ABSTRACTS OF PAPERS PRESENTED AT THE SEVENTH ANNUAL MEETING OF THE *SOCIETY OF NEMATOLOGISTS*, COLUMBUS, OHIO, SEPTEMBER 3–6, 1968

BALASUBRAMANIAN, M. and R. F. MYERS. *Amino acid composition of Aphelenchoides sp. cultured on the fungus Pyrenochaeta terrestris.*

Protein bound amino acids in *Aphelenchoides* sp. cultured on the fungus *Pyrenochaeta terrestris* were identified by two dimensional thin layer chromatography. The thin layer plates were coated with 0.25 mm thickness of Avicel (microcrystalline cellulose powder), spotted with protein hydrolysate, and run in the solvents n-butanol : acetic acid : water (4 : 1 : 5) and phenol : ammonium hydroxide : water (75 : 4 : 21), respectively. Plates were sprayed with 0.3 gm ninhydrin dissolved in 100 ml n-butanol and 3 ml glacial acetic acid, and heated to 105 C for 10 min to bring out the amino acid spots. Amino acids present were alanine, arginine, aspartic acid, glutamic acid, glycine, histidine, leucine/isoleucine, lysine, phenylalanine, proline, serine, threonine, and tyrosine. Since the hydrolysate was taken in 3% H_2O_2 cystine and cysteine appeared as cysteic acid and methionine as methionine sulphate. Besides these amino acids, two unidentified ninhydrin positive spots were detected. Tryptophan would have been destroyed by the acid hydrolysis used. Using the same method of amino acid separation, labeled amino acids were detected when *Aphelenchoides* sp. were incubated in sodium salt of acetic acid 1-C^{14} in distilled water for 24 hr. After the second directional run with phenol mixture, the plate was dried and pressed against Kodak no screen x-ray film for four weeks. The spots on the radioautograph were compared with the spots on the same plate which had been sprayed with ninhydrin. The following amino acids were labeled: alanine, arginine, aspartic acid, cysteic acid, glutamic acid, glycine, leucine/isoleucine, lysine, proline, hydroxyproline, serine, threonine and valine. The results indicate that *Aphelenchoides* sp. can synthesize many amino acids and may not require them in an otherwise adequate diet. Whether they synthesize required amounts or not, is not known. Further work is in progress.—*Supported by USDA Grant 12-14-100-9124(34). Department of Entomology and Economic Zoology, Rutgers University, New Brunswick, New Jersey.*

BARKER, K. R. and C. J. NUSBAUM. *Horizontal distribution patterns of four plant-parasitic nematodes in selected fields.*

Horizontal distribution patterns of *Meloidogyne incognita, Tylenchorhynchus claytoni, Helicotylenchus dihystera,* and *Xiphinema americanum* were investigated in four fields in eastern North Carolina. Fields designated A, B and C were in tobacco in 1966 but were in fescue at sampling time (June or July 1967); the field designated D was planted in tobacco in 1967 and was in barley when sampled. A square area of 1.8 ha in each field was divided into 576 plots of 5 × 5 m for sampling. Soil samples from each plot consisted of five borings, about 15 cm deep, at pre-selected points with a 2.5-cm sampling tube. Field D was sampled twice (April 4 and 25, 1968) to measure reliability of sampling procedure. Individual borings were collected within a radius of 15 cm of those of the first sampling. Soil samples were assayed for nematodes by a sugar flotation-sieving method. Populations of *M. incognita* were variable in all fields.

Larvae were recovered from 93% of plots in field A with the highest count being 6250 larvae per 500 cc of soil. In fields B, C, and D, 54 to 62% of plots yielded *Meloidogyne* larvae with highest counts ranging from 450 to 1500 larvae per 500 cc of soil. Although *M. incognita* occurred in all fields, horizontal distribution was erratic. *T. claytoni* was recovered in substantial numbers from fields A and D; whereas only occasional individuals were found in fields B and C. Distribution of this species was very uniform in fields D and A, and nematodes were recovered from 98 to over 99% of the plots, respectively. *H. dihystera* was limited primarily to fields C and D. Recoveries ranged up to 5200 specimens per 500 cc of soil, but horizontal distribution was erratic. This species was recovered from 22 and 60% of grids in fields C and D, respectively. *X. americanum* was present in all four fields, but horizontal distribution was irregular. This species was apparently absent in many sections of each field as indicated by the low percentages of plots from which individuals were recovered (17–43). In the duplicate sampling of field D, *M. incognita* gave the most variable results. Fifty-four percent of plots yielded this nematode in both samplings, but the detection discrepancies (within each of the 576 plots) was 38%. Variation in distribution of *T. claytoni* was low as indicated by 3.6% detection discrepancies in the two samplings. Recoveries of *H. dihystera* and *X. americanum* in this test were intermediate with 22 and 15% detection discrepancies, respectively. Detection discrepancies for a given species occurred primarily in plots yielding 25 to 50 individuals per 500 cc of soil. Generally, horizontal distribution patterns for a given nematode were similar in these fields, but probably would vary in fields with different cropping histories. More information on horizontal distribution patterns of nematodes should facilitate development of sampling procedures and experimental designs that would increase precision in field experiments as well as general assays for nematodes.—*North Carolina State University, Raleigh, North Carolina.*

