

ABSTRACTS

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EXAMPLES AND MECHANISMS OF ACQUIRED RESISTANCE TO *BACILLUS THURINGIENSIS* INSECTICIDAL PROTEINS IN PLANTS. **Adang, Michael J.** Department of Entomology, University of Georgia, Athens, GA 30602-2605.

Cotton, corn, and potato plants engineered to express *Bacillus thuringiensis* (Bt) insecticidal proteins are in commercial production. A challenge to using these valuable crops is to avoid acquired resistance to the insecticidal proteins. Our approach to addressing issues of Bt-resistance is to investigate the mechanisms by which insects are adapting to Bt. Researchers have selected strains of Indian meal moth, tobacco budworm, beet armyworm, Colorado potato beetle, and diamondback moth for resistance to Bt. *Bacillus thuringiensis* resistant diamondback moths have been reported at various locations worldwide. We examined midgut brush border membranes from susceptible and resistant insects for biochemical changes that may cause resistance to Bt proteins. Several resistance species have eliminated toxin binding sites, whereas other insects display no apparent changes in the brush border membrane. It has been reported that resistant Indian meal moth larvae have altered midgut proteinases. Although still at early stages of investigation, these examples and mechanisms of acquired resistance have implications on Bt gene deployment.

ROTATION OF *HETERODERA GLYCINES* RESISTANT AND SUSCEPTIBLE CULTIVARS FOR SUSTAINABLE SOYBEAN YIELDS. **Anand, S. C., S. B. Sharma, S. R. Koenning, and J. A. Wrather.** Delta Center, University of Missouri, Portageville, MO 63873.

Twenty-three crop rotation treatments, consisting of soybean cyst nematode (SCN) resistant (Hartwig, Bedford, and Forrest) and susceptible (Essex) soybean cultivars, were evaluated in an SCN race 14 infested field between 1989 and 1992. Yields of soybean cultivars in monoculture were compared with those in rotation with the resistant and susceptible cultivars. Yield of Hartwig in monoculture and in rotation with other cultivars was similar. Performance of Essex, Bedford, and Forrest was markedly superior in rotation than in continuous monoculture. In all 4 years, average yields of Bedford were higher in rotation systems than in monoculture. Yield of Essex was 2.5-times greater in a Hartwig-Essex rotation than in monoculture. At-harvest in 1992, the cyst population densities were below detectable levels in many plots planted to Hartwig. The phenomenal decline in SCN population density over the years where Hartwig was planted was an important finding of these studies.

EVALUATION OF DROUGHT-TOLERANT SOYBEAN LINES FOR TOLERANCE TO *HETERODERA GLYCINES*. **Barker, K. R., and S. R. Koenning.** Department of Plant Pathology, North Carolina State University, Box 7616, Raleigh, NC 27695-7616.

Drought-tolerant soybean lines N90-7241 and N90-7199, were compared to the *Heterodera glycines* (SCN) resistant cv. Kirby, *H. glycines* tolerant line GA88-20092, and two susceptible cv. Cook and Coker 237, in a microplot, moisture-level study. The experiment was a factorial arranged in a RCBD with three SCN (race 1) inoculum levels (Pi) and two moisture regimes in a Fuquay sand. Midseason and final nematode numbers were greater in irrigated than nonirrigated microplots ($P = 0.05$). Population densities of SCN were greatest on the drought-tolerant soybean lines ($P = 0.10$). Reproduction of *H. glycines* was less on the SCN-tolerant line, GA88-20092 than on susceptible cultivars. Drought-tolerant soybean lines were similar to susceptible cultivars in

yield response to *H. glycines* Pi. Yield of the drought-tolerant lines, however, differed ($P = 0.01$) in response to the moisture by Pi interaction. The type of drought tolerance present in the soybean lines N90-7241 and N90-7199 does not seem to confer tolerance to *H. glycines*. Furthermore, supplemental irrigation failed to circumvent the damage caused by SCN.

RESISTANCE IN SOYBEAN MATURITY GROUPS IV - VI TO A *HETERODERA GLYCINES* RACE 3 POPULATION FROM MISSISSIPPI. Barnett, J. W., and G. W. Lawrence. Department of Entomology and Plant Pathology, Mississippi State University, Mississippi State, MS 39762.

Greenhouse tests were conducted to evaluate 140 soybean cultivars in maturity groups IV through VI for resistance to *Heterodera glycine* race 3. Included in the study were 39 group IV, 60 group V, and 41 group VI cultivars. Plants were inoculated with 5,000 eggs and second-stage juveniles/plant, and allowed to grow for 50 days. At harvest plant growth parameters were measured and nematodes were counted. For each cultivar, a female index was calculated by dividing the number of females on that cultivar by the number of females on Lee 74. Resistance to the *H. glycines* nematode was exhibited in 89 cultivars as follows: 24 group IV, 43 group V, and 22 group VI. Six maturity group V cultivars showed moderate resistance. Resistance ratings for 14 cultivars differed from ratings previously assigned in another location using a non-Mississippi *H. glycines* population.

PLANTIBODIES - A POTENTIAL APPROACH TO ENGINEERING NEMATODE RESISTANCE. Baum, T. J.,¹ W. A. Parrott,¹ A. Hiatt,² and R. S. Hussey.¹ ¹University of Georgia, Athens, GA 30602, and ²Rose-Hiatt Biotech, San Diego, CA 92121.

Esophageal gland secretions of *Meloidogyne* have putative roles in establishing and maintaining giant-cells in susceptible host plants. Inactivation of stylet secretions by plant-produced antibodies (plantibodies) should interfere with secretion function. Coding regions of the heavy and light chains of a monoclonal antibody (6D₄) specific to esophageal gland secretions were isolated and transformed separately into Xanthi tobacco using the nominally constitutive 35S promoter. Transgenic plants producing 6D₄ heavy or light chains were crossed and progeny plants were assayed for production and assembly of both chains. Functionality of the plantibody was established by indirect immunofluorescence analyses using nematode sections and ELISA using nematode secretions. Progeny of plants producing functional plantibodies were inoculated with *M. incognita*, but no differences in nematode parasitism or reproduction were detected between plantibody-producing plants and controls. Currently, spatial expression patterns and site of plantibody accumulation in the plant cell are being determined.

ALLIANCES BETWEEN ACADEMIA AND INDUSTRY IN BIOCONTROL PRODUCT DEVELOPMENT. Becker, J. O. Department of Nematology, University of California, Riverside, CA 92521.

There is increasing concern that certain advancements of modern crop production have an overall negative impact on the human population, livestock, and the environment. Biological control, despite its popular appeal and acceptance, has not yet measured up to the public expectations of providing feasible and effective alternatives for chemical pesticides. The discrepancy between a rapidly increasing wealth of literature on biological disease control and the apparent lack of commercial biocontrol products is a growing source of frustration for many academic researchers and industry professionals. There are many conceptual and practical differences between academia and industry goals that inhibit or slow product development. Recognition of each other's strengths and weaknesses may foster innovative approaches for cooperative interactions.

METHYL IODIDE: A POSSIBLE METHYL BROMIDE SUBSTITUTE FOR SOIL

FUMIGATION AGAINST PLANT-PARASITIC NEMATODES. **Becker, J. O.,¹ H. D. Ohr,² and J. J. Sims.²** Departments of ¹Nematology and ²Plant Pathology, University of California, Riverside, CA 92521.

Stratospheric ozone destruction has been related, in part, to the use of methyl bromide. Although methyl bromide is important to United States agriculture, under the terms of the Montreal Protocol and the Clean Air Act, its production, importation and use will be banned in the United States by the year 2001. With an estimated insignificant ozone depletion potential, methyl iodide was evaluated as a possible substitute by comparing its efficacy to methyl bromide against various plant-parasitic nematodes. Using 20 liter buckets filled with sandy soil as experimental test units, soil fumigation with methyl iodide at molar equivalent rates was as effective or better than methyl bromide against *Meloidogyne incognita*, *Heterodera schachtii*, and *Tylenchulus semipenetrans*.

NEMATODE COLONIZATION OF NEWLY EXPOSED LAND SURFACES. **Bernard, E. C.,¹ G. A. Laursen,² D. P. Schmitt,³ and S. L. Stephenson.⁴** ¹Entomology and Plant Pathology, The University of Tennessee, Knoxville, TN 37901-1071; ²Biology and Wildlife, University of Alaska, Fairbanks, AK 99775; ³Plant Pathology, University of Hawaii, Honolulu, HI 96822; and ⁴Science, Mathematics and Health Careers, Fairmont State College, Fairmont, WV 26554.

New land surfaces offer colonization opportunities for nematodes after initial colonization by plants and microflora. Substrate ("soil") progressively exposed over the last 75 years by retreat of the Columbia glacier in Alaska, and soil in vegetation pockets in recent Mauna Loa and Kilauea lava flows, were sampled for nematodes. In both locations, *Criconeema longulum* and *Paratylenchus* spp. were the earliest and most common phytoparasitic taxa found in areas colonized by seed plants. *Paratylenchus* spp. were abundant in Columbia regions uncovered after 1980, while *C. longulum* occurred most abundantly in a post-1923 site. *Psilenchus* sp. occurred sparingly in the post-1980 region. *Prionchulus* sp. (Mononchida) was abundant in the post-1923 and post-1935 areas. Colonization patterns in Hawaii were similar. Efficient movement of weakly migratory Criconeematina suggests previously unrecognized dispersal strategies.

ECOLOGY OF *HETERODERA CAROTAE* IN MICHIGAN. **Berney, M. F., and G. W. Bird.** Department of Entomology, Michigan State University, East Lansing, MI 48824.

Carrot cyst nematode (*Heterodera carotae*) has been reported only from one area of North America, Michigan. This species has been found only in commercial carrot production regions, and only in histosols. Extensive host range tests conducted under field, greenhouse, and growth chamber conditions revealed no hosts for carrot cyst nematode other than *Daucus carota* L. (wild and cultivated carrot). *Daucus carota* is present throughout much of Michigan and much of North America as "Queen Anne's Lace." It is a common plant in the early succession of disturbed sites. Survey data show that *H. carotae* is not commonly associated with Queen Anne's Lace in Michigan. With one exception, all Michigan sites currently known to be infested with *H. carotae* are also known to be infested with *Meloidogyne hapla*.

COMPARISON OF THE BEHAVIOR AND GENETIC DIVERSITY OF *GLOBODERA* AND *MELOIDOGYNE* SPP. **Blok, V. C., M. S. Phillips, and D. L. Trudgill.** Scottish Crop Research Institute, Invergowrie, Dundee, DD2 5DA, UK.

Tropical *Meloidogyne* spp. are widely distributed and have a wide host range, whereas *Globodera* spp. appear to have coevolved with *Solanum* spp. in South America. The former reproduce by mitotic parthenogenesis while the latter are amphimictic. The amount of variation in these two groups of nematodes differs. *Meloidogyne* spp. display relatively little intraspecific variation when compared to *Globodera* spp, considering the large number of plant species that are hosts for the former. There are differences in the amount of intraspecific variation when *Meloidogyne* spp. are compared particularly within *M. arenaria*, which is the most variable of these

species. This contrasts with the *Globodera* spp. found in Europe, which show greater intraspecific variation than reported in *Meloidogyne* spp., despite the fact they probably arose from only a few introductions from the total gene pool in South America.

THE USE OF PLANT-MICROBE-SOIL SYSTEMS FOR CHARACTERIZING ALLELOPATHIC INTERACTIONS INVOLVING PHENOLIC ACIDS. Blum, U. Department of Botany, North Carolina State University, Raleigh, NC 27695-7612.

The debate about whether phenolic acids are important allelopathic agents in no-till crop systems focuses on the fact that concentrations of individual phenolic acids recoverable from soil are below levels required for inhibition of germination or seedling growth. For example, the highest concentration observed for p-coumaric acid, one of seven phenolic acids extracted from wheat no-till soils, was $<5 \mu\text{g/g}$ soil. However, reduction of morning-glory seedling biomass by 20% required $16 \mu\text{g/g}$ soil under laboratory conditions. Soils, moreover, contain a variety of phenolic acids and other organic acids. We therefore hypothesized that, under field conditions, the inhibitory effects of phenolic acids might result from mixtures of phenolic acids and other organic molecules in the soil. This hypothesis was tested using a model system in the laboratory. Experiments from this indicated that: a) as the number of phenolic acids added to the soil increased, the concentrations of the individual phenolic acids required to cause a specific growth inhibition declined; b) the effects of individual phenolic acids in a mixture were either additive or partially antagonistic; and c) the addition of either carbohydrates or amino acids to the soil also reduced the concentration of a given phenolic acid required for a specific growth inhibition.

PRELIMINARY STUDIES OF THE INTERRELATIONSHIP OF NEMATODES AND *PYTHIUM* ON SUGARCANE IN LOUISIANA. Bond, J. P., E. C. McGawley, and J. W. Hoy. Department of Plant Pathology and Crop Physiology, Louisiana State University Agricultural Center, Baton Rouge, LA 70803.

Studies included a field survey, a greenhouse inoculation study with nematodes and five cultivars, and a microplot test designed to evaluate nematode-*Pythium* interactions. Survey data revealed the presence of diverse and abundant ($>6,000/\text{kg}$ soil) nematode communities containing *Hoplolaimus*, *Tylenchorhynchus*, *Trichodorus*, *Criconemella*, and *Pratylenchus*. Additionally, ratoon cane usually harbors greater numbers of nematodes than do plant cane. Eight treatment combinations representing the application of aldicarb, metalaxyl, both chemicals or untreated to pots (35 kg capacity) of field soil not fumigated or fumigated 1 month earlier with metham sodium were established in a microplot (a 14-m \times 9 m quonset hut frame covered with 6 mm clear polyethylene sheeting) as a RCBD with six replications. Uniform, germinated cuttings were established in pots and grown full season. Growth parameters were recorded at eight intervals during the season. At harvest, root and top weights of plants grown in nonfumigated soil were about half that of plants from fumigated soil. Nematode reproduction was abundant and root colonization by *Pythium* was extensive. However, chemicals did not provide substantial nematode or fungus control and growth increases resulting from selective pesticides treatments were not significant.

THE OCCURRENCE OF A SECOND PATHOTYPE OF POTATO CYST NEMATODE IN NEW YORK. Brodie, B. B. USDA ARS, Department of Plant Pathology, Cornell University, Ithaca, NY 14853.

Populations of *Globodera rostochiensis* that occur in New York are considered to consist solely of pathotype Ro1. Resistance to Ro1 is controlled by the single dominant gene H_1 bred into several commercial potato cultivars. Since the early 1980's, cultivars with the H_1 gene have been grown repeatedly in *G. rostochiensis*-infested soils of New York. These cultivars annually reduce population densities of Ro1 by 90% or more, resulting in nematode densities in infested fields

below detection levels after 2-4 years. Several cysts of *Globodera* spp. with viable eggs recently were recovered from a field on Long Island and at the golden nematode research farm in Steuben County, New York, where potato cultivars carrying the H₁ gene were planted six or more years. Nematodes from these cysts reproduced equally as well on cultivars with the H₁ gene as on Ro1-susceptible cultivars, whereas pathotype Ro1 reproduced only on susceptible cultivars. These data suggest that a pathotype of potato cyst nematode other than *G. rostochiensis* Ro1 occurs in these fields. The identity of this pathotype is being investigated.

PRELIMINARY STUDIES ON THE GENETIC DETERMINANTS OF VECTOR TRANSMISSIBILITY OF TOBRAVIRUSES. Brown, D. J. F.,¹ S. A. MacFarlane,¹ C. Hernandez², and J. F. Bol.² ¹Scottish Crop Research Institute, Invergowrie, Dundee, DD2 5DA, Scotland, U.K., and ²Institute of Molecular Plant Sciences, Gorlaeus Laboratories, Einsteinweg 55, 2333 CC Leiden, The Netherlands.

Pea early-browning (PEBV) and tobacco rattle (TRV) tobnaviruses, which cause diseases to several vegetable and ornamental crops in Europe, are transmitted by *Paratrichodorus* and *Trichodorus* nematodes. The viruses have bipartite genomes in which the nucleotide sequence of RNA-1 is highly conserved, whereas the RNA-2 is more genetically variable. Vector transmissibility was associated with the RNA-2 segment of the virus genome. Full-length, infectious clones have been constructed for nematode-transmissible isolates of both PEBV and TRV. Deletions and point mutations have been introduced into these clones to identify virus genes, encoding both structural and nonstructural proteins, which are involved in the transmission process.

MOLECULAR MARKERS ASSOCIATED WITH RESISTANCE TO MELOIDOGYNE ARENARIA IN PEANUT. Burow, M.,¹ A. H. P. Paterson,¹ C. E. Simpson,² and J. L. Starr.³ ¹Department of Soil and Crop Science, Texas A&M University, College Station, TX 77843, ²Texas Agricultural Experiment Station, Stephenville, TX 76401, and ³Department of Plant Pathology and Microbiology, Texas A&M University, College Station, TX 77843.

RAPD analysis is being used to identify polymorphisms associated with *M. arenaria* nematode resistance in peanut. Resistant and susceptible BC4F2 tetraploid plants bred from the interspecific hybrid-derived breeding line TxAG-7 and susceptible recurrent parents are being used for molecular analysis. In an initial screen, 344 primers were tested for polymorphisms distinguishing a pooled DNA sample of eight resistant plants from the recurrent parent cv. Florunner. Upon rescreening, several putative markers have been found to differentiate the pooled DNA sample of resistant plants from a pooled DNA sample of four susceptible BC4F2 plants. Screening is continuing with the objectives of confirming the usefulness of these markers, testing additional candidate markers, and determining the number of resistance genes introduced.

REPRODUCTION OF ROTYLENCHULUS RENIFORMIS ON WEED SPECIES COMMON TO LOUISIANA SOYBEAN FIELDS. Carter, C. H., E. C. McGawley, and J. S. Russin. Department of Plant Pathology and Crop Physiology, Louisiana State University, Agricultural Center, Baton Rouge, LA 70803.

The status of 10 weed species as hosts for *R. reniformis* was determined in two greenhouse tests. Inoculum level was 500 vermiform (juveniles, males, motile females) *R. reniformis*/pot, and numbers of vermiform stages were determined after 76 days. Reproduction (R) values were calculated to determine host status. Across tests, analyses of R values showed that pitted morning-glory (*Ipomoea lacunosa*, R=423) was a good host, northern jointvetch (*Aeschynomene virginica*, R=216) and sicklepod (*Cassia obtusifolia*, R=86) were intermediate hosts, and hemp sesbania (*Sesbania exaltata*, R=36), barnyardgrass (*Echinochloa crus-galli*, R=9), prickly sida (*Sida spinosa*, R=8), johnsongrass (*Sorghum halepense*, R=7), wild poinsettia (*Euphorbia heterophylla*, R=7), and

large crabgrass (*Digitaria sanguinalis*, R=2) were poor hosts. The R value for susceptible soybean cv. Davis was 61. The R values were much greater in test 1 (summer) than in test 2 (winter). In test 1, R values on poor weeds hosts remained low or increased when grown in combination with soybean. Values on good and intermediate weed hosts, however, were reduced when combined with soybean. In test 2, R values on all weed hosts were similar in both the presence and absence of soybean.

