population of *P. penetrans* was estimated by bioassay. Plots were ranked and grouped into 10 groups based on the mean number of endospores attached to a second-stage juvenile (J2). Over a period of 2 years, the number of endospores per J2 in the bioassay increased from 0, 0, 1.1, 2.5, 3.6, 4.9, 6.5, 9.1, 11.7, and 17.5 to 1.1, 1.2, 6.2, 22.5, 45.2, 40, 53.2, 56, 67.5, and 63.2, in groups 1 to 10, respectively. The number of endospores/J2 in groups 1 to 3 was lower than in group 4, which was lower than in group 5 to 10 ($P \leq 0.05$). The rate of attachment by *P. penetrans* endospores increased from 0, 0, 33, 69, 87, 96, 99, 99, 99, and 99% to 34, 48, 48, 88, 98, and 100% for the 5 highest groups, respectively. The percentage attachment in groups 1 to 3 was lower than in group 4, which was lower than in groups 5 to 10 ($P \leq 0.05$). At the end of the intercropping season, the average number of 1,636 J2/100 cm^3 soil in plots of groups 4, 7, 8, 9, and 10 was significantly lower than the 2,160 J2/100 cm^3 soil observed in groups 1, 3, 5, and 6 ($P \leq 0.05$). After the following peanut crop, the nematode populations of groups

5 to 10 were suppressed to an average of 37 J2/100 cm^3 soil, compared with 194 J2/100 cm^3 soil in group 4, and 630 J2/100 cm^3 soil in groups 1 to 3 ($P \leq 0.05$). Galling rates of peanut roots and pods were generally low in all plots, averaging 0.5 and 0.6 on a scale from 0 to 5.

COMPETITION BETWEEN *TYLENCHORHYNCHUS ANNULATUS* AND *CRICONEMELLA XENOPLAX* ON GRAIN SORGHUM (*SORGHUM BICOLOR*). I. Wenefrida, J. S. Russin, and E. C. McGawley, Department of Plant Pathology and Crop Physiology, Louisiana State University Agricultural Center, Louisiana Agricultural Experiment Station, Baton Rouge, Louisiana 70803-1720, U.S.A.—Competition between *T. annulatus* and *C. xenoplax* on grain sorghum colonized by *Macrophomina phaseolina* was studied using a replacement series technique. Soil in pots containing Pioneer hybrid 8333 grain sorghum was infested with nematodes in the following *T. annulatus*: *C. xenoplax* ratios: 100:0, 75:0, 50:0, 25:0, 0:100, 0:75, 0:50, 0:25, 25:75, 50:50, and 75:25. Nematode density at 100 was 1 000 vermiform individuals/pot. *M. phaseolina* was either absent or present at 10 colony forming units/g soil. The test was conducted twice in 1995. Results indicate that relative yields of *T. annulatus* and *C. xenoplax* in single culture were significantly higher than those of a hypothetical model representing equal inter- and intraspecific competition. *M. phaseolina* affected relative yields of *C. xenoplax* but not *T. annulatus*. Respectively, root and stem weights were reduced 38 and 31% by *M. phaseolina*. In the absence of *M. phaseolina*, *T. annulatus* reduced root weight by 43%, whereas *C. xenoplax* had no affect. In the absence of *M. phaseolina*, increases in nematode numbers increased damage to roots. In the presence of *M. phaseolina*, nematode damage was uniform across nematode inoculum levels.

EVIDENCE FOR BIOLOGICAL SUPPRESSION OF *HETERODERA SCHACHTII* IN A CALIFORNIA FIELD. A. Westphal, and J. O. Becker, University of California, Riverside, Department of Nematology, Riverside, California 92521, U.S.A.—Naturally occurring suppression of *Heterodera schachtii* was observed in a field at the Agricultural Operations Field Station, University of California, Riverside. In 1975 the Hanford sandy loam soil was inoculated with *H. schachtii*-infested field soil from the Moreno Valley area and has since been continuously cropped to host plants. Following the initial establishment, the population declined over several years and remained at a low level. During 1994 and 1995, field experiments with Swiss Chard (*Beta vulgaris*) were conducted to investigate the cause of suppression. Each experiment had a Latin square design with 5 replicates. Treatments included soil pesticides applied to differentially suppress various parts of the soil biota. Several weeks later greenhouse-reared *H. schachtii* were reintroduced into the treated plots. The biological nature of the suppressiveness was demonstrated in both years by the increased nematode reproduction after pre-plant soil fumigation with metam sodium. While the reintroduced populations of *H. schachtii* failed to increase in non-treated plots, the numbers of cysts and eggs per g soil were significantly higher in the fumigated treatment. Soil treatments with a fungicide, tolclofos methyl and a nematicide, fenamiphos, caused an increase in the number of cysts per g soil, but had no significant effect on numbers of eggs.

EFFECT OF ALTITUDE AND SOIL TYPE ON NEMATODES ASSOCIATED WITH BANANAS IN THE WINDWARD ISLANDS. J. A. Williams, C. Lubin, and H. J. Fagan, WINBAN Research and Development Division, Roseau, St Lucia, West Indies.—A survey of banana farms in 3 of the Windward Islands (Dominica, St. Lucia and St. Vincent) was conducted to determine the incidence and population levels of parasitic nematodes on roots of bananas. Farm locations were selected by parameters of altitude (elevations above and below 228.6m and broad soil type). *Radopholus similis, Helicotylenchus multicinctus, Pratylenchus coffeae, Meloidogyne* sp., *Rotylenchulus reniformis* and *Hoplolaimus* sp. were the nematode species observed. In Dominica, the highest population of *R. similis* and *P. coffeae* was observed in the broad soil types, Ultisol/Inceptisol and Ultisol/Alfisol/Inceptisol, respectively. In comparing population levels of *H. multicinctus* and *P. coffeae*, the population of *H. multicinctus* was higher at low altitude, whilst at high altitude the population level of the 2 species was similar. In St. Lucia, *H. multicinctus* population was higher at low altitude, whilst *P. coffeae* population was higher at the high

altitude. The highest population of *R. similis* and lowest population of *P. coffeae* were observed in St. Vincent where all sites were at low elevation. There was no evidence of an altitude effect on population levels of *R. similis* nor on extent of root or corm damage caused by nematodes. The effect of soil types on nematode populations in St. Lucia and St. Vincent is yet to be established from data being processed.

RFLP MARKERS CLOSELY LINKED TO A GENE CONFERRING RESISTANCE TO *GLOBODERA PALLIDA* POPULATION D236 IN DIPLOID AND TETRAPLOID POTATO POPULATIONS. P. J. C. C. Wolters,[1] J. N. A. M. Rouppe van der Voort,[2] D. G. Esselink,[1] R. T. Folkertsma,[2] and R. Janssen,[1] DLO-Centre for Plant Breeding and Reproduction Research (CPRO-DLO), P.O. Box 16, 6700 AA Wageningen, The Netherlands,[1] and Department of Nematology, Wageningen Agricultural University, P.O. Box 8123, 6700 ES Wageningen, The Netherlands.[2]—Monogenic resistance to *Globodera pallida* population D236 (Pa2) previously has been found in the potato cultivar Multa (2n = 4x = 48). To facilitate the mapping of the resistance gene, a diploid population segregating for the resistance gene was used. 'Multa' and the diploid population have *Solanum tuberosum* ssp *andigena* CPC 1673, the source of the monogenic resistance to D236, as a common ancestor. Using the AFLP-technique, the resistance gene was mapped on chromosome 12. The position of the resistance gene was verified with RFLP-probes specific for chromosome 12. These RFLP-probes also were used in the tetraploid Maritta × Multa population in which the resistance gene was reported originally. The same RFLP-probes, with which markers linked to the resistance gene were identified in the diploid population, could be used to identify markers linked to the resistance gene in the tetraploid population. The use of these RFLP-probes was further extended to 2 additional populations (one diploid, one tetraploid) segregating for the resistance gene. Results of all 4 populations were compared and the possible use of molecular markers in a tetraploid population, based on extrapolation of markers linked to a resistance gene in a diploid population, was discussed.