DYNAMICS OF *HIRSUTELLA RHOSILIENSIS* AND *HETERODERA SCHACHTII*: A COMPUTER SIMULATION MODEL. Caswell-Chen, E. P., and B. A. Jaffee. Department of Nematology, University of California, Davis, CA 95616.

A BASIC computer simulation model of the interaction between *Hirsutella rhossiliensis* and the sugarbeet cyst nematode, *Heterodera schachtii*, was developed to determine how characteristics of the fungus, nematode, and environment affect biological control. The model uses a series of difference equations, iterated weekly, to describe nematode-fungus interaction. The model universe of 17 cm³ is used as an appropriate scale for the interaction. Temperature was constant (20 C), and water potential moderate. Root growth was not specifically modeled, but nematode carrying capacity was defined and increased nematode numbers resulted in increased root damage and intraspecific competition. Dynamics over 168 weeks of host availability, or a 24-week season of host availability followed by 24-week winter, were simulated. Nematode suppression varied and depended strongly on spore density, transmission rate, and nematode carrying capacity.

INFLUENCE OF EARLY-SEASON BELOW-GROUND ARCHITECTURE OF *SOLANUM TUBEROSUM* ON *PRATYLENCHUS PENETRANS*. Chen, J., and G. W. Bird. Department of Entomology, Michigan State University, East Lansing, MI 48824.

The below-ground architecture of *Solanum tuberosum* consists of root and shoot tissue divided into eight components (basal roots, nodal roots, stolon roots, tuber roots, below-ground stems, stolons, tubers, and a seed piece). The influence of this structure on *Pratylenchus penetrans* was studied using cv. Superior grown under greenhouse conditions in Montcalm sandy loam soil from a potato field infested with *P. penetrans*. Plants were destructively sampled every 5 days from 10 to 45 days after planting. *Pratylenchus penetrans* was recovered from basal, nodal, stolon, and tuber roots, and stolons 15, 25, 35, 45, and 30 days after planting, respectively. The nematode completed a life cycle in stolon tissue. The final dry weights of basal, nodal, stolon, and tuber roots, stolons, tubers, and below ground stems were 0.03, 1.18, 0.01, <0.01, 0.15, 0.03, and 1.05 g/plant, respectively; compared with 0.88, 2.20, 0.02, <0.01, 0.26, 3.69, and 2.13 under pathogen-free conditions.

FUNGAL ANTAGONISTS OF THE SOYBEAN CYST NEMATODE. Chen, S. Y.,¹ D. W. Dickson,¹ and D. J. Mitchell.² ¹Entomology and Nematology Department, and ²Department of Plant Pathology, University of Florida, Gainesville, FL 32611-0620.

The mycoflorae in females and cysts of *Heterodera glycines* were investigated in a Florida soybean (*Glycine max*) field, and their influences on the nematode were estimated in the laboratory and greenhouse. More than 40 fungal species were isolated from the females, cysts, and eggs. In the field, the frequencies of fungi colonizing white and yellow females were low, but a high frequency of fungi was encountered in brown cysts, and the frequency increased with time of exposure of the cysts to the soil; the similarity of mycoflora in brown cysts for two sampling dates decreased with the days between the two sampling dates. A greenhouse bioassay showed that the nematode populations were suppressed by the fungi colonizing females, cysts, and eggs in soils collected from five field sites. The fungi isolated from the females and cysts differed in pathogenicity to the eggs, and some fungal species showed a high pathogenicity. The hatching rate of the nematode was correlated negatively with the percentage of eggs parasitized. Light, scanning

electron, and transmission electron microscopy studies revealed that 10 of 12 fungi studied penetrated the cyst wall, and at least three species penetrated through the cyst wall from the outside.

BIOLOGICAL CONTROL OF *MELOIDOGYNE ARENARIA* USING *PASTEURIA PENETRANS* IN FLORIDA. Chen, Z. X.,¹ D. W. Dickson,¹ D. J. Mitchell,² and R. McSorley.¹ ¹Entomology and Nematology Department, and ²Department of Plant Pathology, University of Florida, Gainesville, FL 32611-0620.

The biological control of *Meloidogyne arenaria* race 1 on peanut (*Arachis hypogaea* cv. Florunner) using *Pasteuria penetrans* was investigated in field microplots. *Pasteuria penetrans* was introduced either by direct infestation of microplots with endospores (Test 1) or by attaching the endospores to second-stage juveniles (J2) of the nematode (Test 2). In Test 1, *P. penetrans* reduced the root galling index and number of galls on pods of peanut ($P \leq 0.05$). At different inoculation levels of 0 (control), 1,000, 3,000, 10,000, and 100,000 endospores/g soil, the average numbers of galls on pods were 65.3, 32.6, 45.9, 30.1, and 8.8 galls/pods/plant, respectively, whereas the root galling indices were 6.3, 6.2, 6.7, 5.0, and 2.6 on a scale of 0 (none) to 10 (severe), respectively. In Test 2, the results indicated that the number of galls on roots and on pods were reduced with an increasing percentage of juveniles with attached endospore ($P \leq 0.05$). No effect on plant growth or yield occurred in either test.

EFFECT OF CROPPING SEQUENCES ON *HETERODERA GLYCINES* POPULATIONS. Cloud, G., and R. T. Robbins. Department of Plant Pathology, University of Arkansas, Fayetteville, AR 72701.

For several years a 3-year rotation that uses resistant cultivars, susceptible cultivars, and nonhost crops has been used to manage *Heterodera glycines*. This study used three cultivars (Walters, Agro A5885, and Hartwig) with *H. glycines* resistance from different sources, one susceptible cultivar (Hutcheson), and nonhost grain sorghum (Deltapine 522DR). At the conclusion of the 1994 growing season 25 rotational combinations were achieved with six replications per rotation combination. At the 1994 harvest, *H. glycines* egg population levels (eggs/100 cm³ soil) were lower ($P = 0.05$) in plots planted to Hartwig and grain sorghum in 1994 despite the crop planted in 1993. Soybean yields of Hutcheson, Walters, and A5885 were higher ($P = 0.05$) when planted after Hartwig and grain sorghum compared to following Hutcheson and Walters.

WHY *CAPILLARIA HEPATICA* (NEMATODA) IS UNABLE TO CONTROL MOUSE OUTBREAKS IN AUSTRALIA. Cooper, W. R. Division of Biochemistry and Molecular Biology, School of Life Sciences, Australian National University, Canberra A.C.T. 0200, Australia.

Australian agriculture suffers from irregular outbreaks of house mice *Mus domesticus* (up to 1,000 mice/ha), which cause extreme income loss due to damage to crops and stored products. *Capillaria hepatica* (Nematoda) was investigated as a potential biological control agent. Preliminary experiments with laboratory mice suggested that the nematode decreased the number of young produced and increased the interlitter time in infected female mice. A host-parasite model based on this experiment and some estimated parameters of life histories of mice and *C. hepatica* suggested that the parasite can dampen outbreaks. However, two small scale introductions of the nematode failed to establish, and no effect on host numbers could be detected. It was possible to establish infections in captive wild-type mice, indicating the failed introductions were not due to host resistance. Further, experiments have shown that there is no effect on number of young produced or time between litters in wild mice. The persistence of the soil-dwelling stages was studied using a fluorescence-based viability test. Refinement of the model, incorporating these data, showed that the *Mus-Capillaria* system is too unstable to be of use in biological control.

RESISTANCE AND TOLERANCE OF STRAWBERRY CULTIVARS TO *PRATYLENCHUS PENETRANS* IN ONTARIO. Dale, A.,¹ and J. W. Potter.² ¹H.R.I.O., Box 587, Simcoe ON N3Y 4N5, and ²Agriculture Canada Research Station, Vineland Station ON L0R 2E0, Canada.

In 1990 and 1991, tolerance of 19 strawberry cultivars to the lesion nematode, *Pratylenchus penetrans*, and their effect on nematode reproduction was compared in fumigated and infested field soil. Highly tolerant cultivars (Guardian, Bounty, and Veestar) grew as well in infested as in fumigated soil. Dry top weight of intolerant cultivars (Selva, Sparkle, and Annapolis) was reduced by 25% in infested soil. Most cultivars that supported large nematode population densities were in a family related to the cross Sparkle x Valentine. All cultivars supporting the fewest nematodes (Annapolis, Chandler, Micmac, Pajaro) were in a family related to Tioga. No immune cultivar was found. Variability for both resistance and tolerance among cultivars was consistent over the 2 years. The continuous nature of the variation with the relation together with the relatively distinct family groupings for resistance to *P. penetrans* suggests that resistance can be improved by breeding.

EVALUATION OF MULTI-PURPOSE SOIL FUMIGANTS AS ALTERNATIVES TO METHYL BROMIDE. Dickson, D. W.,¹ and S. L. Locascio.² Departments of ¹Entomology and Nematology, and ²Horticultural Sciences, University of Florida, Gainesville, FL 32611.

The efficacy of two formulations of methyl bromide (MBr 98-2, 450 kg/ha and MBr 67-33, 390 kg/ha) was compared with five other multipurpose soil fumigants for control of *Meloidogyne* spp., *Sclerotium rolfsii*, *Cyperus rotundus*, and *C. esculentus* on tomato at two sites in drip irrigated polyethylene mulch culture in Florida. Treatments included chloropicrin (390 kg/ha), 1,3-D + 17% chloropicrin (325 liters/ha), dazomet (450 kg/ha), metham sodium (935 liters/ha), and tetrathiocarbonate (1,870 liters/ha). Pebulate (4.5 kg/ha) was included with some treatments to suppress weeds. Tomato fruit was harvested at the breaker stage of growth and graded into marketable size categories of extra-large, large, and medium. At the Green Acres site in 1994 relative marketable fruit yields were 100% for MBr treatments, 77% for 1,3-D-pic + pebulate, 74% for chloropicrin, 72% for 1,3-D-pic, 60-65% for chloropicrin + pebulate and metham sodium, 56% for the untreated control, and 51-54% for dazomet and tetrathiocarbonate treatments. At this site *Sclerotium rolfsii*-infected plants were reduced ($P \leq 0.05$) by all treatments except tetrathiocarbonate and the untreated control. *Meloidogyne arenaria* galling indices were positively correlated ($P \leq 0.01$) with marketable yield ($r = 0.74$, $n = 66$), and plots treated with MBr provided greater marketable yields and lower ($P \leq 0.05$) galling indices compared with other treatments. At the Hort Unit site, nutsedge counts were 30 plants/0.1 m² for the untreated control, 8/0.1 m² with the MBr treatments, 13/0.1 m² with the pebulate containing treatments, and 28/0.1 m² with the remaining treatments. Relative yields were 100% for MBr, 86% with pebulate containing treatments, 60-70% with chloropicrin, 1,3-D-pic, dazomet, and metham sodium drip applied, 45% with soil applied metham sodium, 47% with tetrathiocarbonate, and 40% with the untreated control.

NEMATODE POPULATION DYNAMICS ON BENTGRASS. Donald, P. A., and B. S. Fresenburg. Plant Science Unit, 108 Waters Hall, Columbia, MO 65211.

Effects of a nematicide, biocontrol product, and nitrogen fertilizer were measured by monitoring the population dynamics of bentgrass soil nematodes. Fenamiphos (1 kg/90 m²) was applied once, and both Zap (Torro, Minn., MN) (0.8 L/90 m²), and 8% nitrogen (0.45 kg/90 m²) were applied twice, ca. 1 month apart. Fertility and fungicide treatments were applied uniformly across all plots. Comparisons were made against the untreated control plots. Soil samples were collected weekly from 18 May through 16 October 1994 and nematodes were extracted and counted. *Meloidogyne graminicola* and *Criconebella* sp. were the only plant-parasitic nematodes found in the plots and both were found in all plots. Plant-parasitic nematode levels were low at the time treatments were applied. Non-plant-parasitic nematodes were also counted from each plot. Mid-summer levels of

Criconemella sp. exceeded our damage threshold of 500 individuals/100 cm³ of soil. The ratio of plant-parasitic nematode to other nematodes and the numbers of plant-parasitic nematode found per species varied in relation to treatment.

GENETIC ANALYSIS OF VIRULENCE IN LINES OF THE SOYBEAN CYST NEMATODE, *HETERODERA GLYCINES*. Dong, K., C. H. Opperman, and K. R. Barker. Plant Pathology Department, North Carolina State University, Raleigh, NC 27695-7616.

Three inbred *Heterodera glycines* lines, OP20, OP25, and OP50, were developed and used as parents for testing virulence gene(s) on resistant soybean cultivars. Male and female nematodes were separated by hydroponic cultures. Crosses and back crosses were made on water agar medium. Host-range tests of F1 generation nematodes indicated that at least one virulence gene in OP20 and OP50 for host PI88788 is dominant. Virulence genes in OP50 for host Peking and PI90763 are recessive. Three types of single female descent populations, (random mating, back crossed once [BC1F2] and back crossed twice [BC2F1]), were established on susceptible cultivar Lee to determine the number of genes involved in inheritance of virulence. Virulence test results of these lines demonstrated the presence of two independent genes in OP20 for host PI88788, and two independent genes in OP50, one for host PI88788 and another for host PI90763.

INDUCTION OF SECRETIONS IN *GLOBODERA PALLIDA* BY PHYTOHORMONES. Duncan, L. H.,¹ W. M. Robertson,² and J. R. Kusel.¹ ¹Department of Biochemistry, University of Glasgow, Glasgow, G12 800, and ²Scottish Crop Research Institute, Invergowrie, Dundee, DD2 5DA UK.

Isolation of secretions from *G. pallida* population Luffness has allowed the production of antiserum that binds to the head and body of the nematode. Exposure of nematodes to the serotonin agonist 5-methoxy-N, N-dimethyltryptamine hydrogen oxalate increases secretions resulting in an altered pattern of antibody binding. To determine the stimulus by which these secretions may be induced in planta, the effects of various phytohormones were investigated. Results from these studies implicate phytohormones as possible triggers for the induction of secretions.

COLONIZATION OF HEAT-TREATED PINE LOGS BY *BURSAPHELENCHUS XYLOPHILUS* AND ITS *MONOCHAMUS* VECTORS. Dwinell, L. D. U.S. Forest Service, Southern Research Station, 320 Green St., Athens, GA 30602.

To find out if heat-treated pine logs remain susceptible to colonization by pine sawyers (*Monochamus* spp.) and pinewood nematodes (*Bursaphelenchus xylophilus*), Virginia and eastern white pine logs were heated in a 77/66 C (dry bulb/wet bulb) kiln until the wood temperature reached 60 C 2.5 cm deep. Sixteen 91-cm-long by 15.8-cm-d (mean) logs were treated in two runs. Treated and untreated logs were placed in the field during the flight period of the pine sawyers. The logs were debarked and the number of pine sawyer entrance holes was counted. The Baermann funnel technique was used to assay wood samples taken from the logs for nematodes. Within pine species, differences were not significant in either the number of pine sawyer entrance holes or population density of pinewood nematodes. Heat-treating Virginia and eastern white pine logs with attached bark until the outer sapwood temperature reached 60 C did not alter their susceptibility to attack by pine sawyers or colonization by pinewood nematodes.

ULTRASTRUCTURE OF *PRATYLENCHUS PENETRANS*. Endo, B. Y.,¹ U. Zunke,² and W. P. Wergin.¹ ¹USDA ARS, Nematology Laboratory, Beltsville, MD 20705, and ²Institut für Angewandte Botanik, Marseiller Str. 7, 2000 Hamburg, Germany.

Various developmental stages of the lesion nematode *Pratylenchus penetrans* were observed with transmission (TEM) and scanning electron microscopy (SEM) to define structural anatomy of the

esophagus, intestine, and facets of the reproductive system. The esophageal lumen wall is circular through the procorpus and triradiate in cross-section through the metacorpus, where it is extendable as part of a pump valve. Subventral gland valves open into the central lumen, which then extends posteriad to form the esophago-intestinal valve. Intestinal lumen is bounded by irregular membrane folds of adjacent intestinal cells. Testes contained spermatocytes that transform into amoeboid spermatids with electron-dense, nonmembrane-bound nuclei surrounded by fibrous bodies and spermatozoa. Comparisons were made of the nematode and host structures observed with video-enhanced light microscopy and low temperature SEM.

REPLACEMENT SERIES: A NEW APPROACH TO STUDY COMPETITION BETWEEN PHYTOPARASITIC NEMATODES. Erwin, S. R., 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, LA 70803-1720.

Competition between *Meloidogyne incognita* (MI) and *Rotylenchulus reniformis* (RR) was examined in two replacement series experiments in a greenhouse. Soil in pots containing seedlings of 'Davis' soybean was infested with 1,000 vermiform nematodes in the following MI:RR ratios: 1,000:0, 750:250, 500:500, 250:750, and 0:1,000. After 86 days, the relative yield of each species (ratio of yield in mixed culture to yield in single culture) was calculated based on nematodes in soil per gram of dry root tissue. In both experiments, relative yields of RR did not differ from those proposed in a hypothetical noncompetition model (lack of fit regression, $F = 0.9944$, $P > F = 0.3990$), indicating that competition with MI did not affect RR populations. However, competition with RR stimulated reproduction of MI (lack of fit regression, $F = 7.3683$, $P > F = 0.0002$). This was most evident at the 500 MI:500 RR and 250 MI:750 RR infestation ratios.

GENES FOR RESISTANCE TO *HETERODERA GLYCINES* IN THE SOYBEAN CULTIVAR HARTWIG. Faghihi, J.,¹ R. A. Vierling,² J. M. Halbrecht,³ V. R. Ferris,¹ and J. M. Ferris.¹ ¹Department of Entomology, Purdue University, West Lafayette, IN 47907-1158, ²Indiana Crop Improvement Association and Adjunct, Department of Agronomy, Purdue University, West Lafayette, IN 47907-1150, and ³Fruit Research and Extension Center, Pennsylvania State University, Biglerville, PA 17307-0309.