IMPROVING THE PLACEMENT OF VYDATE™ FOR THE CONTROL OF PLANT-PARASITIC NEMATODES. S. Woods, P. P. J. Haydock, K. Evans, and T. C. K. Dawkins, Crop and Environment Research Centre, Harper Adams University Sector College, Newport, Shropshire, TF10 8NB, U.K., Entomology and Nematology Department, IACR-Rothamsted, Harpenden, Herts., AL5 2JQ, U.K., and DuPont (U.K.) Limited, Wedgwood Way, Stevenage, Herts., SG1 4QN, U.K.—Oxamyl (Vydate 10G:10% gr, DuPont) is a nematicide used for the control of a variety of plant-parasitic nematodes in both temperate and tropical countries. When formulated as granules, oxamyl, whilst being mobile in the soil solution, often performs best when incorporated into the area of the soil profile where nematode damage predominates. The timing and depth of nematicide incorporation is varied depending on the nematode species and crop involved. In U.K. potato production, placement of a nematicide in the top 20 cm of soil prior to planting gives the best control of the potato cyst nematodes, *Globodera rostochiensis* and *G. pallida*. Damage to peanut by *Meloidogyne* and *Pratylenchus* species can be decreased by using a granular nematicide and best results are achieved when the granules are incorporated into the top 5-7 cm of the soil. The Vytest™ diagnostic kit is a qualitative assay developed by DuPont to detect oxamyl in soil. The kit has been used by DuPont advisors in field situations for 3 years to aid the efficient application and incorporation of oxamyl by potato growers. The Vytest™ could help improve placement of nematicides in tropical countries by matching data on the vertical distribution of economically important nematode species with the placement of oxamyl. The paper outlined the practical use of Vytest in the field and its implications for nematicide use.

APPLICATION OF ENTOMOPATHOGENIC NEMATODES ON FOLIAGE: AN ASSESSMENT OF APPLICATION TECHNOLOGY. D. J. Wright,[1] E. R. Lello,[1] M. N. Patel,[1] and G. A. Matthews,[2] Department of Biology[1] and International Pesticide Application Research Centre,[2] Imperial College of Science, Technology and Medicine, Silwood Park, Ascot, Berkshire, SL5 7PY, U.K.[2]—The number of

infective juveniles (IJs) applied and the volume of water used during nematode application are important determinants in the efficacy of these nematodes against foliar pests. In the present study, the performance of a number of different spray methods (standard fan and full cone hydraulic nozzles, and a spinning disc) for the application of *Steinernema carpocapsae* (All) IJs was assessed. Larvae of diamondback moth, *Plutella xylostella*, on Chinese cabbage were used as a model system. The greatest number of IJs were applied using the hydraulic nozzles and subsequently gave the highest insect mortality (up to 98%). However, the spinning disc (which used the least amount of liquid) gave nearly 50% insect mortality while applying less than 9% of the IJs compared with the most effective of the hydraulic nozzles. This suggests that further work on low volume (spinning disc) systems is justified and may lead to cost effective applications of nematodes. The work was also discussed in relation to the timing of foliar applications of nematodes, and the effects of various abiotic and biotic factors on IJ survival and efficacy.

PRELIMINARY OBSERVATIONS OF THE FIRST IDENTIFIED SUGARBEET ROOT-KNOT NEMATODE RESISTANCE. M. H. Yu, USDA, Agricultural Research Service, Salinas, California, 93905, U.S.A.—Under *Meloidogyne* spp. infested conditions, sugarbeet (*Beta vulgaris*) plants generally suffer varied degrees of root gall and protuberance symptoms. A sea beet (*B. maritima*) germplasm that segregated for resistance to root-knot nematode, *M. incognita* Race 1, has been identified. The nematode resistance was transmissible to sugarbeet and its hybrid derivatives through pollination in the greenhouse. Resistant plants were produced in progeny of interspecific hybridization, backcrosses, and self-pollination of resistant genotypes. Intensity of certain undesirable traits of the sea beet, e.g. fibrous root systems, decreased as the number of backcross generations increased. Among the resistant progeny populations, one mutant leaf phenotype was discovered. Based on preliminary test results, this sea beet source was resistant to at least 4 species of root-knot nematode, *M. incognita, M. javanica, M. arenaria* and *M. hapla.*

RESTRICTION FRAGMENT LENGTH POLYMORPHISMS IN POLYMERASE CHAIN REACTION AMPLIFIED RIBOSOMAL DNAS OF FIVE *PRATYLENCHUS* SPECIES. Q. Yu, and J. W. Potter, Pest Management Research Centre, Agriculture and Agri-Food Canada, Box 6000, Vineland Station, Ontario L0R 2E0, Canada.—Restriction fragment length polymorphisms (RFLPs) from polymerase chain reaction (PCR) amplified ribosomal DNAs of 5 *Pratylenchus* species, *P. crenatus, P. neglectus, P. penetrans, P. thornei,* and *P. zeae,* were studied. Length variation in the internal transcribed spacers (ITS) was observed. The ITS region of *P. crenatus* is about 783 base pairs (bp) in length, *P. neglectus* 941 bp, *P. penetrans* 603 bp, *P. thornei* 737 bp, and *P. zeae* 692 bp. Restriction enzyme digestions of these regions showed a unique pattern for each species. The results indicate that these species of *Pratylenchus* can be clearly differentiated from one another by rDNA markers.

A RELIABLE, PRECISE METHOD TO DIFFERENTIATE SPECIES OF ROOT-KNOT NEMATODES IN MIXTURES ON THE BASIS OF ITS-RFLPs. C. Zijlstra, DLO Research Institute for Plant Protection (IPO-DLO), P.O. Box 9060, 6700 GW Wageningen, The Netherlands.—To arrive at an effective way of studying host range, genetic variation, virulence and plant nematode interactions in general, it is essential to work with characterized nematode populations preferentially consisting of one species only. Therefore, it is desirable to have methods to determine the species constitution of populations to be studied. We established a way to differentiate species of root-knot nematodes based on RFLPs of the ITS region of rDNA. Recent research showed that this method can also be applied to sensitively detect species in mixtures. DraI, EcoRI and RsaI restriction patterns of ITS PCR products from mixtures of *Meloidogyne hapla, M. chitwoodi, M. fallax* and *M. incognita* can detect these species when their presence in a mixture is 5% or more. The ratio of the intensities of the bands of each species-specific restriction pattern observed corresponded with the ratio of the species present in the mixture, indicating that the nematodes of the populations of the different species tested each contain

a similar number of ribosomal cistrons. This approach could be the basis for the development of a practical approach for determining the root-knot nematode species composition of field isolates to be used for routine analysis.

RISHITIN IN ROOTS OF TOMATO INDUCED BY *MELOIDOGYNE INCOGNITA*. S. V. Zinovieva, Institute of Parasitology, Russian Academy of Sciences, Leninskii prospect, 33, Moscow, 117071, Russia.—The effect of the root-knot nematode, *Meloidogyne incognita*, on production of the phytoalexin, rishitin, was investigated on tomato plants. Twenty-day-old seedlings of a resistant and a susceptible cultivar were infested with 10 000 *M. incognita* juveniles per plant. Accumulation of rishitin in the infected root has been correlated with the incompatible response of tomato to *M. incognita*. Quantitation of rishitin in the root tissues was made at intervals up to 25 days after inoculation with nematodes. Rishitin localization was detectable about 2-5 cm from the root tip. Rishitin accumulated in the resistant cultivar from 1 to 5 days after inoculation with *M. incognita* (45 μ/g fresh root tissue) and in the susceptible cultivar from 15 days after inoculation (10 μ/g fresh root tissue). The motility of second-stage larvae of *M. incognita* was inhibited by incubation in rishitin. All second-stage larvae exposed to 50 ppm solution of rishitin for 3 hr became rigid, made no movement, and appeared dead.

MANAGEMENT OPTIONS FOR CONTROL OF TRICHODORIDAE AND TOBACCO RATTLE VIRUS. F. C. Zoon, A. de Heij, A. T. Ploeg, C. J. Asjes, and P. W.Th. Maas, DLO Research Institute for Plant Protection, PO Box 9060, NL-6700 GW Wageningen, The Netherlands.—Problems due to tobacco rattle virus (TRV) in flower bulbs and potato are dealt with by removal of diseased plants and control of the vector nematodes by soil fumigation. Due to the restriction of pesticide use, there is a need for alternative management strategies and risk assessment. In flower bulb fields with primary TRV infection, *Paratrichodorus teres* was found most frequently, followed by *P. pachydermus* and *Trichodorus similis*. TRV serotypes obtained from the bulbous crops and from *Petunia* bait tests on soil from the same locations supported the importance of these vector species. In laboratory tests, the green manure crops Fodder Radish, White Mustard and Phacelia were non-hosts for *P. teres*. In soil containing viruliferous *P. teres*, roots of all tested crops and weeds, except Fodder Radish, gave a strong accumulation of naturally transmitted TRV. However, on soils with either *P. pachydermus* or *T. primitivus* as virus vectors, a large proportion of the tested crops showed some resistance to TRV accumulation. Fodder Radish was the only 'safe' crop for all 3 vector-virus combinations. In a field with viruliferous *P. teres* and *P. pachydermus*, a much lower percentage of TRV-symptomatous *Gladiolus* plants was found after a crop of Fodder Radish (3%) than after Italian Ryegrass (19%) or Fallow (16%). A sandwich method was developed to study nematode activation and attraction towards plant roots. Without plants, there was hardly any migration of *P. teres*, whereas with sugarbeet seedlings, the majority of these nematodes moved to the root compartment within 4 days. An intermediate layer of soil amended with 2% organic household waste compost inhibited nematode attraction, apparently due to interaction with plant root signals. Organic amendments placed below the plants may protect them during an initial period of susceptibility to TRV infection, or sensitivity to nematode feeding. Rational use of intercropping, weed control and organic amendments provides promising management tools to reduce damage due to trichodorid nematodes and TRV.