The number of genes for resistance to *Heterodera glycines* in progeny from the cross of Williams '82 × Hartwig (derived from Forrest × PI 437.654) was estimated by screening 220 F₂:F₃ lines with a fourth generation true inbred population of *H. glycines*, followed by assignment of lines to phenotype cells using a minimum variance cluster analysis. The ratio of phenotypes (resistant: segregating: susceptible) was not different from 1:8:7, suggesting a two-gene system. The ratio from screening 183 F₂ plants was not different from a 3:13 (resistant: susceptible) ratio. This suggests that resistance in this cross is conferred by both a dominant (Rhg) and a recessive (rhg) resistance gene.

SYNOPSIS OF A DEVELOPING DATABASE OF THE HOST STATUS OF PLANTS TO NEMATODES. Ferris, H., E. P. Caswell-Chen, and R. L. Sloan. Department of Nematology, University of California, Davis, CA 95616.

Initial development of the database has been from the major journals in nematology, with incorporation of earlier records from previous compilations. There are currently over 37,000 records in the database that include information on the host status of 5,344 taxa (to the variety level) of plants to 686 taxa (to the race level) of nematodes. The data include information from 66 countries. Approximately 63% of the records are from North America, 17% from Asia, 10% from Europe, 8% from Africa, 1% from South America, and 1% from Australia. The records are derived from 3,772 literature sources. For individual nematode genera, there are 15,700 host and 446 nonhost records for *Meloidogyne*, 5,322 hosts and 522 nonhost records for *Heterodera*, 343

host, and 18 nonhost records for *Globodera*, 3,570 host and 21 nonhost records for *Pratylenchus*, and 870 host and 15 nonhost records for *Helicotylenchus*. Percentages of hosts indicated as resistant or moderately resistant are 15% for *Meloidogyne*, 25% for *Heterodera*, 23% for *Globodera*, 8% for *Pratylenchus*, and 1% for *Helicotylenchus*, providing a crude measure of the frequency of resistance to those genera in plant germplasm.

PHYLOGENETIC RELATIONSHIPS OF CACTODERA BASED ON RIBOSOMAL DNA SEQUENCE DATA. Ferris, V. R., J. M. Ferris, and J. Faghihi. Department of Entomology, Purdue University, West Lafayette, IN 47907-1158.

Cactodera is distinctive among plant parasitic cyst nematodes by having morphological characteristics of both *Heterodera* and *Globodera*. The globose adult body has a terminal cone (as in *Heterodera*); but the vulval terminal is surrounded by circumfenestration (as in *Globodera*), and a D-layer (like *Globodera*) is present in most species. Ribosomal DNA (rDNA) sequence data for the two internal transcribed spacers (ITS1 and ITS2) and the 5.8S rRNA gene from *C. milleri* and *C. weissii* were compared with similar data for other species of Heteroderidae. In a maximum parsimony (PAUP) analysis and a maximum likelihood analysis of the rDNA sequence data, both *Cactodera* species grouped with *Globodera virginiae*, with *C. milleri* grouping more closely with *G. virginiae* than with *C. weissii*. These three species grouped more closely with other *Globodera* species than with schachtii-group species of *Heterodera*.

DEVELOPMENT AND HOST ATTACHMENT STUDIES USING PASTEURIA FROM BELONOLAIMUS LONGICAUDATUS FROM FLORIDA. Giblin-Davis, R. M.,¹ D. Williams,² T. E. Hewlett,³ and D. W. Dickson.³ ¹Fort Lauderdale R. E. C., ²Department of Microbiology, and ³Department of Entomology and Nematology, University of Florida, Gainesville, FL 32611-0620.

Ultrastructural studies with transmission electron microscopy (TEM) show that *Pasteuria* (S-1 isolate) from *Belonolaimus longicaudatus* is a new species. The epicortical wall of *Pasteuria* (S-1) surrounds the endospore in a sublateral band and the basal cortical wall thins to expose the inner endospore, similar to *P. thornei* but different from the other described species. The greatest outer cortical wall thickness is 1/3 the endospore diameter for *Pasteuria* (S-1) compared with 1/4-1/15 for the other described species of *Pasteuria*. The life cycle is similar to the other described species and involves attachment of a mature endospore to the cuticle of the host, penetration, formation of a mycelial microcolony, fragmentation, and sporogenesis. Laboratory host attachment studies with *Pasteuria* (S-1) using J2 of *Meloidogyne incognita*, *M. javanica*, *M. arenaria*, *B. longicaudatus*, *Hoplolaimus galeatus*, and *Pratylenchus penetrans* showed that this bacterium attaches only to *B. longicaudatus*.

CHROMOSOME NUMBER AND ISOZYME PHENOTYPES OF RADOPHOLUS IN HAWAII. Goo, M. Y. C., and B. S. Sipes. Department of Plant Pathology, University of Hawaii, Honolulu, HI 96822.

Populations of burrowing nematode collected from banana (Ba1), calathea (Ca1), and anthurium (An1 and An2) in Hawaii were evaluated for chromosome number, esterase, and malate dehydrogenase phenotypes. Fresh, egg-laying females were smeared on glass slides, stained with orcein, and observed under the microscope. Haploid chromosome numbers ranged from 4 to 7 in each population. Karyotypes of greatest frequency were n=4 for Ca1 (68%), n=5 for Ba1 (34%), and n=4 or 5 in An1 or An2 ($\leq 50\%$). PAGE esterase phenotypes were similar among all four populations. Two malate dehydrogenase phenotypes among the four populations were identified. A two-band phenotype was found in Ba1 and An1. A three-band malate dehydrogenase phenotype was detected in Ca1 and An2. Differences in chromosome number and isozyme patterns for each of the field populations studied suggested that the populations were no as uniform as anticipated.

THE REGULATION OF PATHOGEN-TRIGGERED PROGRAMMED CELL DEATH AND OTHER DEFENSE RESPONSES IN PLANTS. **Greenberg, Jean T.** Department of Molecular, Cellular and Developmental Biology, University of Colorado at Boulder, Campus Box 347, Boulder, CO 80309.

Many plant-pathogen interactions are accompanied by plant cell death. Recent evidence suggests that this cell death is often programmed, thus it results from an active process by the host and is not simply the result of the action of pathogen-derived toxins. We have isolated and characterized mutants of *Arabidopsis thaliana* that bypass pathogen exposure to induce pathogenic symptoms and multiple defenses. These mutants, called *acd1* and *acd2* (for accelerated cell death), must activate at least three independent signals to induce different signal transduction pathways. Progress on characterizing the *acd* mutants and determining the mechanism of activation of defenses will be discussed.

FACTORS AFFECTING THE PATHOLOGICAL RELATIONSHIP BETWEEN PLANT-PARASITIC NEMATODES AND ALFALFA. **Griffin, G. D.,** USDA ARS, Forage and Range research Laboratory, Utah State University, Logan, UT 84322-6300.

Pathological relationships between plant-parasitic nematodes and alfalfa are affected by environmental and biological factors such as soil moisture, temperature, soil texture, plant resistance, and differences in virulence of nematode races and populations. Factors that are conducive to nematode control under one nematode-alfalfa relationship may not be conducive under another relationship. The pathogenicity of the alfalfa stem nematode, *Ditylenchus dipsaci*, is affected more by soil water than temperature and soil texture, and nematode pathogenicity is affected by time and frequency of cutting. Root-knot nematodes, *Meloidogyne hapla* and *M. chitwoodi*, and root lesion nematode, *Pratylenchus penetrans* and *P. neglectus*, are more pathogenic at high temperatures and in sandy loam soil. Virulent differences in races and populations have been observed in root-knot and lesion nematodes, but not in the alfalfa stem nematode.

ALLELOPATHY AND HOST DEFENSE RESPONSES. **Gwinn, K. D., and J. F. Green.** Departments of Entomology and Plant Pathology, and Chemistry. University of Tennessee. Knoxville, TN 37901-1071.

Plants use low molecular weight chemicals synthesized via pyruvate and shikimic acid pathways for defense against pathogens (plant pathogen allelochemicals [PPA]) and in competition with other higher plants (higher plant allelochemicals [HPA]). Ecologists postulate that HPA are defense compounds that confer a competitive advantage in the environment and are therefore selectively maintained. Some chemicals have been implicated as both HPA and PPA (e.g., juglone); localization prevents overall phytotoxicity. Other HPA are structurally similar to PPA and have similar mechanisms of phytotoxicity. Conversely, some HPA predispose hosts to plant disease. In this review, we will examine the roles of chemical structure, localization, and mechanism of several HPA and PPA. Particular emphasis will be given to the allelochemicals produced in the Poaceae and to the roles endophytic fungi play in allelopathy.

ALLELOPATHY IN THE MANAGEMENT OF PLANT PARASITIC NEMATODES. **Halbrendt, J. M.** The Pennsylvania State University, Fruit Research and Extension Center, P.O. Box 309, Biglerville, PA 17307.

Many studies have confirmed the presence of nematicidal chemicals in plant tissues, extracts, and decomposing residues. Some compounds have been identified and determined to be secondary metabolites that presumably serve as a natural chemical defense against parasites and (or) disease. Most studies, however, have not identified active compounds. Regardless of biochemical data, the potential to exploit naturally occurring allelochemicals for nematode control is attractive; and there have been many attempts to utilize this approach either by rotation, intercropping, or as green

manure. Results have met with mixed success. Although proof of allelochemical involvement is difficult under field conditions, it is evident that some rotation crops are significantly better at reducing nematode populations than others. Presumably non-host rotations simply starve the nematode population (passive suppression) while others effectively increase nematode mortality by the production of allelochemicals (active suppression). Progress toward a sustainable agriculture should benefit from studies on allelopathic nematode control. However, farmer acceptance of new rotation techniques are based on economic and logistical considerations as well as efficacy. Practical aspects of production cannot be overlooked. Dagger nematode control with rapeseed green manure is presented as a potential practical application of this idea.

RESPONSE OF *MELOIDOGYNE* SPP. AND *HETERODERA GLYCINES* TO TANNIC ACID. **Hewlett, T. E.,¹ E. M. Hewlett,² and D. W. Dickson.¹** ¹Entomology and Nematology Department, University of Florida, Gainesville, FL 32611, and ²Buchholz High School, Gainesville, FL 32606.

Tannic acid was tested for its efficacy on control of *Meloidogyne arenaria* race 1 and its effect on the response of *M. arenaria*, *M. incognita*, and *Heterodera glycines*. Three rates of tannic acid (0.1, 1.0, and 10 g/500 cm³ soil) were applied preplant (powder) and at plant (powder and drench) on tomato inoculated with *M. arenaria* second-stage juveniles (J2) in greenhouse experiments. Nematode galling was reduced ($P \leq 0.05$) at the rate of 1.0 g tannic acid/500 cm³ soil in all treatments, 0.1 g had no effect, and 10 g was phytotoxic to tomato seedlings. Second-stage juveniles of *Meloidogyne* spp. were attracted in large numbers ($P \leq 0.05$) to areas of agar plates containing 0.3 ml of tannic acid (ca. 10,000 ppm), whereas *H. glycines* J2 were not attracted to tannic acid.

A GENE ASSOCIATED WITH RESISTANCE OF *GLYCINE MAX* CV. HARTWIG TO *HETERODERA GLYCINES*. **Huang, Y. R.,¹ G. Brown,¹ P. Kozlowski,¹ S. Anand,² and R. I. Bolla.¹** ¹Department of Biology, Saint Louis University, St. Louis, MO 63103-2010, and ²Delta Center, University of Missouri, Portageville, MO 63873.

Induction and repression of specific genes at different times after infection of soybean cultivars Hartwig and Essex with *Heterodera glycines* race 3 were investigated by the Differential Display procedure. Soybean was infected in the two-leaf stage with 3,000 second-stage juveniles of SCN. Infected and noninfected plants were harvested beginning 48 hours later. RNA was isolated from root tissue in the presence of an RNase inhibitor and DNase. After reverse transcription of mRNA, the cDNAs were amplified by PCR using Differential Display primers. These fragments were separated on 6% polyacrylamide sequencing gels. A cDNA fragment amplified from the infected Hartwig mRNA population has been isolated and cloned. Authenticity of this fragment as uniquely expressed in infected Hartwig has been verified by a procedure developed in our laboratory (Brown et. al., unpubl.). The mRNA encoded by this gene is expressed in root tissue as early as 3 days after infection. This gene is not expressed in uninfected Hartwig or in Essex. It is a member of a small gene family, other members of which are found in Essex. Preliminary information from sequence analysis and hybridization suggest this gene has a similarity to HMGCoA reductase.

MORTALITY OF PLANT-PARASITIC NEMATODES CAUSED BY BACTERIAL (*XENORHABDUS* SPP. AND *PHOTORHABDUS LUMINESCENS*) CULTURE MEDIA. **Hu, K., J. Li, and J. M. Webster.** Center for Pest Management, Simon Fraser University, Burnaby, Vancouver, B.C. V5A 1S6, Canada.

Second-stage juveniles (J2) of *Meloidogyne incognita* and a mixed population of *Bursaphelenchus xylophilus* were killed when exposed to the cell-free culture filtrate of *Xenorhabdus bovienii*, a bacterial symbiont of the entomopathogenic nematode *Steinernema* spp. The percentage mortality of J2 *M. incognita* was negligible when exposed to 25 or 50%

concentrations of 12 hour and 24-hour culture filtrate, but was 100% when exposed to 48 hour-120 hour culture filtrate at concentrations of 50% or more. The percentage mortality of *B. xylophylus* was only about half that of *M. incognita* at high concentrations (50% or more) of the culture filtrate. Neither water nor uninoculated tryptic soya broth culture medium, corrected to the same pH as the cell-free culture filtrate, resulted in a mortality greater than 2%. The major nematocidal compound of the culture filtrate, as detected by evaporation, neutralization, and infrared spectroscopy of the corresponding salt, was ammonia.

BIOCHEMICAL AND MOLECULAR CHARACTERIZATION OF *NACOBBUS ABERRANS* ISOLATES. Ibrahim, S. K.,¹ J. G. Baldwin,¹ P. A. Roberts,¹ and B. C. Hyman². Departments of Nematology¹ and Biology², University of California, Riverside, CA 92521.

Nacobbus aberrans is widely distributed in tropical and temperate regions, but there remains disagreement about the taxonomic status of *Nacobbus* spp. Isolates of *N. aberrans* from Argentina, Mexico, and two from Peru were analyzed for intraspecific variation. Comparisons were made using both individual and bulked females. The isoenzymes acid phosphatase, malate dehydrogenase, and esterase revealed reproducible banding patterns that differed in number and relative mobility for all the isolates. PCR-rDNA amplification of the internal transcribed spacers and the 5.8S gene from all four isolates generated a single fragment of about the 900 base pairs. Amplified rDNA fragments were digested with 13 restriction enzymes, generating a combination of size and restriction site polymorphisms useful for discrimination among the isolates. Nucleotide sequences from the internal transcribed spacer (ITS) were also compared for the four populations. Ten different random primers yielded reproducible differences in the number and size of the RAPD-PCR products among these samples.

CONTROL OF *MELOIDOGYNE CHITWOODI*, *PARATRICHODORUS ALLIUS*, AND CORKY RINGSPOT IN POTATO WITH FUMIGANT AND NONFUMIGANT NEMATOCIDES. Ingham, R. E.,¹ P. B. Hamm,² and B. Swanson.³ ¹Department of Botany and Plant Pathology, Oregon State University, Corvallis, OR 97331-2902, ²Hermiston Agriculture Research and Extension Center, Hermiston, OR 97838, and ³Crop Production Services, Umatilla, OR 97882.

Metham sodium (MS), 1,3-dichloropropene (1,3-D), 1,3-D plus chloropicrin (C-17), ethoprop, and oxamyl at various rates and combinations were evaluated during 1992-94 for control of *M. chitwoodi* (MC) and *P. allius* (vector for tobacco rattle virus) affecting potato in the Columbia Basin of Northeast Oregon. Metham sodium alone did not control corky ringspot (CRS) or MC. At low population densities of *P. allius* (2/250 g soil), 1,3-D controlled CRS at 94 liters/ha and at higher population densities (30/250 g soil), 140 liters/ha was adequate. However, rates of 1,3-D less than 234 liters/ha did not always control MC. Combinations of 1,3-D at 94 liters/ha plus MS at 374 liters/ha controlled both CRS and MC. Efficacy of C-17 was variable. Metham sodium plus ethoprop at 13 kg a.i./ha controlled MC and CRS under light nematode pressure. Metham sodium plus 2 or 3 post-plant applications of oxamyl at 1.1 kg a.i./ha was sometimes sufficient for CRS but did not control MC.

INTERFERENCE BY ENTOMOPATHOGENIC AND FUNGIVOROUS NEMATODES OF ROOT INVASION BY PLANT-PARASITIC NEMATODES. Ishibashi, N., and T. Matsunaga. Department of Applied Biological Sciences, Saga University, Saga 840, Japan.

Six species of entomopathogenic nematodes and *Aphelenchus avenae* were tested for their effects on root invasion by *Meloidogyne incognita* and *Pratylenchus coffeae*. The beneficial nematodes were applied before, at the same time, or after the inoculation of plant nematodes onto cucumber transformed hairy roots in a petri dish or onto seedlings in pots. All beneficial nematodes tested were suppressive to root invasion by plant nematodes. *Steinernema carpocapsae* and *A. avenae* reduced invasion by 97% on agar plates and 76% in soil. *Steinernema anomali*, *S. glaseri*,

Steinernema sp., *Heterorhabditis bacteriophora*, and *A. avenae* were most effective following simultaneous application with plant nematodes, whereas *S. carpocapsae* and *S. kushidai* gave the best results by preapplication. *Pratylenchus coffeae* was generally more susceptible than *M. incognita*. In nonautoclaved soil, the effect of beneficial nematodes was not so marked as in autoclaved soil.

PATHOGENICITY OF ENTOMOPATHOGENIC NEMATODES FOR THIRD INSTARS OF THE WESTERN CORN ROOTWORM. Jackson, J. J. USDA ARS, Northern Grain Insects Research Laboratory, Route 3, Brookings, SD 57006.

Pathogenicity of five *Steinernema* spp. and two *Heterorhabditis* spp. were compared using concentration-mortality data from bioassays on third instars of the western corn rootworm, *Diabrotica virgifera virgifera*. *Steinernema glaseri* and *S. feltiae* caused occasional mortality without a predictable concentration-mortality response. The median lethal concentration for the remaining test species and strains ranged from 26 to 1,772 nematodes/insect. Two strains of *H. bacteriophora* (Lewiston and Oswego), *H. argentinensis*, and two *Steinernema* species (*S. scapterisci* Argentinean and *S. carpocapsae* Mexican) were most pathogenic with LC₅₀ values near 60 nematodes per insect. The remaining species and strains were 7-50 times less pathogenic. Nematode strain and the size of the infective stage were significant factors influencing pathogenicity.