NEMATODES OF AN ITALIAN VOLCANIC LAKE. A. Zullini & M. Maggioni, University of Milan, 20133 Milano, Italy.—Bolsena is a small volcanic lake in central Italy with a maximum depth of 151 m. Its nematode fauna differs from that of other Italian fresh waters. Main aquatic plants are *Phragmites*, *Potamogeton* and especially *Chara*. Shallow benthic animals are mainly Naididae, Chironomidae, Gastropoda, Turbellaria, Hirudinea, Isopoda and the nematode *Tripyla glomerans* (dominant species). Deep benthos consists mainly of Tubificidae and Lumbriculidae. Samples of sediment were taken from 0 to 133 m depth. In all, 31 nematode species were recognized (7 of them are new). Some nematode species, such as *Tobrilus pellucidus*, *Aquatides aquaticus* and *Chromadorita leuckarti*, seem larger on

the average, than expected. In particular, *Tripyla glomerans* is significatively larger, on the average, than most specimens found until now in the world (females: 3.01 mm vs. 2.48 mm; males 2.94 mm vs. 2.54 mm). The Bolsena lake is inhabited by some marine related animals such as Acari (*Copidognatus hephaestios*: Halacaridae s.st., a taxon typical of sea waters including a few species adapted to fresh waters), and Crustacea (*Parapseudoleptomesochara italica* and *Schizopera* sp., thalassoids taxa). In addition, the Bolsena lake, unlike most freshwater Italian habitats, is rich in Chromadoria (*Chromadorina, Punctodora, Chromadorita, Paracyatholaimus*), a marine related nematode group. The marine-related nematode fauna and the large body dimension of some species may be a consequence of ancient geologic conditions of this area, which was submerged by the sea during the Pliocene.

ULTRASTRUCTURE OF *PRATYLENCHUS PENETRANS*. U. Zunke,[1] B. Y. Endo,[2] and W. P. Wergin,[2] University of Hamburg, Institute of Applied Botany, 20355 Hamburg, Germany,[1] and Nematology Laboratory, Plant Sciences Institute, USDA,[2] Beltsville, Maryland 20705, U.S.A.[2]—Observations were made on the ultrastructural anatomy of various developmental stages of *Pratylenchus penetrans* using transmission (TEM) and low temperature scanning electron microscopy (LTSEM) with emphasis on the esophagus, intestine, and reproductive system. The two subventral glands are delineated by cell membranes. At the base of the metacorpus, the lumen of the esophagus branches and terminates in quadriradiate valves in each of the subventral gland ampullae. The secretory granules are small and have moderate electron opacity. In comparison, secretory granules in the dorsal gland extension in the metacorpus may appear uniformly small but show a wide range in size and electron opaqueness within the procorpus. Sphincter muscles occur at each end of the metacorpus. Spermatogonial cells in the germinal zone of the testis develop into amoeboid spermatids with spherical nuclei that appear to lack a membrane and contain uniform concentrations of chromatin. The vaginal cuticle forms a flat contoured channel which is convoluted near the vulva and is continuous with the body cuticle. Two pairs of muscles with longitudinal-tangential orientations are attached to the cuticular wall of the vulva. Comparisons were made on observations of isolated nematodes and those made on nematodes within plant host cells using video-enhanced light microscopy (VECM).