REPLACING METHYL BROMIDE WITH NATURAL ENEMIES OF PLANT-PARASITIC NEMATODES. Jaffee, B. A. Department of Nematology, University of California, Davis, CA 95616-8668.

Natural enemies of nematodes are numerous, but none seems able to replace major nematicides. Why? Have we (researchers) emphasized the wrong kinds of natural enemies? Is biological control difficult to achieve or are we still early in our studies? Are promising natural enemies identified and in need of development? Have we lacked sufficient optimism? Have we spent too much time studying fundamental biology and too little time screening agents under realistic conditions? Consideration of these questions may help us understand where we are and where we need to go to achieve biological control of plant-parasitic nematodes.

EFFICACY OF PELLETIZED HYPHAE OF *HIRSUTELLA RHOSSILIENSIS* AGAINST *HETERODERA SCHACHTII* IN FIELD MICROPLOTS. Jaffee, B. A.,¹ G. S. Abawi,² A. E. Muldoon,¹ and B. B. Westerdahl.¹ ¹Department of Nematology, University of California, Davis, CA 95616, and ²Department of Plant Pathology, NYSAES, Cornell University, Geneva, NY 14456.

Alginate pellets, with and without hyphae of the nematophagous fungus *Hirsutella rhossiliensis*, were added to field microplot soil (1 or 2 pellets/cm³ soil) that was also infested with *Heterodera schachtii* (5-20 eggs or juveniles/cm³ soil). After 2 weeks, the microplots were planted with cabbage seedlings (in California) or table beet seedballs (in New York). After two more weeks, roots were removed, weighed, and stained. Many juveniles penetrated the roots whether the fungus was added or not. To determine the extent of *H. rhossiliensis* growth in soil, pellets were placed in observation chambers with untreated and heated (60 C for 0.5 or 2 hours) microplot soil. The fungus grew well in heated soil but grew poorly in unheated soil, suggesting that other organisms in the microplot soil were inhibitory. Similar inhibition was not observed in a peach orchard soil.

PROTEINS IN THE ADHESIVE MATERIAL ON THE CONIDIA OF THE NEMATOPHAGOUS FUNGUS *DRECHMERIA CONIOSPORA*. Jansson, H.-B., and E. Friman. Department of Microbial Ecology, Lund University, Ecology Building, S-223 62 Lund, Sweden.

The conidia of the nematophagous fungus *Drechmeria coniospora* adhere to the sensory structures of nematodes purportedly by means of protein-protein binding. The adhesive pad can

be seen as a thick material covering the conidial "bud" and has been suggested to contain proteins that bind to proteinaceous sensilla exudates from the nematodes. We have used proteases and detergents to dissolve the adhesive material from the conidia. The material has been isolated using high performance liquid chromatography (HPLC) and electrophoretic techniques with and without biotin surface labelling. The effects of each treatment on the conidia were assessed using transmission electron microscopy. The material consists of several proteins, with molecular weights ranging from 18-100 kDa, and carbohydrates.

EFFECT OF SIMULATED RAINFALL ON EFFICACY AND LEACHING OF FENAMIPHOS. Johnson, A. W., R. D. Wauchop, and D. R. Sumner. USDA ARS, Coastal Plain Experiment Station, University of Georgia, Tifton, GA 31793.

The objective of this 2-year field study was to determine the degradation, movement, and efficacy of fenamiphos (3SC and 15G) in the soil and residue in squash fruit following different simulated rainfall treatments after nematicide application. Rainfall treatments of 0, 2.4, and 5.0 cm water had little effect on fenamiphos concentrations in the soil except for some transfer from the 0-8 cm of soil to 8-15 cm deep. From 57-100% of the fenamiphos, 98-100% of the fenamiphos sulfoxide, and 98-100% of the total fenamiphos residues remained in the 0-15 cm soil layer in all plots; after rainfall, trace amounts (≤ 0.329 ppm) of fenamiphos residue occurred in the 15-30 cm soil layer. Concentrations of total residue in squash fruit ranged from 1 to 4 μg fenamiphos sulfone/g (ppb), which is far below the tolerances established for fenamiphos on other vegetable crops.

GENE EXPRESSION CHANGES DURING THE HATCHING PROCESS OF *GLOBODERA ROSTOCHIENSIS*. Jones, J. T. Scottish Crop Research Institute, Invergowrie, Dundee, DD2 5DA UK.

Second-stage juveniles of *G. rostochiensis* are stimulated to hatch by root diffusates of their host. Potato root diffusate (PRD) generates structural changes in the unhatched nematode and is thought to induce other changes, which prepare the J2 for a parasitic mode of existence. Such effects are likely to require changes in gene expression. We have used a range of techniques, including differential display, to examine changes in gene expression during the hatching process of *G. rostochiensis*. We have compared dormant (dried) cysts with those soaked in water and those exposed to the host stimulus (PRD) for various lengths of time. We have also included the hatched nematodes, at two ages, in our comparisons. Our results show that few, if any, changes in gene expression or protein profile are induced directly by host stimuli before hatching has occurred. Changes in gene expression apparently occur during or immediately after the natural hatching process. These results are discussed in the host-parasite relationship.

LONG-TERM STUDY ON THE VALUE OF NEW CROPS FOR THE MANAGEMENT OF ROOT-KNOT AND SOUTHERN BLIGHT IN 'FLORUNNER' PEANUT. King, P. S., R. Rodríguez-Kábana, D. G. Robertson, and C. F. Weaver. Department of Plant Pathology, Auburn University, Auburn, AL 36849.

The value of rotations of peanut with cotton, castor (*Ricinus communis*), American jointvetch (*Aeschynomene americana*), partridge pea (*Cassia fasciculata*), and sesame (*Sesamum indicum*) for the management of *Meloidogyne arenaria* and southern blight (*Sclerotium rolfsii*) was studied for 9 years in a field in southeast Alabama. In 1994, peanut following any of the rotation crops yielded higher ($P \leq 0.01$) than monoculture peanut. Numbers of *M. arenaria* juveniles in soil following the rotations were lower than those in peanut monoculture soil. Incidence of southern blight was lowest in peanut following castor, partridge pea, sesame, and cotton; the jointvetch-peanut rotation had no effect on the blight. Application of tebuconazole (Folicur) to control *S. rolfsii* increased yields in all cropping systems; the highest increase was in monoculture peanut and

the lowest in peanut following castor.

CROPPING OF COTTON AND PEANUT AND THEIR INFLUENCES ON POPULATIONS OF RENIFORM AND ROOT-KNOT NEMATODES IN FLORIDA. Kinloch, R. A. University of Florida, REC, Jay, FL 32565.

Growers' fields were assayed for population changes of *Rotylenchulus reniformis* and *Meloidogyne* spp. Peanut following cotton was monitored 84 times. Among these, 30 sites were infested with reniform nematode, whose population densities declined on average from 1,550-260/100 cm³ soil and root-knot nematode, whose J2 remained unchanged at 50-60. In 54 non-reniform nematode sites, root-knot nematode declined from 160-40. Cotton following peanut was monitored 55 times. Among these, 20 sites were infested with reniform nematode whose populations increased from 160-1,160, and root-knot nematode, which remained unchanged at 70. In 35 non-reniform sites, root-knot nematode populations increased from 40-90. Reniform nematode infested sites averaged 72% sand, 15% silt, and 13% clay, whereas non-reniform nematode sites averaged 81% sand, 11% silt, and 8% clay.

MELOIDOGYNE INCOGNITA-THIELAVIOPSIS BASICOLA INTERACTIONS ON COTTON. Kirkpatrick, T. L.,¹ and C. S. Rothrock.² ¹Southwest Research and Extension Center, Hope, AR 71801, and ²Department of Plant Pathology, University of Arkansas, Fayetteville, AR 72701.

Meloidogyne incognita (Mi) is frequently found in association with *Thielaviopsis basicola* (Tb), the causal agent of black root rot, in Arkansas cotton fields. Seedling vigor has been particularly low in many of these fields, and low yields are generally seen. Microplots (76 cm-d × 0.75 m deep) were used to evaluate the effects of these two organisms individually and in combination on cotton growth and yield. Treatments included Mi at 5,000 eggs and juveniles/500 cm³ soil, Tb at 13,130 propagules/500 cm³, both Mi and Tb at the same rates, and a control where neither organism was present. A completely random design with 10 replications of each treatment was used. The combination of Mi + Tb resulted in greater seedling mortality during the first 4 weeks of the season. Dry weight of surviving plants following this treatment was lower than for the other treatments. This combination also decreased plant height at 7 and 9 weeks after planting and resulted in fewer fruiting branches formed during the season and fewer bolls retained. The Mi + Tb treatment also delayed maturity of the crop and resulted in lower seed cotton yield.

INFLUENCE OF ELEVATION AND IRRIGATION ON NEMATODE POPULATION DENSITIES AND PINEAPPLE YIELDS. Ko, M. P.,¹ D. P. Schmitt,¹ H. Fleisch,² and B. S. Sipes.¹ ¹Department of Plant Pathology, University of Hawaii, Honolulu, HI 96822, and ²Maui Pineapple Company, Haliimaile, HI 96768.

Four to five levels (0-12.5 eggs/cm³ soil) of *Meloidogyne javanica* (Mj) were added to microplots grown to pineapple at three elevations (L-46 m, M-305 m, and H-518 m), and two moisture regimes (irrigated and nonirrigated). Population densities of inoculated Mj and indigenous *Helicotylenchus dihystera*, *Paratylenchus* spp., and *Rotylenchulus reniformis* (Rr), and the growth response of pineapple in microplots were then monitored at timed intervals. *Meloidogyne javanica* established readily at H- but only slowly at L-elevation. A dose-response of growth and pineapple yield to Mj occurred only in the irrigated plots. Pineapple growth and yield were highest at M-elevation, where Mj and Rr densities were lowest. *Meloidogyne javanica* and the indigenous nematodes reduced yields of ratoon- more than plant-crop and influenced yields of irrigated plots more than nonirrigated plots. Nematode densities were generally higher at ratoon- than at plant-crop harvest, and higher in irrigated than in nonirrigated plots. *Meloidogyne javanica* was more prevalent at H- than at L-elevation sites, whereas Rr's site prevalence was the reverse.

GREENHOUSE EVALUATION OF THREE SOIL AMENDMENTS FOR CONTROL OF

MELOIDOGYNE INCOGNITA. Krusberg, L. R., S. Sardanelli, and J. G. Kantzes. Botany Department, University of Maryland, College Park, MD 20742-5815.

Three soil amendments formulated from combinations of crabshell meal, soybean meal, guano, and urea, supplied by Igene Biotechnology, Inc., were evaluated in greenhouse pot tests for their ability to suppress *Meloidogyne incognita*. Untreated loamy sand field soil was used. Amendments were mixed into the soil at 1,800 and 3,600 kg/ha. Mixed nematode eggs and J2 were added to the soil at 8,000/pot 9 days before planting with susceptible tomato (transplants) or green bean (direct seeded). Experiments were terminated 60 days after planting. The high application rate of all three amendments suppressed nematode egg production in tomato plant roots as did the high rate of 2 of the 3 amendments in bean roots; the low rate gave erratic results on both plants. All three amendments increased bean and tomato plant growth at both rates applied, and no phytotoxicity was observed.

BALANCING THE PRODUCT PORTFOLIO: THE AGROCHEMICAL INDUSTRY'S INVESTMENT IN BIOLOGICAL CONTROL. Lackey, B. A. Zeneca Ag Products, Southern Regional Technical Center, Leland, MS 38756.

Current growth in the biopesticide sector of the plant protection industry is estimated at 20-25% per annum, primarily supported by small biotechnology-based companies. Such growth is fostered by public concern about chemical pesticides, stringent regulations, and a decline in novel technology with traditional chemical synthesis. In response to these issues, the agrochemical industry recognizes biotechnology as a necessary investment. Resources are increasingly being allocated to develop biotechnological approaches to pest and disease control through in-house research programs, contractual grants, and acquisitions. Direct financial reward from this investment is, however, a minor expectation. The potential for long-term benefits (improved public image, downstream applications of novel technology, resistance management, and product life extension of current chemical pesticides) better describes the agrochemical industry's motivation to enter the biopesticide market.

COTTON PLANT DEVELOPMENT IN A RENIFORM NEMATODE INFESTED FIELD. Lawrence, G. W.,¹ and K. S. McLean.² ¹Department of Entomology and Plant Pathology, Mississippi State University, Mississippi State, MS 39762, and ²Department of Agriculture, Northeast Louisiana University, Monroe, LA 71209.

The effect of the reniform nematode (*Rotylenchulus reniformis*) on the growth of cotton (Deltapine 50) was examined. Cotton plant growth and development was assessed biweekly during the growing season. At harvest cotton boll position, boll number, and boll weights were recorded. Treatments included aldicarb (Temik 15G) at 3.9, 5.6 and 7.8 kg product/ha and an untreated control. During the growing season, cotton plants were larger, had a greater leaf area, produced more squares and green bolls in the aldicarb-treated plots. At harvest more open bolls and bolls with a greater total weight were produced on aldicarb-treated plants. Total boll weight was increased 12.8 g/plant in the aldicarb treated plots compared with the untreated control. Reniform nematode numbers, averaged across the growing season, were lower in the aldicarb treated plots compared with the control.

MECHANISMS OF MORPHOLOGICAL STRUCTURES OF SWEET POTATO RESISTANCE TO *DITYLENCHUS DESTRUCTOR*. Lin, M. S.,¹ He, L. M.,¹ Wen, L.,¹ Fang, Z. D.,¹ and Song, B. F.² ¹Department of Plant Protection Nanjing Agricultural University, Nanjing, China 210095, and ²International Potato Center Regional Office for China, Beijing, China 100081.

The phellem was found to consist of 4 to 5 layers of cells in the root tuber periderm of resistant varieties of sweet potato 'Lushu-78066' and 'Shengli-100'. There was more suberin in the cell wall of phellem of resistant than susceptible varieties. The morphological structure of the sweet

potato can inhibit invasion of potato rot nematode effectively. The periderm of susceptible varieties was poorly developed, consisting of 1 to 2 layers of phellem cells with little suberin. The nematodes may invade the epidermis of susceptible varieties within 24 hours. The pith of stem and subterranean stems in both resistant and susceptible varieties of sweet potato may be invaded by the nematode. In the resistant varieties Lushu-78006 and Shegli-100, the cell walls of xylem parenchyma were thicker and more lignified and the top of the root tubers had more vessels linking the subterranean stem than in the susceptible varieties 'Li Zixiang' and 'Xushu-18'. This structure may limit invasion and spread of the nematode. Wounding of the epidermis allowed nematodes to invade both resistant and susceptible varieties, causing the epidermis and cortex to become black, brown, and spongy. The nematode cannot invade tissue inside phellogen of the root tuber, suggesting that the phellogen may be a barrier to nematode infection.

RELATIONSHIP BETWEEN THE STRUCTURE OF U.S. AGRICULTURE AND NEMATODE MANAGEMENT IN POTATO PRODUCTION. Mather, R. L., and G. W. Bird. Department of Entomology, Michigan State University, East Lansing, MI 48824.

The number of farm sector enterprises in the United States has declined during the past 50 years. The impact of this decline on applied nematology is not easily understood. The results of a potato research project were used to quantify differences in nematode management practices among Michigan potato farms. The farms were stratified into size classes of <20, 20-101, and >101 ha, and 40 of the 550 Michigan potato farms were selected at random for the study. These enterprises collectively represented 23.6% of Michigan potato production acreage. Simple linear regression models were used to assess the impact of irrigation, crop rotation, and nematicide (fumigant, chemigant, and nonfumigant) use on expected tuber yields by farm size. A multiple regression model was used to assess the joint impact of these factors. This model explained 68.92% of the variability in expected yield. Changes in the structure of U.S. agriculture appear to have potential to significantly impact applied nematology.

REPRODUCTION AND PATHOLOGICAL VARIATION IN POPULATIONS OF ROTYLENCHULUS RENIFORMIS. McGawley, E. C.,¹ and C. Overstreet.² ¹Department of Plant Pathology and Crop Physiology, and ²Louisiana Cooperative Extension Service, Louisiana State University Agricultural Center, Louisiana Agricultural Experiment Station, Baton Rouge, LA 70803-1720.

Seventeen populations of reniform nematode (9 from Louisiana, 2 from Arkansas, 3 from Hawaii, 2 from Mississippi, and 1 from Texas) were studied in greenhouse and laboratory tests to determine the extent of variation among populations with respect to reproduction on and (or) damage to cotton (*Gossypium hirsutum* cv. Deltapine [DP] 90 and DP 41) and soybean (*Glycine max* cv. Bragg and Hartz [H] 6686). Each of these populations was derived from a single egg mass. High and low R-values ($R = Pf/Pi$, $Pi = 518-630$ vermiform individuals/15-cm-d pot) at 95 days after inoculation were 68.5 and 8.2 for DP 90, 29.4 and 9.3 for DP 41, 185 and 57.2 for Bragg, and 176.3 and 43.9 for H6686, respectively. Hatch of reniform nematode eggs was evaluated in distilled, deionized water (pH 6.8-7.0) and in fumigated soil. In water, hatch during a 72-hour period ranged from 42-90%. In soil, hatch at 26 C ranged from 45-93%. Eleven populations of reniform nematode caused reductions ($P \leq 0.05$) in cotton root weights and 14 caused reductions ($P \leq 0.05$) in soybean root weights.

FORMULATION OF BIOPESTICIDES: A CASE HISTORY OF TECHNOLOGY TRANSFER. McGuire, M. R. USDA ARS, Bioactive Constituents Research Unit, 1815 North University, Peoria, IL 61604.

Biopesticides are being touted as tools to replace some of the synthetic organic pesticides. However, for a number of reasons, commercial products are not meeting market expectations.

Production, shelf life, and field activity are all aspects of microbial pesticides that must be considered when developing new products. We have focused on formulation as an issue and emphasized naturally occurring polymers from surplus farm commodities as matrix or film forming materials. Our data clearly demonstrate the utility of these polymers for extending residual activity and increasing efficacy of *Bacillus thuringiensis* in both granular and sprayable formulations. However despite extensive efforts in technology transfer by the scientists involved in the project and by staff from the Biotechnology Research and Development Corporation, industry has been reluctant to commercialize these inventions. Possible reasons include the following: The "not invented here syndrome"; lack of knowledge of suitable industry standards for flowability, mixing, etc.; cost and bulk of additional ingredients; market size; and company size.

FUNGICIDE SUPPRESSION OF *FUSARIUM SOLANI*, THE CAUSAL AGENT OF SUDDEN DEATH SYNDROME OF SOYBEAN. McLean, K. S., and G. W. Lawrence. Assistant Professor, Department of Agriculture, Northeast Louisiana University, Monroe, LA 71209, and Associate Professor, Department of Entomology and Plant Pathology, Mississippi State University, Mississippi State, MS 39762.

Pentachloronitrobenzene (PCNB), benomyl, captan, carboxin, and metalaxyl were examined in vitro and in microplots for their efficacy in suppression of *Fusarium solani*. In vitro suppression was determined by incorporation into potato dextrose agar by filter sterilization at rates of 5, 10, and 15 ppm. A 5-mm agar disk of *F. solani* was inverted on the agar surface and placed at 25 C. Microplots were inoculated with a cornmeal sand-*F. solani* inoculum at a rate of 0.5% (w/w) and 100 eggs of *Heterodera glycines* race 3/100 cm³ soil. In vitro, all chemicals reduced ($P \leq 0.05$) fungal growth compared to the control at 7 and 14 days. The greatest reductions of 66% and 45% were observed in the benomyl and carboxin treatments, respectively. The incidence and severity of visual foliar symptoms in the microplots were reduced by all fungicides compared to the control. Plant height, the number of pods per plant and plot yields were greatest in the PCNB and carboxin treatments.

CULTURAL PRACTICES FOR MANAGING NEMATODES. McSorley, R. Entomology and Nematology Department, University of Florida, Gainesville, FL 32611-0620.

The most widely used and successful cultural practices for managing plant-parasitic nematodes are the use of resistant cultivars and some elements of cropping systems, such as rotation crops, cover crops, or nematode-antagonistic crops. Site selection, sanitation, and weed management are probably useful practices in many situations, while organic amendments, fallow, flooding, timing of planting dates, or tillage practices may be helpful in some cases. Most methods emphasize reduction of nematode numbers, but improvement of crop tolerance to nematodes should not be overlooked. Integration of several practices may be required for maximum nematode suppression. Most of these methods require detailed knowledge of nematode and plant biology and ecology for their successful use. Although the principles are known, detailed data on specific nematode-host cultivar combinations are often lacking, and therefore the ability to obtain such data will likely determine the probability of success or failure in managing nematodes in a particular region.

NEMATODE COMMUNITY STRUCTURE IN DECLINING AGRICULTURAL FIELDS. McSorley, R. Entomology and Nematology Department, University of Florida, Gainesville, FL 32611-0620.

Densities of nematode genera in soil were determined on four sampling dates in a soybean (*Glycine max*) field and on three sampling dates in a subsequent crop of rye (*Secale cereale*). At planting of the soybean crop, the nematode community consisted of 50.4% bacterivores (B), 22.0% plant parasites (P), and 15.4% fungivores (F). Composition changed to 44.0% B, 37.3% P, and 6.6% F at the end of the soybean crop, and 51.6% B, 31.8% P, and 10.6% F at the end of the rye

crop. Above-ground biomass of rye was correlated ($P \leq 0.05$; 10 df) with numbers of F in the November ($r = 0.746$), December ($r = 0.761$), and February ($r = 0.689$) samples, and with B in November ($r = 0.695$) and December ($r = 0.734$) but not February. Several indices of nematode community structure were calculated on each sampling date, however, except for F/B in December ($r = 0.632$) and February ($r = 0.594$) and (F+B)/P in November ($r = 0.594$), none were correlated ($P \leq 0.05$; 10 df) with rye biomass. Evidence suggests a strong influence of plant biomass on certain components of the nematode community, particularly bacterivores and fungivores.

EFFECT OF *HETERODERA GLYCINES*, *MELOIDOGYNE INCOGNITA*, AND *PRATYLENCHUS PENETRANS* ON SOYBEAN CULTIVARS WITH VARYING DEGREES OF SUSCEPTIBILITY TO *H. GLYCINES*. Melakeberhan, H. Department of Entomology, Michigan State University, East Lansing, MI 48824.

The response of *Heterodera glycines*-resistant ('Brian'), -tolerant-susceptible ('G88-2002'), and -intolerant-susceptible ('Tracy') soybean cultivars to *Meloidogyne incognita*, *Pratylenchus penetrans*, and *H. glycines* infection was determined. *Heterodera glycines* and *M. incognita* (eggs), and *P. penetrans* (all vermiform stages) were inoculated at 0, 1,000, 3,000, or 9,000 (experiments 1 and 2), and at 6,000 (experiments 3 and 4) individuals per 15-cm clay pot. Plants were placed randomly on greenhouse benches (25 ± 2 C) and watered daily to saturation and twice per week with normal strength Hoagland solution over a 6-7 week-period. Shoot dry weight and number of nematodes per g fresh root weight were determined. With the exception of *H. glycines* on Brian, the numbers of all three nematodes increased with increasing inoculum levels in experiments 1 and 2. In experiments 3 and 4, the numbers of *H. glycines* were fewer than *M. incognita* and *P. penetrans* on Brian, whereas the numbers of all three nematodes were similar on the tolerant and susceptible cultivars. Only *H. glycines* and *M. incognita* affected the dry weights of Tracy in all experiments.

EFFECT OF SOIL pH AND NUTRIENTS ON *PRATYLENCHUS PENETRANS* INFECTION ON *PRUNUS AVIUM*. Melakeberhan, H., and G. W. Bird. Department of Entomology, Michigan State University, East Lansing, MI 48824.

Four *Prunus avium* L. rootstocks ('Mazzard', 'Mahaleb', 'GI148-1', and 'GI148-8') were planted into soils with pH of 5.2 or 7.0 and nutrients (commercial fertilizer) applied at planting only (deficient) or twice per week (optimal). *Pratylenchus penetrans* was inoculated at 1,500 nematodes/g fresh root weight and plants were grown for 3 months in greenhouse (25 ± 2 C). More nematodes per g fresh root weight were recovered in GI148-8 than in either GI148-1 or Mahaleb. Fewer ($P \leq 0.05$) nematodes were recovered from treatments at pH 5.2 than at pH 7.0 for all rootstocks and, for most rootstocks, in the deficient compared with optimal fertilization. *Pratylenchus penetrans* did not affect plant growth or macronutrient (N, P, K, Mg, and Ca) concentrations in the leaves. The low soil pH almost invariably affected plant growth and leaf and soil concentrations of macro nutrients, whereas regular nutrient application increased plant growth and leaf and soil nutrient concentrations.

TELONE® SOIL FUMIGANTS AS A METHYL BROMIDE ALTERNATIVE. Melichar, M. W., DowElanco, 9330 Zionsville Road, Indianapolis, IN 46268.

The liquid soil fumigant, 1,3-dichloropropene (1,3-D) is used for preplant soil treatment to control plant-parasitic nematodes and certain other soil pests and plant diseases. DowElanco has three 1,3-D containing products - Telone II, Telone C-17, and Condor® - registered globally. Telone II, Telone C-17, or Telone C-17 alone or formulated with additional chloropicrin (up to 45% chloropicrin) applied singly or in combination with pebulate herbicide have been field tested as methyl bromide alternatives. For nematodes, all Telone soil fumigant treatments provided equal control compared with methyl bromide. For soil-borne diseases, Telone C-17 alone or with

additional chloropicrin provided equal to or slightly less control than methyl bromide. For nutsedge and other weeds (purple cudweed, cutleaf evening primrose, and old field tonelflax), Telone II and Telone C-17 alone or with additional chloropicrin provided less control than methyl bromide; however, Telone C-17 combined with pebulate provided equal control compared with methyl bromide. For the crop species tested (cantaloupe, tomato, pepper, and tobacco), no significant differences in yield and vigor were noted.

EFFECTS OF *VERTICILLIUM LECANII* STRAINS ON *MELOIDOGYNE INCOGNITA* POPULATIONS. Meyer, S. L. F., USDA ARS, Nematology Lab, BARC-West, Beltsville, MD 20705-2350.

A wild type strain of *Verticillium lecanii* (ATCC 58909) and four mutant strains (selected for increased benomyl tolerance) were tested in the greenhouse (space provided by Crop Genetics International, MD) for antagonism to *Meloidogyne incognita* on tomato. Tomato was sown in flats containing sand and transplanted 3 weeks later into pots, each pot containing one seedling in 538 cm³ soil. Before transplanting, nematode eggs were mixed into the soil at rates of 1,000 and 5,000 eggs/pot, and fresh mycelia and conidia of each fungus strain were suspended in water and poured into transplant holes (10 pots/fungus treatment). Water without fungus and water containing autoclaved fungus were used as controls. Plants were then grown 45 days. Only application of mutant strain M1S1 to pots treated with 5,000 eggs caused a high reduction in root-knot nematode egg numbers (41% decrease compared to pots receiving water only). Strain M2S1, which reduced egg numbers in some trials but not others when applied in alginate prills during a previous study, did not cause a large population decrease in this study.

MODELLING ESTABLISHMENT OF *MELOIDOGYNE JAVANICA* ON TOMATO ROOTS (*LYCOPERSICON ESCULENTUM*, CV. TINY TIM). Milstein, T. C. Department of Entomology, University of Queensland, Australia, 4072.

The phase of the *Meloidogyne* life cycle most strongly affected by environmental conditions is the period from egg hatch until the second-stage juvenile has established a feeding site in the root. Therefore, construction of a useful computer assisted model of *Meloidogyne* population increase requires a good understanding of this establishment process. Laboratory and field experiments are being used to assess effects of soil texture, soil moisture, distance of inoculum from the root, soil antagonists, and soil temperature on nematode establishment. The results of these experiments are used as input variables for a model. The model GENSECT, is a generic population dynamics and management model. GENSECT uses modules that describe biological functions such as development, reproduction, and mortality. These modules are linked to defined nematode life stages, i.e., eggs, freshly hatched second-stage juveniles (J2), established J2, sub-adults, and adults. Field data will validate model predictions.

ATTACHMENT SPECIFICITY OF *PASTEURIA PENETRANS* ENDOSPORES TO *MELOIDOGYNE* SPP. Narabu, T. Department of Plant Protection, National Agriculture Research Center, Tsukuba, Ibaraki 305, Japan.

Endospores of three isolates of *Pasteuria penetrans*, PPMI-1, PPMA, and PPMH, collected from females of *Meloidogyne incognita* (Mi), *M. arenaria* (Ma), and *M. hapla* (Mh) respectively, were tested for attachment to 106 populations of Mi, 12 of *M. javanica* (Mj), 43 of Ma (phenotypes of A1 and A2), and 19 of Mh. All populations were collected in Japan, cultured from single egg masses, and identified to species based on esterase phenotypes. Second-stage juveniles were exposed to 10⁴ endospores of PPMI-1, PPMA, or PPMH in 0.35-ml cell culture wells. After incubating 24 hours, PPMI-1 attached only to 83% of the Mi and all the Mj, PPMA attached only to all the Ma, and PPMH attached only to all the Mh populations tested ($P \leq 0.01$). Another isolate, PPMI-2, attached to the Ma, Mh, and Mi populations to which PPMI-1 did not attach, but

not to the Mj and Mi populations to which PPMI-1 attached. PPMI-2 also attached to an Mi population that had over seven generations developed an apparent resistance to attachment with PPMI-1.

SOYBEAN YIELD AND POPULATIONS OF *HETERODERA GLYCINES* AS AFFECTED BY TILLAGE, DATE OF PLANTING, AND CULTIVAR. Niblack, T. L., G. S. Smith, J. A. Wrather, and H. C. Minor. Plant Sciences Unit, University of Missouri, Columbia, MO 65211.

Soybean field plots were established in 1991 at three Missouri locations infested with *Heterodera glycines*. At each location, a split-split plot arrangement consisted of three tillage treatments (conventional-, ridge-, and no-till), three planting dates (May, June, and July), and four maturity-adapted *H. glycines*-resistant or -susceptible cultivars. Cultivars were planted in the same plots in 1991 and 1993 or 1992 and 1994, with corn planted in the intervening years. As a result, isolates of *H. glycines* from one location showed evidence of "race shift," but it was irrespective of cultivar. The overwhelming importance of the location effect in the data demands that caution be used in generalizing for recommendations. However, most of the results were unsurprising: Soybean yields were higher for resistant than susceptible cultivars; and population densities of *H. glycines* were higher on susceptible than resistant cultivars. Relative yields and nematode densities were mostly unaffected by planting date or tillage until the fourth year, when densities were lower in reduced-tillage treatments.

INSECT-PARASITIC NEMATODE FORMULATIONS AND PRODUCTS. Nickle, W. R. USDA ARS, Nematology Laboratory, Bldg. 011A, BARC-W., Beltsville, MD 20705.

There are now at least six companies in the United States selling 15 different nematode products. Steinernematid nematodes can be reared cheaply in liquid fermentation and are exempt from EPA regulations under FIFRA as they are considered to be macro-parasites rather than microbials. Next to *Bacillus thuringiensis*, there are probably more nematode boxes for sale off-the-shelf than any other group of biological control organisms. Formulations include fine clay, clay granules, coarse sand, and vermiculite. Shelf-life at room temperature, UV protection and preservatives to keep down bacterial and fungal contaminants are critical. Expiration dates should appear on all off-the-shelf products as efficacy of current products drops after 3 months.

PHYSICAL METHODS OF NEMATODE CONTROL. Noling, J. W. University of Florida, Citrus Research and Education Center, 700 Experiment Station Road, Lake Alfred, FL 33850.

Because of the relatively low thermal sensitivity of nematodes, physical approaches that employ heat continue to be the most effective and widely used. Steam and hotwater are used for soil treatment in greenhouse or nursery operations or for plant product disinfestation. New technological advances in steam or hotwater generation, delivery, distribution, and soil incorporation must be developed to adapt these methods for field use. Further research is needed to determine field soil treatment regimes and lethal exposure times required to compensate for temperature buffering environmental factors. With additional research, there appears to be expanding potential for use of soil solarization, particularly in combination with other nematode control strategies. With minor exceptions, use of electromagnetic forms of radiation for treatment of soil or plant materials is impractical because of the high dose and lengthy exposures required to induce nematode sterility and/or mortality. In general, commercial development and expanded use of physical methods of nematode control will depend on overcoming many technical, environmental, and economic constraints.

FACTORS AFFECTING GENE STRUCTURE CHANGES IN SMALL POPULATIONS. Novitski, Charles E. Department of Biology, Central Michigan University, Mt. Pleasant, MI 48859.

The accumulation of nucleotide substitutions and larger changes in DNA during evolution, occurring due to selection, random drift, and other known phenomena, is affected by various features of the population. In those nematode populations in which the effective population size is small, the interactions of that size with selection intensity, mode of reproduction of the organism, multiple allelism of loci, organelle location of the genetic material, and of other factors are potentially important. Consideration of these effects on amplification and alteration of gene families is also addressed.

INTERACTIONS BETWEEN *MESOCRICONEMA XENOPLAX* AND *MELOIDOGYNE INCOGNITA* AND THE INCIDENCE OF PEACH TREE SHORT LIFE. Nyczepir, A. P., and B. W. Wood. USDA ARS, Southeastern Fruit and Tree Nut Research Laboratory, Byron, GA 31008.

Mesocriconema xenoplax (Mx) and *Meloidogyne incognita* (Mi), singly and together, were studied for their effect on peach trees. 'Lovell' peach seedlings were established in 24 field microplots in August 1989 in soil that had been preplant fumigated with methyl bromide. In November 1989, the nematode species alone (10,000 Mx or Mi/microplot) and together (10,000 Mx + 10,000 Mi) were added. Net photosynthesis levels increased in leaves of trees growing in the presence of Mi in September 1993 but were suppressed ($P \leq 0.05$) in Mi+Mx treatment trees relative to the control in September 1994. The population density of Mi J2 was not affected by the presence of Mx; however, Mx was suppressed ($P \leq 0.01$) in the Mx + Mi treatment relative to Mx-alone 16, 37, 40, and 52 months after inoculation. In April 1994, 80% of the trees in Mx-alone infested soil developed short life symptoms and died, and the remaining tree succumbed to short life in March 1995. No other trees, including those with Mi+Mx, died.

SYSTEMIC INDUCTION OF RESISTANCE OR SUSCEPTIBILITY FOLLOWING INCOMPATIBLE OR COMPATIBLE PLANT-NEMATODE INTERACTIONS. Ogallo, J. L., and M. A. McClure. Department of Plant Pathology, University of Arizona, Tucson, AZ 85721.

Advance inoculations of tomato cv. Celebrity or pyrethrum clone '223' with incompatible *Meloidogyne incognita* or *M. javanica* suppressed reproduction ratio (Pf/Pi) of compatible *M. hapla* in pot and field experiments. Advance inoculations with *M. hapla* enhanced the Pf/Pi of *M. incognita* and *M. javanica*. A split-root assay was used to demonstrate the systemic nature of induction factors in root systems. Induction intensity increased with longer intervals between induction and challenge inoculations and with greater densities of the induction inoculum, to a saturation point. Optimal induction of resistance occurred within 10 days with an inoculum density of 5,000 infective juveniles per plant in 500 cm³ potted soil. Reproduction ratio of *M. hapla* in plants was suppressed 84% on tomato and 72% on pyrethrum in pot tests, and 55% on pyrethrum seedlings in field plots, within 60 days of infection. Pyrethrum seedlings with induced resistance were not stunted by *M. hapla* within 90 days of infection, whereas the number of leaves produced by unprotected seedlings were fewer by 33% in pots and 36% in field plots compared to nematode-free controls. These results show that initial incompatible or compatible plant-nematode interactions conditioned plants to increased resistance or susceptibility, respectively, to subsequent infection by either nematode genotype.

FIELD EVALUATIONS OF TRANSGENIC ROOT-KNOT NEMATODE RESISTANT TOBACCO. Opperman, C. H., and M. A. Conkling. Departments of Plant Pathology and Genetics, North Carolina State University, Raleigh, NC 27695.

We have identified the tobacco root-specific gene, TobRB7, as being up-regulated during root-knot nematode infection. We have further defined the Nematode Responsive Element (NRE) of the TobRB7 promoter. Full length cDNA antisense constructs of TobRB7 driven by either the NRE or the 35S CaMV promoter were transformed into tobacco. When these plants were infected

with root-knot nematode a substantial reduction in galling and egg production was observed. Greenhouse and field trials with the NRE-TobRB7 antisense plants provided strong evidence that nematode infection was substantially reduced. In all trials, root galling was reduced by approximately 70% compared to control treatments. The field trials indicated that protection lasted for the entire growing season (approximately 4 months). Galls that were observed on the roots of antisense plants were small and tended to appear as discrete entities compared to the large and clustered galls on the controls.

VARIETAL REACTION OF COTTON TO *ROTYLENCHULUS RENIFORMIS* IN A MULTI-SITE STUDY. Overstreet, C.,¹ and E. C. McGawley.² ¹Louisiana Cooperative Extension Service, and ²Department of Plant Pathology and Crop Physiology, Louisiana State University, Baton Rouge, LA 70803.

Six cultivars of cotton (*Gossypium hirsutum* cvs. Deltapine [DPL] SI, DPL 5690, Hyperformer HS 46, Stoneville [STN] 132, STN 311, and STN 887) were planted at five locations in 1993 and seven locations in 1994 within the major cotton-producing parishes of Louisiana. The cultivars were arranged as strip plots 4-8 rows wide (1-m centers) × ca. 460 m in length and were treated at planting with aldicarb (0.58 kg a.i./ha). Soil at all locations was a silt loam infested with *Rotylenchulus reniformis* and trace levels of other plant-parasitic nematodes. Soil samples were collected before planting and at harvest and yield of seed cotton was determined for each plot. Differences ($P \leq 0.05$) in cultivar yields were observed within locations at four sites in 1993 and at three sites in 1994. When yield and nematode data were analyzed across both sites and years, however, differences in cultivar performance and nematode reproduction were not significant.

COMPARISON OF TWO TECHNIQUES FOR EXTRACTING NEMATODES FROM SOIL. Perez, E. E.,¹ R. McSorley,¹ and D. P. Weingartner.² ¹Department of Entomology and Nematology, University of Florida, Gainesville, FL 32611, and ²Agricultural Research and Education Center, University of Florida, Hastings, FL 32145.

Nematode recovery with centrifugal flotation depends on adequate suspension of the subsample in water. The purpose of this experiment was to compare two techniques for suspending the soil subsample (by hand or mechanically with a high-pressure water nozzle) when using the centrifugal flotation technique. Sets of 16 soil samples each were collected from potato and cabbage fields. Two subsamples of 100 cm³ soil were taken from each sample, and each subsample was processed by suspending the soil by mixing manually or by water pressure. Numbers of nematodes recovered by the two techniques were counted and analyzed using a paired *t*-test. Recovery numbers of *Tylenchorhynchus* sp., *Belonolaimus* sp., and *Criconemella* sp. were higher ($P = 0.05$) when the soil was suspended by hand. Recovery of *Criconemella* sp. was 75-100% greater when the subsample was suspended by hand. The two techniques did not show any difference when nematodes were recovered in low (<5 nematodes/100 cm³ soil) numbers.

SEPARATION OF NICHEs PRECLUDES COMPETITION BETWEEN TRICHODORIDS AND *BELONOLAIMUS LONGICAUDATUS*. Perez, E. E.,¹ D. P. Weingartner,² and R. McSorley.¹ ¹Department of Entomology and Nematology, University of Florida, Gainesville, FL 32611, and ²IFAS Agricultural Research and Education Center, Hastings, FL 32145.

Plots of either potato or cabbage, fumigated with 1,3-dichloropropene or nonfumigated, were used to determine if resurgence of trichodorids (*Paratrichodorus* spp. and *Trichodorus* spp.) is enhanced by the lack of competition with *Belonolaimus longicaudatus* (BL). Soil samples were taken 0-20 cm and 20-40 cm deep from each of 32 plots before fumigation, at planting, and at monthly intervals after planting. In the 1994-95 season, trichodorids (T) were found at higher numbers at 20-40 cm deep (30 T vs. 1 BL/100 cm³ soil), whereas higher numbers of *B. longicaudatus* were found at 0-20 cm (34 BL vs. 2 T/100 cm³ soil). Trichodorid numbers were

reduced after fumigation in potato, whereas numbers increased after fumigation in cabbage. Results from 1993-94 were similar to 1994-95 in that the two nematodes were also separated by depth. Competition between these two species is limited in this system because their niches are separated vertically.

METHODS TO QUANTIFY NEMATOPHAGOUS FUNGI IN SOIL: MICROSCOPY OR GUS GENE ACTIVITY. Persmark, L., Y. Persson, and H.-B. Jansson. Department of Microbial Ecology, Lund University, Ecology Building, S-223 62 Lund, Sweden.

A soil dilution method in combination with a most probable number (MPN) estimation has been used to quantify nematophagous fungi in soil. In field soils mostly between 10-100 propagules/g soil was obtained using this method. *Arthrobotrys oligospora* was transformed with the β -glucuronidase (GUS) gene. The transformant was stable through several one-spore isolations, and also after re-isolation from nonsterile soil, where it had been grown for 1 month. The transformant did not differ from the wild type in growth rate, sporulation or production of traps. The GUS activity was 15 nmol/MU (minute-mg protein)⁻¹ in pure culture. In a comparison between the methods we added alginate-pelletized *A. oligospora* transformants to soil. The growth of the fungus was detected using the MPN method, which gave ca. 1,000 propagules/g soil, whereas the GUS-method failed to show any activity above the background level. Further development of the GUS method is in progress.

DISTRIBUTION OF PLANT-PARASITIC NEMATODES IN OREGON VINEYARDS. Pinkerton, J. N.,¹ T. A. Forge,² and R. E. Ingham.² ¹Horticultural Crops Research Laboratory, USDA ARS, Corvallis, OR 97330, and ²Department of Botany and Plant Pathology, Oregon State University, Corvallis, OR 97330-2902.

The number of small vineyards in western Oregon has grown rapidly. Currently, winegrapes are planted on over 6,600 acres and generate annual revenues of \$10 million. In 1994 a survey was conducted to determine the association of nematodes with vine health in Oregon vineyards. Thirty-four vineyards were surveyed in five regions of western Oregon, with 4-9 vineyards per region. Vineyards were selected and partitioned into blocks by cultivar, age of the planting, crop history, and soil characteristics. *Criconebella xenoplax*, *Xiphinema americanum*, *Pratylenchus* spp., and *Paratylenchus* spp. were recovered from over 85% of the vineyards; only 15% of vineyards had detectable populations of *Meloidogyne* spp. Only population densities of *C. xenoplax* exceeded levels that have been found damaging in California vineyards. *Criconebella xenoplax* was found at the greatest population densities in vineyards greater than 10-year-old and on old *Prunus* orchard sites in the Northern Willamette Valley. Populations of *C. xenoplax* were associated with stunted plants in only two vineyards. The long-term impact of *C. xenoplax* and other nematodes in vineyards has not yet been determined in Oregon.

NEMATODE COMMUNITY STRUCTURE IN THE CITRUS SOIL ECOSYSTEM. Porazinska, D. L.,¹ R. McSorley,¹ and L. W. Duncan.² ¹Entomology and Nematology Department, University of Florida, Gainesville FL 32611-0620, and ²Citrus Research and Education Center, Lake Alfred, FL 33850-2299.

Composition of the nematode fauna was determined in a 7-year-old citrus grove (a perennial model system). Thirty-two trees (Valencia sweet orange on Carrizo citrange rootstock), maintained under standard practices, were sampled in a 0.2-ha area on 2 February 1995. Nematode community structure, organic matter, microbial respiration, *Phytophthora* levels, root biomass, and soil moisture were examined. Several ecological measures (community structure indices, diversity indices, and modified maturity index) were assessed, correlated with the above listed soil characteristics, and compared with values obtained in other studies with annuals and perennials. Bacterivores were the most abundant trophic group in the nematode community (mean=74% of

total nematodes). Total nematode number was strongly correlated with moisture ($r = 0.497$, $P = 0.01$) and *Phytophthora* ($r = 0.504$, $P = 0.01$). Predators, omnivores, and plant parasites showed greater correlations with soil moisture than did other trophic groups.

ITS1 SIZE VARIATION AND NEMATODE IDENTIFICATION. Powers, T. O. Department of Plant Pathology, University of Nebraska, Lincoln, NE 68583-0722.

The ITS1 region of the nuclear ribosomal DNA repeating unit was PCR-amplified from over 20 genera of nematodes, including tylenchid and adenophorean plant parasites. The size of the amplified region ranged from 350 bp to 1.1 kb. Congeneric species had similarly or identically sized amplification products. Digestion of amplified product with restriction enzymes has produced a number of "species-specific" RFLP patterns. Attempts are underway to link specific RFLP patterns with described species in *Helicotylenchus*, *Tylenchorhynchus*, *Belonolaimus*, and *Filenchus*, by measuring nematodes on temporary slide mounts before PCR amplification. Most species examined to date exhibit discrete RFLP patterns that appear to be conserved among geographically distant populations.

HATCHING BEHAVIOR OF *HETERODERA GLYCINES* EGGS EXPOSED TO LOW TEMPERATURE. Qiu, J., and A. E. MacGuidwin. Department of Plant Pathology, University of Wisconsin, Madison, WI 53706.

Sucrose-gradient centrifugation was used to separate eggs of *Heterodera glycines* into two bands. Band 2 was composed of more than 95% mature (vermiform juvenile) eggs. Band 1 was composed of 56% immature eggs and 44% mature eggs. Cumulative percentage of hatch over 42 days for the band 1 and band 2 eggs, respectively, was 52 and 61% in 3 mM ZnCl₂ (pH=5.3) and 22 and 1% in deionized water. In a second experiment, eggs were treated in 5% polyethylene glycol at 4 or 24 C for 10 days or at 4 or 24 C for 5 days and then frozen at -10 C for 5 days. After the temperature treatment, eggs were incubated in ZnCl₂ for 42 days at room temperature. Cumulative hatch of band two eggs ranged from 55-60% and did not differ among the temperature treatments. Cumulative hatch of band one eggs was 52% for eggs held at 24 C for 10 days, 20% for eggs held at 4 C for 10 days, and 7 and 3%, respectively, for eggs held at 4 and 24 C for 5 days and then frozen.

IDENTIFICATION OF A YELLOWING DISEASE IN BARLEY IN SWITZERLAND CAUSED BY ARABIS MOSAIC NEPOVIRUS TRANSMITTED BY *XIPHINEMA DIVERSICAUDATUM*. Ramel, M.-E.,¹ P. Gugerlei,¹ R. Vallotton,¹ and D. J. F. Brown.² ¹Federal Agricultural Research Station of Changins, CH-1260 Nyon, Switzerland, and ²Scottish Crop Research Institute, Invergowrie, Dundee, DD2 5DA, Scotland, U.K.

Nepoviruses transmitted by longidorid nematodes have not been associated with a disease in graminaceous plants. However, a yellowing disease in field crops of barley cv. Express growing in western Switzerland has recently been shown to be associated with a serologically distinct strain of arabis mosaic nepovirus (ArMV-B), naturally transmitted by *Xiphinema diversicaudatum* present in moderate numbers at these field sites. Attempts to mechanically infect barley plants with the virus were unsuccessful; however, *X. diversicaudatum* from Scotland transmitted ArMV-B but not ArMV-type to the individual barley plants, which subsequently developed yellow foliage, and to cv. Robur and Athena, from which Express had been bred.

EVIDENCE FOR PROTEOLYTIC PROCESSING OF A CUTICLE COLLAGEN PROTEIN IN A PLANT-PARASITIC NEMATODE. Ray, C., and R. S. Hussey. Department of Plant Pathology, University of Georgia, Athens, GA 30602-7274.

Cuticles of females of *Meloidogyne incognita* contain a 76-kDA collagen protein that comprises over 50% of the β -mercaptoethanol-soluble cuticle proteins. The N-terminal amino acid sequence

of this collagen protein was found to be identical to the predicted amino acid sequence, starting at amino acid number 66, of the *M. incognita* collagen Lemmi 5 cDNA clone (Gene 151:237-242). A putative subtilisin-like protease recognition site occurred immediately upstream of the region of amino acid homology between LEMMI 5 and the N-terminal sequence of the 76 kDa collagen protein. These data support previous speculation about the existence of this novel method of collagen maturation and provide further evidence that this mechanism has been conserved during nematode evolution. Expression of the *Lemmi 5* gene was transcriptionally regulated. *Lemmi 5*-specific transcripts were present in females but not in eggs or second-stage juveniles. *Lemmi 5* analogs were present in four *Meloidogyne* species, but not in *Caenorhabditis elegans* or *Heterodera glycines*.

ISOLATION OF A GENE ENCODING A SECRETED *MELOIDOLOGYNE* ESOPHAGEAL GLAND PROTEIN. Ray, C., and R. S. Hussey. Department of Plant Pathology, University of Georgia, Athens, GA 30602-7274.

A monoclonal antibody specific for an antigen in the subventral esophageal glands and stylet secretions of both second-stage juveniles and females of *Meloidogyne incognita* was used to immunoscreen a random-primed cDNA expression library. The cDNA library was constructed from mRNA isolated from preparasitic second-stage juveniles. Two positive clones were identified having cDNA inserts of approximately 2.3 kb and 2.2 kb long. Initial DNA sequence analysis indicated that the two inserts were derived from the same gene, which was named *sec-2*. In low-stringency genomic Southern blot analysis, the 2.3 kb *sec-2* cDNA insert bound to analogous sequences in *M. incognita*, *M. arenaria*, *M. javanica*, and *M. hapla*. No binding was observed to DNA from *Heterodera glycines*, *Caenorhabditis elegans*, or tomato. Preliminary analysis of the predicted amino acid sequence of 144 residues of SEC-2 protein showed that it was rich in leucine, phenylalanine, and histidine.

ENZYME-LINKED IMMUNOSORBENT ASSAY (ELISA) FOR *PASTEURIA NISHIZAWAE*. Reise, R. W.,¹ T. A. Chen,² D. J. Chitwood,¹ K. J. Hackett,³ R. M. Sayre,¹ and R. N. Huettel.¹
¹USDA ARS, Nematology Laboratory, Bldg. 011A, Room 153, BARC-West, Beltsville, MD 20705, ²Department of Plant Pathology, Rutgers University, New Brunswick, NJ 08903, and ³USDA ARS, Insect Biocontrol Laboratory, Bldg. 011A, Room 214, BARC-West, Beltsville, MD 20705.

An ELISA-based procedure for quantifying the number of *Pasteuria nishizawae* endospores was developed with a monoclonal antibody generated in mice against a homogenate of *P. nishizawae*. The method could detect *P. nishizawae* obtained from *Heterodera glycines* cysts in concentrations as low as 1,600 endospores/ml. *Pasteuria penetrans* obtained from *Meloidogyne arenaria* also produced a detectable but somewhat weaker response in the ELISA. Absorbance values obtained during the procedure were near zero for samples of homogenates of uninfected *H. glycines* or *M. arenaria*. Three other bacterial species examined (*Bacillus thuringiensis*, *Streptomyces* spp., and *Escherichia coli*) failed to react with the antibody. A few problems with nonspecific activity associated with endogenous peroxidases in plant root samples were observed but were overcome by procedural modifications.

EFFECT OF *MELOIDOLOGYNE ARENARIA* POPULATION LEVEL AND MULCH TYPE ON OKRA. Ritzinger, C. H. S. P., and R. McSorley. Department of Entomology and Nematology. University of Florida, Gainesville, FL 32611-0620.

Organic amendments have often been used to manage plant-parasitic nematodes, but it is not fully understood whether the mode of action is suppression of nematodes by nematicidal effects or promotion of plant health by beneficial effects on soil, or both. Naturally infested microplots on sandy soil in Florida were blocked into low and high population levels of *M. arenaria* race 1

and planted with seedlings of 'Clemson Spineless' okra (*Hibiscus esculentus*). Mulch treatments consisting of perennial peanut (*Arachis glabrata*) hay, composted yard waste (mainly woodchips), or an unmulched control were applied to four replications at each population level. Nematode level and mulch type each affected yield ($P \leq 0.05$), which was highest in plots with perennial peanut mulch. Plant stands, gall ratings, and final nematode population densities were affected ($P \leq 0.05$) only by initial nematode level. Mulch treatments had no effect on final nematode population densities or gall ratings.

EFFECT OF TEMPERATURE ON EXPRESSION OF RESISTANCE TO *MELOIDOGYNE* SPP. IN CARROT. Roberts, P. A., and W. C. Matthews. Department of Nematology, University of California, Riverside, CA 92521.

Selected carrot genotypes were inoculated with eggs of *M. hapla*, *M. incognita*, and *M. javanica* and were maintained at constant soil temperatures of 22 C, 25 C, 28 C, 31 C, or 34 C. Root galling and egg production assays were used to determine resistance expression. Differential resistance expression was found among nematode species, for both level of resistance and resistance response to high temperature. Brazilia-derived genotypes were most resistant to *M. javanica*, but also were resistant to *M. incognita* and *M. hapla*. Reproduction on resistant genotypes increased at 28 C or at 31 C compared to lower temperatures, depending on genotype-nematode species combination. These results support our preliminary findings that carrot has several root-knot nematode resistance genes.

TOLERANCE TO *ROTYLENCHULUS RENIFORMIS* AND RESISTANCE TO *MELOIDOGYNE INCOGNITA* RACE 3 IN HIGH-YIELDING BREEDING LINES OF UPLAND COTTON. Robinson, A. F.,¹ C. G. Cook,² and N. Namken.³ ¹USDA ARS, College Station, TX 77845, ²USDA ARS, Weslaco, TX 78596, and ³Texas Agricultural Experiment Station, Weslaco, TX 78596.

Ten advanced breeding lines of Upland cotton (*Gossypium hirsutum*) were evaluated for lint yield production with and without 1,3-dichloropropene fumigation in a field infested with *Rotylenchulus reniformis* in the Lower Rio Grande Valley of Texas in 1992, 1993, and 1994. The susceptible control, Stoneville 453 (STV 453), yielded 739 and 400 kg/ha, respectively, with and without fumigation. Nine lines had higher yields ($P \leq 0.05$) than STV 453 with or without fumigation and lower estimated losses to *R. reniformis*. Four lines yielded 57-86% more lint than the partially resistant cv. LA RN1032. In growth chamber experiments examining LA RN1032 and six of the lines, all had *R. reniformis* population multiplication factors of >100 , 10 weeks after inoculation; however, population densities increased more slowly on three of the lines than on STV 453. Three lines also were resistant to *Meloidogyne incognita* race 3, with a multiplication factor of <1.0 , compared with 9.1 for STV 453 and 25.8 for root-knot nematode susceptible Deltapine 16.

REPULSION OF *MELOIDOGYNE INCOGNITA* BY ALGINATE PELLETS OF THREE NEMATOPHAGOUS FUNGI. Robinson, A. F.,¹ B. A. Jaffee,² A. C. Bridges,¹ and A. E. Muldoon.² ¹USDA ARS, College Station, Texas 77845, and ²Department of Nematology, University of California, Davis, CA 95616.

Columns (38-mm-d, 40 or 72 mm long) of sand (94% $<250\text{-}\mu\text{m}$ particle size) wetted with a synthetic soil solution (10-12% moisture) were used to examine responses of second-stage juveniles (J2) of *Meloidogyne incognita* race 3 to calcium alginate pellets of *Monacrosporium cionopagum*, *M. ellipsosporum*, and *Hirsutella rhossiliensis*. A layer of 10 or 20 pellets was placed 4 or 20 mm from one end of the column. After 0, 3, or 14 days, J2 were put on both ends, on one end, or in the center. Second-stage juveniles were extracted from 8-mm sections 1 or 2 days later. All three fungal pellets were repellent; pellets without fungi were not. Aqueous extracts of pellets and of sand in which pellets had been incubated were repellent, but acetone extracts redissolved in water

were not. Injection of CO₂ (20 µl/minute) into the pellet layer attracted J2 and increased mortality. However, parasitism of *M. javanica* J2 by all three pelletized fungi, and invasion of cabbage seedling roots in vials containing four pellets and 17-cm³ loamy sand indicated repellency had little effect on efficacy.

LIGHT AND CO₂ RESPONSE OF POTATO AS INFLUENCED BY *PRATYLENCHUS PENETRANS* AND *VERTICILLIUM DAHLIAE*. Saeed, I., A. E. MacGuidwin, and D. I. Rouse. Department of Plant Pathology, University of Wisconsin, Madison, WI 53706.

Gas exchange studies were conducted on potato plants cv. Russet Burbank infected with *Pratylenchus penetrans* (Pp) or *Verticillium dahliae* (Vd) or both pathogens. Treatments were three levels of Pp, one level of Vd, the combinations of Vd and Pp, and a non-treated control. Measurements were made at 90-94 days after planting on young, fully-expanded leaves. Relationships between photosynthesis and intercellular CO₂ (C_i) at three light levels (200, 400, and 1,000 micromol m⁻² s⁻¹ of photosynthetically active radiation [PAR]) were determined. Nematode infection reduced carbon assimilation rates at medium or high population densities but not at the low level. Leaves from *Verticillium*-infected plants exhibited a slight reduction in assimilation rates. Effects of either pathogen alone on assimilation rates were more pronounced at high light intensities. A marked reduction in assimilation rates over virtually the entire photosynthetic light curve was caused by joint infection specifically at the high level of the combination treatment. This was manifested in reduced light-saturation levels in leaves from diseased plants.

EFFECTS OF SELECTED GREENHOUSE INSECTICIDES ON *HETERODERA GLYCINES* REPRODUCTION. Souhrada, S. K., D. M. Volkers, and G. L. Tylka. Department of Plant Pathology, Iowa State University, Ames, IA 50011.

Three experiments were conducted to determine the effects of avermectin, dienochlor, endosulfan, fluvalinate, kinoprene, fatty acid potassium salts, and resmethrin insecticides, alone and in some dual combinations, on reproduction of *Heterodera glycines*. Distilled and tap water were control treatments. Susceptible soybean seedlings were transplanted into sand:soil mix, inoculated with *H. glycines* eggs, and incubated at 27 C. Treatments were sprayed on foliage and soil surfaces 4 to 8 days and 13 to 18 days after transplanting; a third application was made 22 days after transplanting in one experiment. Females were dislodged from soybean roots 28 days after transplanting and counted. Mean numbers of females ranged from 57 to 1,158/root. Large differences in numbers of females were observed among experiments, but not among treatments within experiments. However, mean numbers of females on roots of plants treated with endosulfan, kinoprene, fatty acid potassium salts, and dual combinations of these three insecticides were greater ($P \leq 0.05$) than those on roots of the control treatments in one experiment. None of these greenhouse insecticides adversely affected *H. glycines* reproduction.

MORPHOMETRICS OF *HETERORHABDITIS* POPULATIONS (NEMATA: HETERORHABDITIDAE) FROM CALIFORNIA: A MULTIVARIATE ANALYSIS. Stock, P.,¹ H. K. Kaya,¹ and S. L. Gardner.² ¹Department of Nematology, University of California, Davis, CA 95616, and ²University of Nebraska, Lincoln, NE 68588.

Nine populations of the genus *Heterorhabditis* (Nemata:Heterorhabditidae) from northern and southern California were studied. Using multivariate morphometrics, 8 of 9 populations were identified as *H. bacteriophora*, with the population from Bodega Bay identified as distinct. A total of 270 individuals representing 9 populations and 29 morphometric variables were subjected to analysis. Males and infective juveniles (IJ) were measured and the data summarized using measures of central tendency, principal component analyses, and canonical and stepwise discriminant analyses. Based on this analysis, the Bodega Bay population stands apart and is distinct from all other populations of Californian *Heterorhabditis*. It is evident from this and

additional studies that the Bodega Bay population represents a new species (at present being described).

CYST NEMATODE RELATIONSHIPS INFERRED FROM NUCLEAR AND MITOCHONDRIAL NUCLEOTIDE SEQUENCES. Sui, D., and T. O. Powers. Department of Plant Pathology, University of Nebraska, Lincoln, NE 68583-0722.

PCR amplified DNA from the nuclear encoded ITS region and the mitochondrially encoded COII gene have been used to evaluate phylogenetic relationships among 19 cyst nematode species and subspecies. Approximately 700 bp of the ITS region and 220 bp of the COII gene have been sequenced from each species. Preliminary analyses of the ITS region alone show little support for conventional species groupings; however, these results may change as additional weighing schemes and different alignments are attempted. Strong support exists for the monophyly of *Globodera*, and *G. tabacum* was found to be distinct from *G. pallida* and *G. rostochiensis*.

EFFECTS OF TEMPERATURE AND RESISTANCE IN *PHASEOLUS VULGARIS* ON DEVELOPMENT OF *MELOIDOGYNE ARENARIA* RACE 1. Sydenham, G. M., R. McSorley, and R. A. Dunn. Entomology and Nematology Department, University of Florida, Gainesville, FL 32611-0620.

Penetration and development of *M. arenaria* race 1 in *P. vulgaris* germplasm G1805 were studied in a growth room (22-25 C). Numbers of juveniles penetrating roots of susceptible Black Valentine and resistant G1805 were similar up to 7 days after inoculation. More ($P \leq 0.05$) nematodes were present in susceptible roots 14-49 days after inoculation. Nematodes developed more rapidly and in greater numbers in susceptible than in resistant plants. Resistance in G1805 was expressed by delayed nematode development rather than by differential penetration of second-stage juveniles. In a second experiment, plants were grown in growth chambers for a total of 99 accumulated heat units at 24 or 28 C before inoculation, at which time they were moved to the post-inoculation temperatures of 24 or 28 C. The temperature regimes were set at pre/post inoculation temperatures of 24/24, 24/28, 28/24, and 28/28 C. Fewer ($P \leq 0.05$) nematodes were present in roots of G1805 than in Black Valentine at all temperature regimes. Pre-inoculation temperature did not affect numbers of nematodes in Black Valentine roots. However, at 1 and 2 days after inoculation, more ($P \leq 0.05$) nematodes had entered roots of G1805 plants grown at the pre-inoculation temperature of 28 C than of 24 C. Post-inoculation temperatures did not affect numbers of nematodes in roots of either germplasm, but advanced developmental stages were reached most rapidly in both at 28 C. More rapid nematode development at 28 C was due to temperature effect on nematode development rather than temperature effect on resistance expression.

THE GENETIC POPULATION STRUCTURE OF POTATO CYST NEMATODES. Szalanski, A. L.,¹ C. C. Fleming,² D. Sui,¹ and T. O. Powers.¹ ¹Department of Plant Pathology, University of Nebraska, Lincoln, NE 68583-0722, and ²Department of Agriculture for Northern Ireland, Falden, Mill Road, Newtownabbey, Co. Antrim, BT36 7ED, Northern Ireland, UK.

The ITS region of the nuclear ribosomal DNA repeating unit is being sequenced to determine the genetic population structure of *Globodera pallida* and *G. rostochiensis*. Preliminary analyses of eight PCN populations indicated that greater genetic variation existed in South American populations compared to European populations, and the majority of nucleotide variation was found in ITS1. Examination of 13 additional South and North American isolates continues to support these observations and suggests that some Mexican cyst populations share a genetic affinity with *G. tabacum* than with PCN. To date, the two PCN species have the highest observed level of intraspecific sequence variation among nematodes.

CHARACTERIZATION OF RESISTANCE OF CAROLINA CAYENNE PEPPER TO *MELOIDOGYNE INCOGNITA*. Thies,¹ J. A., J. D. Mueller,² and R. L. Fery.¹ ¹USDA ARS, U.S. Vegetable Laboratory, Charleston, SC 29414, and ²Department of Plant Pathology, Clemson University, Blackville, SC 29817.

Carolina Cayenne pepper (*Capsicum annuum*) was released by the USDA and Clemson University in 1985. The major attribute of this cultivar is its exceptionally high resistance to the southern root-knot nematode. Methyl bromide is currently used to control this pest in pepper, but the pending withdrawal of this fumigant from the United States market has resulted in a need for an alternative control measure. We evaluated the efficacy of Carolina Cayenne's resistance in greenhouse, microplot, and field studies. In all tests, Carolina Cayenne plants exhibited exceptional resistance, with minimal nematode reproduction, minimal galling, and no yield reduction. In a test conducted in an infested field, for example, Carolina Cayenne outyielded a root-knot nematode susceptible isolate by 339%. We conclude that the resistance exhibited by Carolina Cayenne is a suitable alternative to methyl bromide for controlling root-knot nematodes in pepper.

INFLUENCE OF WEED-CHILE PEPPER ASSOCIATIONS ON THE POPULATION DYNAMICS OF *MELOIDOGYNE INCOGNITA*. Thomas, S. H.,¹ J. Schroeder,¹ and L. W. Murray.² ¹Department of Entomology, Plant Pathology and Weed Science, and ²Department of Experimental Statistics, New Mexico State University, Las Cruces, NM 88003-0003.

Field experiments were conducted during 1993 and 1994 to compare *M. incognita* (RKN) population development on chile peppers (*Capsicum annuum* cv. NM 6-4) growing in the presence or absence of the annual weeds spurred anoda (*Anoda cristata*), Palmer amaranth (*Amaranthus palmeri*), or Wright groundcherry (*Physalis wrightii*), or the perennial weeds yellow nutsedge (*Cyperus esculentus*) or purple nutsedge (*C. rotundus*). Root-knot nematode eggs were extracted from chile roots and associated weeds at 2-week intervals after chile emergence and populations compared using analysis of variance. Root-knot nematode reproduction increased and chile root weights decreased in association with either perennial weed during both seasons and with *A. palmeri* in 1994. Neither *A. cristata* nor *P. wrightii* influenced RKN reproduction on chile. All annual weeds supported greater RKN reproduction than perennials, with *A. palmeri* being the best overall host.

THE LEGISLATIVE STATUS OF METHYL BROMIDE. Thomas, W. B. EPA -6205J, 401 M St. S.W., Washington, DC 20460.

Methyl bromide is used extensively on a global basis as a pesticide against nematodes, weeds, insects, fungi, bacteria, and rodents. As a soil fumigant, it is used in significant quantities in the production of strawberry and tomato, as well as other agriculture commodities. Grain, fresh fruit, forestry products, and other materials are fumigated with methyl bromide to control pest infestations during transport and storage. Structures are also treated with this chemical to control wood destroying insects and rodents. However, methyl bromide has been identified as a significant ozone depleting substance, resulting in regulatory actions being taken by the U.S. Environmental Protection Agency and by the United Nations Environment Program (Montreal Protocol). The science linking methyl bromide to ozone depletion is strong, and was reinforced by the 1994 UNEP Montreal Protocol Science Assessment on Ozone Depletion, which states: "Methyl bromide continues to be viewed as a significant ozone depleting compound."

REDUCTION IN THE ABUNDANCE OF *HIRSUTELLA RHOSILIENSIS* CONIDIA BY RESISTANT DAUERLARVA. Timper, P., and B. B. Brodie. U.S. Plant, Soil and Nutrition Laboratory, Tower Road, Cornell University, Ithaca, NY 14853.

The retained cuticle (sheath) of the dauerlarva can protect rhabditid nematodes from infection by nematode-pathogenic fungi. Conidia of the fungus *Hirsutella rhossiliensis* adhere to the sheath

but do not infect the nematode. To test the hypothesis that dauerlarva could deplete the supply of *H. rhossiliensis* conidia in soil, we added different proportions of resistant (with a sheath) and susceptible (sheath removed with 0.05% sodium hypochlorite) *Heterorhabditis bacteriophora* to soil infested with the fungus. We found that with an increasing proportion of resistant nematodes added to soil, there was a decrease in abundance of conidia. When more than 60% of the nematodes were resistant, there was a net decrease in conidia compared with soil in which no *H. bacteriophora* were added.

THERMAL TIME AND NEMATODE ECOLOGY. Trudgill, D. L. Scottish Crop Research Institute, Invergowrie, Dundee, DD2 5DA UK.

Where there is a linear relationship between temperature and rate of nematode development it is possible to estimate the base temperature and degree days (thermal constant) requirement. Studies with *Meloidogyne* spp. show that these values are of considerable ecological significance, as the base temperature shows the thermal environment to which nematodes are adapted (temperate species have lower values than tropical) and the thermal constant shows their relative rates of development (greater for tropical species). Embryogenesis takes ca. 40% of the degree days requirement for the total life cycle, which suggests that a substantial amount of the total development requirement occurs in the egg and *Meloidogyne* spp. have a much greater requirement than *Caenorhabditis elegans*. The use of such data in pest risk assessments and its possible physiological basis will be briefly considered.

SYSTEMIC ACQUIRED RESISTANCE SIGNAL TRANSDUCTION. Uknes, S., E. Ward, M. Hunt, K. Weymann, D. Chandler, S. Potter, L. Friedrich, and J. Ryals. Agricultural Biotechnology Research Unit, Ciba Geigy Corporation, Research Triangle Park, NC 27709.

Systemic acquired resistance (SAR) has been well characterized in tobacco and cucumber, however the signal transduction pathway leading to SAR is not well understood. Recently, *Arabidopsis* was shown to display SAR following infection by pathogens or by treatment with immunization compounds. We have taken two approaches to dissect the signal transduction pathway leading to SAR. First, we isolated mutants of *Arabidopsis* with constitutive expression of the SAR genes. Plants with constitutive SAR gene expression are resistant to pathogens. Second, because salicylic acid (SA) increased following pathogen infection and can induce the same spectrum of resistance as pathogen treatment, we engineered transgenic *Arabidopsis* to produce salicylate hydroxylase. Salicylate hydroxylase converts SA to catechol, a compound that does not induce SAR. Pathogens caused more severe disease symptoms on salicylate hydroxylase plants, implicating a general role for SA in the restriction of disease symptoms.

RELATIONSHIPS BETWEEN *MELOIDOGYNE INCOGNITA* RESISTANCE GENES IN *LYCOPERSICON PERUVIANUM* DIFFERENTIATED BY TEMPERATURE AND NEMATODE VIRULENCE. Veremis, J. C., and P. A. Roberts. Department of Nematology, University of California, Riverside, CA 92521.

Inheritance of heat-stable and heat-unstable resistance to isolates of *Meloidogyne incognita* that are (a)virulent to resistance gene *Mi* was investigated on clones, F₁, F₂ and test-cross (TC₁) generations of *Lycopersicon peruvianum* PI 270435-2R2, 270435-3MH, 126443-1MH, and 126440-9MH. Segregation for resistance was determined in experiments carried out at 25 C (*Mi* expressed) and 32 C (*Mi* not expressed). Segregation in TC₁ and F₂ progenies indicated that heat-stable resistance to a *Mi*-avirulent isolate and resistance to a *Mi*-virulent isolate are conferred by different single dominant genes in each of the clones 270435-2R2, 270435-3MH, and 126443-1MH. Additional screening of clones of the same individual TC₁ and F₂ plants suggested that genetic control of resistance to *M. incognita* appears as separate series of loci in *L. peruvianum*.

CYSTATIN-SENSITIVE PROTEINASES IN *MELOIDOGYNE HAPLA*. Vrain, T.,¹ D. Michaud,¹ L. Cantin,¹ M. B. Bottino,² and L. Jouanin.² ¹Agriculture and Agri-Food Canada, 6660 N.W. Marine Drive, Vancouver, B.C., Canada V6T 1X2, and ²Institut National de Recherches Agronomiques, Versailles, 78026, France.

Proteinase activities were monitored throughout the development of *Meloidogyne hapla*. Activity detected in extracts of parasitic juveniles (J2) was optimal at pH 5.5 and was inhibited by the cysteine proteinase inhibitors (PIs) E-64, iodoacetamide, and hen egg cystatin. All the activity measured between pH 3.5 and 7.5 was accounted for by two proteinase forms, Mhp1 and Mhp2, characterized by distinct mobilities in gelatin-containing polyacrylamide gels. Mhps were detected in extracts and stylet exudates of parasitic J2 and females. Mhp expression appears to be constitutive throughout the development of the nematode; they may be involved in the pathogenic process. Two plant cysteine PIs, oryzacystatins I and II (OCI and OCII), had different affinities for Mhp1. Both inhibitors nearly inactivated the proteinase in native conditions but unlike OCII, OCI conserved a high affinity for Mhp1 under denaturing conditions. This suggests the OCI-Mhp1 complex is highly stable.

CHOOSING PROTEINASE INHIBITORS TO CONTROL NEMATODES. Vrain, T., L. Cantin, and D. Michaud. Agriculture and Agri-Food Canada, 6660 N.W. Marine Drive, Vancouver, B.C., Canada V6T 1X2.

We describe a two step strategy to assess the potential of proteinase inhibitors (PIs) for nematode control. The first step is based on the gelatin-polyacrylamide gel electrophoresis procedure, to estimate the degree of affinity between the target proteinases and their putative inhibitors. The second step is to "mildly" denature the target proteinases by heat or acid treatment, and to analyze the ability of PIs to recognize slightly modified proteinase variants. As shown by the differential effects of two plant cysteine PIs, oryzacystatin I and oryzacystatin II, on the major proteinases of *Meloidogyne hapla*, these simple analytical procedures may prove useful for studying the ability of cystatins to form highly stable complexes with nematode cysteine proteinases, without the need for prior purification steps. Estimating the ability of PIs to recognize proteinase variants, the second procedure could predict the ability of PIs to inactivate "newly" appeared proteinase forms during the development of resistance in target pests.

EMBRYOLOGICAL DEVELOPMENT OF *HETERODERA GLYCINES* ON SOYBEAN CULTIVARS OF DIFFERENT MATURITY GROUPS IN IOWA. Wainwright, L. L., and G. L. Tylka. Iowa State University, Ames, IA 50011.

Embryogeny of *Heterodera glycines* was studied in 1994 on an indeterminate, maturity group (MG) II and a determinate, MG VII soybean cultivar in replicate *H. glycines*-infested field microplots. Plants were sampled every 10 days through the growing season, beginning 32 days after planting. Females and cysts were dislodged from roots, and eggs were extracted, fixed in 3% formaldehyde, and stored at 4 C until analyzed for stage of development by flow cytometry. A maturity index (MI) was calculated by dividing the number of mature eggs containing vermiform juveniles by the total number of eggs in each sample. Vegetative shoot development of the cultivars was similar throughout most of the growing season, but the MG II cultivar began flowering about 50 days before the MG VII cultivar. Maturity index of the egg populations was similar ($P > 0.05$) between cultivars at most sampling dates. Eggs recovered from both cultivars were mostly mature by late September, yet the MG VII cultivar remained actively growing. Two *H. glycines* generations likely occurred during the 123-day experiment.

RESISTANCE OF *MENTHA X PIPERITA* AND *MENTHA SPICATA* LINES TO ROOT-KNOT NEMATODES. Walker, J., and J. Melin. Department of Plant Pathology, University of Georgia, Georgia Station, Griffin, GA 30223.

Six peppermint (*Mentha x piperita*) and six spearmint (*M. spicata*) accessions from the USDA Clonal Germplasm Laboratory Plant Introduction (PI) collection were evaluated for resistance to *Meloidogyne incognita* race 3 (Mi) and *M. arenaria* race 2 (Ma) in 8-week greenhouse tests. No root-knot nematode galls formed on peppermint or spearmint exposed to initial densities of five Mi or Ma eggs/cm³ soil. Fewer than two galls/plant formed on three peppermint accessions (PI 557950, 557968, 557976) and none on accessions PI 557937, 557972, 557973 exposed to 15 Mi eggs/cm³. Only one peppermint accession (PI 557950) and none of the spearmint accessions developed root-knot nematode galls when exposed to 15 Ma eggs/cm³. No eggs were detected on any mints, but numerous galls and eggs were present on 'Rutgers' tomato.

USING VARIOUS DAMAGE FUNCTIONS TO ASSOCIATE TOBACCO CYST NEMATODE POPULATIONS WITH YIELD OF FLUE-CURED TOBACCO. Wang, J., and C. S. Johnson, Virginia Polytechnic Institute and State University, Southern Piedmont Agricultural Research and Extension Center, Route 3, Box 60, Blackstone, VA 23824.

Field experiments were conducted in 1993 and 1994 to examine the relationships between population densities of tobacco cyst nematodes (*Globodera tabacum solanacearum*) and flue-cured tobacco yield and quality. Flue-cured tobacco cultivars resistant (NC 567) or susceptible (K 326) to tobacco cyst nematodes were transplanted into untreated soil or soil treated with 6.8-7.5 liters/ha fosthiazate. Treatments were arranged in a randomized complete block design with 12 replications. Nematode population densities were monitored by collecting soil samples every week for the first 11 weeks of the growing season. Fosthiazate reduced nematode populations throughout the growing season ($P \leq 0.05$) for both cultivars in both years. A minimum yield level was not reached, preventing use of Seinhorst's damage function to describe the relationship between tobacco cyst nematode population densities and flue-cured tobacco yield. Reductions in yield were best described by linear regression models based upon population levels 5 or 6 weeks after transplant. Yields of K 326 appeared to be more highly correlated with nematode population densities ($P \leq 0.04$; $R^2 = 0.32-0.63$) than were yields of NC 567, especially in 1993.

IN VITRO SCREENING FOR BURROWING NEMATODE, *RADOPHOLUS* SP., RESISTANCE AND TOLERANCE IN ANTHURIUM. Wang, K. H.,¹ B. S. Sipes,² and A. R. Kuehnle.¹
¹Department of Horticulture, and ²Department of Plant Pathology, University of Hawaii, Honolulu, HI 96822.

Burrowing nematode, *Radopholus* sp. can reduce anthurium flower yield by 50%. Resistance and tolerance are alternative methods to chemical control of this disease. Seventeen commercial anthurium varieties were cultured in vitro and screened for burrowing nematode resistance or tolerance. Four plantlets were placed in cultural vessels, replicated six times, and inoculated with 400 burrowing nematodes per vessel. Three months later, plant damage and nematode reproductive factor on each plant variety was determined. Symptoms of infection were quantified to assess tolerance, whereas nematode reproduction was measured to assess resistance. Results showed that resistance and tolerance were independent of each other. 'Mauna Kea' and 'Midor' were among the most tolerant varieties, and 'Anuenue' was among the most resistant. These varieties will serve as a guide for future breeding.

ABG-9008- A NEW BIOLOGICAL NEMATICIDAL COMPOSITION. Warrior, P., L. A. Rehberger, and P. A. Grau. Abbott Laboratories, 6131 RFD, Oakwood Road, Long Grove, IL 60047.

Several years of biological screening specifically against plant-parasitic nematodes resulted in the discovery of a nematicidal composition designated ABG-9008 produced by an isolate of a soil fungus originally isolated from nematode cadavers. Laboratory and greenhouse studies demonstrated significant levels of nematode control; field studies on carrot, tomato, and cauliflower

indicated that ABG-9008 provided commercially acceptable control of *Meloidogyne incognita* and *Heterodera schachtii* while enhancing crop yields. The toxicology work in support of product registration have shown the product to be safe to mammals and to the environment. The product is being developed for commercial application.

BAHIAGRASS-COTTON ROTATIONS FOR THE MANAGEMENT OF *MELOIDOGYNE INCOGNITA* AND OTHER NEMATODE PATHOGENS. Weaver, C. F., R. Rodríguez-Kábana, and D. G. Robertson. Department of Plant Pathology, Auburn University, Auburn, AL 36849-5409.

A 5-year experiment was conducted in a field infested with *Meloidogyne incognita*, *Hoplolaimus galeatus*, *Paratrichodorus minor*, and *Pratylenchus* spp. to determine the value of 'Pensacola' bahiagrass (*Paspalum notatum*) as a rotation crop for the management of nematode diseases in cotton (*Gossypium hirsutum*). The experiment was Yields of seven cotton cultivars were assessed in the following rotation schemes: cotton monoculture, 1, 2, and 3 years of cotton following 2 years of bahiagrass and 1 and 2 years of cotton following 3 years of bahiagrass. First- and second-year cotton yields following bahiagrass were higher ($P \leq 0.05$) than yields in cotton monoculture. Third-year cotton yields following bahiagrass were not different from yields obtained in the monoculture system. The magnitude of yield response to the rotation system was cultivar dependent. At-plant applications of aldicarb (17 g a.i./100 m row) improved cotton yields in both the monoculture and the rotation systems. The effect of the nematicide was more evident in the monoculture system.

ANALYSIS OF FACTORS INFLUENCING APPLICATION OF *STEINERNEMA* SPP. IN THE PACIFIC NORTHWEST. Webster, J. M., A. E. Hayes, and A. E. Ingraham. Centre for Pest Management, Simon Fraser University, Burnaby, Vancouver, B.C. V5A 1S6.

Commercial formulations of *Steinernema carpocapsae* (All strain) are used to control *Otiorynchus sulcatus*, the black vine weevil, on cranberry, but in the dry-pick areas of British Columbia growers obtain inconsistent results. Field and laboratory experiments, using a *Galleria mellonella* bioassay, showed that nematodes from a commercial source had infectivity levels of 95-100% immediately before and after application through a sprinkler system or boom sprayer. Infective nematodes were distributed to all areas of the field plots by both application systems and were detectable in the soil 8 months following application but at progressively diminishing levels. Soil type generally did not appear to adversely effect nematode infectivity. *Steinernema feltiae* 27, and *S. feltiae* Umea were the most effective of four isolates of *Steinernema* spp. tested at low temperatures (8-10 C). Such strains, if produced and marketed at competitive prices, could enhance the efficacy of entomopathogenic nematodes in regions where soils are cool at the time of field application.

INTERACTIONS BETWEEN RING AND STUNT NEMATODES AND *MACROPHOMINA PHASEOLINA* ON GRAIN SORGHUM. Wenefrida, I., E. C. McGawley, and J. S. Russin. Department of Plant Pathology and Crop Physiology, LSU Agricultural Center, Baton Rouge, LA 70803.

Effects of *Tylenchorhynchus annulatus* and *Criconemella xenoplax*, alone and in combination with *M. phaseolina* on grain sorghum, were examined. In a microplot experiment, two grain sorghum hybrids (Dekalb-Pfizer 50 and Pioneer 8333), three levels of *M. phaseolina* (0, 10, and 100 cfu/g fumigated soil), and two nematode infestation levels (1,445 and 2,890 nematodes/pot) were used. Plants were harvested after 105 days. Dry weights of roots and heads were reduced by *M. phaseolina* at 10 cfu/g. Populations of both nematode species decreased as *M. phaseolina* level increased. A consistent antagonistic relationship occurred between *M. phaseolina* and nematodes on plant growth and nematode colonization on roots. Pathogenicity tests with these

nematode species alone and in combination were conducted in a greenhouse on Pioneer 8333. Root dry weight was reduced (20-27%, $P = 0.001$) by *T. annulatus*. The reduction was greater (35%, $P = 0.001$) when it was combined with *C. xenoplax*, although *C. xenoplax* alone did not reduce root dry weight.

APPLICATION OF LOW TEMPERATURE FIELD EMISSION SCANNING ELECTRON MICROSCOPY TO STUDIES IN NEMATOLOGY. Wergin, W. P., and E. F. Erbe. USDA ARS, Nematology Laboratory, Beltsville, MD.

Preparation of samples for conventional scanning electron microscopy (SEM) requires fixation, dehydration, and critical point drying. Although these procedures are widely used, they cause artifacts by: (1) failing to preserve cellular constituents; (2) solubilizing fats and carbohydrates; (3) causing considerable shrinkage and distortion. To avoid these problems a field emission SEM has been equipped with a cryosystem. With this combined instrumentation, samples can be rapidly cryofixed in liquid nitrogen (LN). The frozen samples, which are maintained at LN temperatures, can then be coated, observed and photographed in the frozen hydrated state. The procedure allows observations of intact nematodes, whose surfaces can be examined, as well as fractured specimens, whose internal structural features are revealed. The field emission SEM, on which the cryosystem is installed, can magnify more than $\times 100,000$. Therefore, this technique appears to be a valuable research tool that could add considerable information to nematological studies involving taxonomy, morphology, anatomy, biology, as well as host-parasite interactions.

"NATURAL" PRODUCTS FOR NEMATODE MANAGEMENT ON ANNUAL CROPS. Westerdahl, B. B.,¹ C. A. Anderson,¹ and J. D. Radewald.² ¹Department of Nematology, University of California, Davis, CA 95616, and ²Department of Nematology, University of California, Riverside, CA 92521.

A number of "natural" products have been tested or used in California for nematode suppression or control. Some products have achieved U.S. EPA but not California registration, while others are in the process of obtaining United States and California registrations. Other products are sold as organic fertilizers or soil amendments and so are not required to be registered as nematicides. Three trials on tomato and two on carrot have been conducted in a field containing *Meloidogyne javanica* to compare the efficacy of 16 "natural" products to six chemical nematicides. The "natural" products were either chitinolytic, microbial fermenters, or plant derivatives. Although none have performed as consistently as the soil fumigants 1,3-dichloropropene, several of the "natural" products have produced differences ($P \leq 0.05$ or $P \leq 0.10$) in nematode population densities, yield, or quality compared to untreated controls in one or more trials.

UTILIZATION OF COVER-CROPPING SYSTEMS WITH ALLELOPATHIC POTENTIAL. Weston, L. A. Department of Horticulture, University of Kentucky, Lexington, KY 40546.

Allelochemicals from leaves, flowers, seeds, stems, and roots of living and (or) decomposing plant materials offer potential for biorational weed and pest control. Under appropriate conditions, allelochemicals may be released in quantities suppressive to developing weed seedlings. Two main approaches for allelopathic weed suppression are described. First, living rotational crops can be used to interfere with the growth of surrounding weeds. Successful examples include tall or creeping red fescue, sorghum, alfalfa, black mustard, buckwheat, and oats. Attempts to select germplasm with enhanced suppressive ability have been limited. Second, cover crop residues or living mulches have been used to selectively suppress weed growth for variable lengths of time. Cover crops frequently used for suppression of weeds include winter rye, wheat, and sorghum. Residues may provide suppression by physical factors or by release of allelochemicals or microbially altered allelochemicals. Elucidation of the physiological basis for allelopathy in a species of weeds may allow for the future selection of traits responsible for pest suppression.

MITOCHONDRIAL DNA DIVERSITY IN CONSPECIFIC *MELOIDOGYNE INCOGNITA* POPULATIONS. Whipple, L. E., and B. C. Hyman. Department of Biology, University of California, Riverside, CA 92521.

Genetic diversity among conspecific *Meloidogyne* populations is important in understanding their diverse phenotypes, including virulence behavior on defined cultivars. Genetic diversity among J2-stage individuals from reproductively isolated *M. incognita* (races 1 and 3) populations was assessed via a polymerase chain reaction (PCR) assay designed to measure size variation within a 63 base pair mitochondrial variable number tandem repeat (VNTR). Amplification products were fractionated by gel electrophoresis based upon number of repeat copies, while densitometrically-determined allele frequencies were used to calculate diversity at different population structure levels. Among the populations assayed, the 63 base pair VNTR ranged in size from 2-21 repeat copies. Diversity indices within J2 individuals ranged from 0-0.93; in contrast, there was no significant difference in genetic diversity at this locus among these same populations. In comparing population structural levels, most of the variation resides within individuals, not within or among populations.

TOWARDS PRACTICAL BIOLOGICAL CONTROL OF PARASITIC NEMATODES IN DOMESTIC ANIMALS. Wolstrup, J.,¹ P. Nansen,² J. Grønvold,³ S. A. Henriksen,⁴ and M. Larsen.² Department of Ecology and Molecular Biology, ¹Microbiology and ³Zoology Sections, Royal Veterinary and Agricultural University, 21 Rolighedsvvej, DK-1958 Frederiksberg C, Denmark, ²Center for Experimental Parasitology, Department Veterinary Microbiology, Royal Veterinary and Agricultural University, 13 Bülowsvej, DK-1870 Frederiksberg C, Denmark, and ⁴National Veterinary Laboratory, 27 Bülowsvej, DK-1790 Copenhagen V, Denmark.

Nematophagous fungi have been investigated in Denmark since 1982 for biological control of nematode parasites in domestic animals. *Arthrobotrys oligospora* added to cow pat may reduce the number of nematode juveniles in feces and in herbage. Conidia and (or) mycelia of this fungus were introduced to cattle per os. Unfortunately, the fungus lost its viability during the passage through the alimentary tract of the calves. An in vitro bioassay was developed to select fungi that were able to resist the digestive processes of the alimentary tract of cattle. One successful fungus isolated by this method was *Duddingtonia flagrans*. *Duddingtonia flagrans* resulted in a substantial decrease in juvenile counts and acquisition of infection in calves grazing naturally contaminated pastures. Similar results were obtained when *D. flagrans* was applied against various nematodes in outdoor reared pigs.

CHANGES IN *HETERODERA GLYCINES* FEMALE INDEX ON A RESISTANT CULTIVAR FOLLOWING CROPPING SEQUENCES. Young, L. D. USDA ARS, 605 Airways Blvd., Jackson, TN 38301.

Change in female index (FI) of *Heterodera glycines* was measured with a bioassay on 'Bedford' soybean (*Glycine max*) following 11 cropping sequences over a 10-year period in a race 14-infested field. The FI was the number of females on Bedford expressed as a percentage of females on susceptible 'Essex'. Cropping sequences included continuous plantings of 'Forrest' and J82-21 (both resistant to race 3) and Bedford and J81-116 (both moderately resistant to races 3 and 14); a rotation of Bedford, corn (*Zea mays*), and Essex; and rotations of Bedford with J81-116 or J82-21. Bedford, J81-116, and J82-21 have different sources of resistance to *H. glycines*. Average FI for continuous plantings of Forrest, J81-116, or J82-21 was <26. The FI for continuous Bedford was 66 after the second year and averaged 85 for the next 8 years. Rotations of resistant cultivars with different sources of resistance or with a nonhost and a susceptible soybean slowed the increase in FI, but after 10 years FI for the rotation treatments were not significantly different than FI for the continuous planting of Bedford.

NEMATOCIDAL ACTIVITY OF AQUEOUS EXTRACT OF SEEDS OF *CUCURBITA MAXIMA*. Yu, Q., and J. Potter. Pest Management Research Center, P.O. Box 6000, 4902 Victoria Ave, Vineland Station, L0R 2E0, Ontario, Canada.

A water extract of seeds of *C. maxima* was found to be selectively nematocidal to *Caenorhabditis elegans* and *Pratylenchus penetrans* in vitro. After 24 hour immersion, the LD₅₀ on *P. penetrans* was 626 ppm of the extract, whereas the LD₅₀ on *C. elegans* was 1,617 ppm. Preliminary tests show that the active compound is thermostable, and its MW is below 8,000 daltons.

NEMATODES IN THE WAIMANALO, HAWAII WATERSHED. Zhang, F., and D. P. Schmitt. Department of Plant Pathology, Honolulu, HI 96822.

The objective of this study was to determine the movement of nematodes in the Waimanalo irrigation system. Samples were collected from Maunawili Mountain (source of water for the reservoir), the reservoir, the outlets of the reservoir, and farms using potable water or reservoir water for irrigation. *Aphelenchus*, *Helicotylenchus*, *Meloidogyne*, *Pratylenchus*, *Rotylenchulus*, *Tylenchorhynchus*, ring (genus not determined), *Tylenchus*, and *Xiphinema spp.* were detected from the mountain and the farms. *Paratylenchus* was collected only from farms. Average numbers of *Rotylenchulus*, *Meloidogyne*, and *Pratylenchus* occurred in low numbers (10, 41, and 10/250 cm³ soil) and the nematodes occurred in low frequency (10, 25, and 8% of the samples) on the mountain. Mean numbers of the same genera were 895, 284, and 170/250 cm³ soil from 88, 78, and 20% of the soil samples collected from farms. No nematodes were collected from the reservoir, but a few plant-parasitic nematodes were obtained from one of the outlets. The numbers of nematodes and genera did not differ ($P = 0.05$) in fields watered with potable water or reservoir water.