CALAWAY, W. T. *Mononchoides associated with waste water treatment.*

The first nematode given the generic name *Mononchoides* was *M. longicauda* described by Rahm in 1929. Several other species have been recognized since then but some of them were described under the generic name *Eudiplogaster*. The genus *Mononchoides* (syn. *Eudiplogaster*) of the Diplogasteridae along with several genera of the Rhabditidae comprise the dominant nematode taxa found in sewage treatment processes. Members of the genus *Mononchoides* have been collected from sewage treatment processes in Florida and Central Ohio. They were proportionally less numerous in Ohio and the predominant species in Florida was not present in any sample from Ohio. The large dorsal tooth present in members of *Mononchoides* indicates predatory habits, and they were grown quite successfully on water agar using *Panagrellus redivivus* as food. The Florida species attacks members of Rhabditidae, the bristle worm *Aelosoma,* insect eggs, and insect larvae. The nematodes attach themselves to the prey in some manner and immediately create an opening in the cuticle. The body fluids then are pumped out, with the median bulb evidently taking an active part, leading to collapse and accordion-like pleating of the prey. The predation by *Mononchoides* on metazoa present in waste treatment, especially on nematodes, insect eggs and larvae, makes it an important factor in maintaining the ecological balance in waste treatment processes. Three interesting features of the predominant species of *Mononchoides* found

in Florida may be pointed out here: (i) The dorsal gland empties through a channel of the dorsal tooth. Two tiny openings have been observed, one just dorsal and the other just ventral of the apex of the big tooth. (ii) Posteriad of the dorsal tooth the dorsal gland channel opens into a wide sinus which lies alongside the posterior stomatal chamber. (iii) The anterior edge of the metarhabdions usually carries fine toothlets in a double arc but on the right metarhabdion these are partially replaced by a large tooth. —*Department of Entomology and Nematology, University of Florida, Gainesville, Florida.*

COLE, R. J. and S. R. DUTKY. *A sterol requirement in Turbatrix aceti and Panagrellus redivivus.*

Biochemical studies indicate that *Turbatrix aceti* cannot biosynthesize sterols *de novo* and therefore probably relies on an exogenous source of sterol for normal growth and development. A nutritional requirement for sterol in the DD-136 nematode (a parasite of insects) has been demonstrated in this laboratory using a sterol-deficient test system in which the bacterium associated with the nematode was placed on peptone-glucose agar slants. The DD-136 nematode failed to develop and reproduce unless sterol was added to the test system. Since both *T. aceti* and *Panagrellus redivivus* can be cultured monoxenically with a number of different bacteria, we used the same technique to evaluate their sterol requirement. The sterol-deficient bacterial test system used consisted of endospores of *Bacillus subtilis* placed on peptone-glucose agar slants. The bacterium was cultured on tryptose phosphate agar slants supplemented with 20 μg manganous sulfate and was transferred to the test system after it sporulated. Studies with this sterol-deficient bacterial test system conclusively demonstrated that both species of nematodes required exogenous sterol for normal growth and development. In *Turbatrix,* the requirement was evident about one week after the nematode was transferred to the sterol-deficient test system. In *Panagrellus,* a second serial transfer to a sterol-deficient system was necessary before the sterol requirement was demonstrated. Thus, *Panagrellus* can either utilize stored and available sterol in an extremely efficient manner or its threshold for growth is just above the amount of sterol available in our test system (which contained trace amounts of sterol). Cholesterol-4-^{14}C added to the test system was not biologically altered by the bacterium. The demonstration of a sterol requirement shows that sterol and/or a sterol metabolite(s) perform a vital function(s) in these species of nematodes.—*Entomology Research Division, Agricultural Research Service, U. S. Department of Agriculture, Beltsville, Maryland.*

COOPER, JR., ALAN F. *Respiration and lipid catabolism of two nematode species.*

Standard Warburg techniques were used to study the respiration of *Aphelenchus avenae* and *Pelodera* sp. after various periods of starvation and of anaerobic exposures. Gaseous N_2 was continuously flushed over the nematode suspensions, held at 28 C, for periods of 1, 2, 4, 8, 16, 24, 48, and 96 hr. All except the 96-hr exposure, which killed the nematodes, resulted in a progressively longer post-recovery period to attain maximum O_2 consumptions of 5.6–5.8 μl O_2 consumed/hr/mg dry wt for *A. avenae* and 6.1–6.3 μl O_2 consumed/hr/mg dry wt for *Pelodera* sp. Anaerobic exposure of 16 hr resulted in a slight postanaerobic oxygen debt with fresh unstarved nematodes. There was no comparable response in starved nematodes. A mixture of 98% N_2 and 2%

CO_2 produced the same results as N_2, but a mixture of 95% N_2, 4% O_2 and 1% CO_2 produced only moderate initial reduction of post O_2 consumption even after an 8-day exposure. Respiratory quotients (RQ = amt CO_2 produced/amt O_2 consumed) of fresh unstarved *A. avenae* (mixture of 4th stage larvae and adults) and *Pelodera* sp. (mixture of 3rd and 4th stage larvae and adults) were .94–.96 for the first 8–16 hours after which it dropped to .41–.43. An RQ of .94–.96 was never observed in starved nematodes of either species. It is postulated that these nematodes initially used a carbohydrate food source, but after a few hours began to use an endogenous lipid reserve which was being incompletely oxidized.—*Supported by National Science Foundation Grant No. GB 6569.* *Department of Nematology, University of California, Riverside, California.*

DUNN, R. A. *Extraction of cysts of Heterodera species from soils by centrifugation in high density solutions.*

A survey of central New York State soils for *Heterodera* species required highly efficient extraction of cysts without reducing the viability of their contents. Many cyst-flotation techniques require thorough drying of the soil to reduce the specific gravity of the egg-laden cysts. *H. trifolii,* and to a lesser extent *H. schachtii,* are sensitive to desiccation; drying of soil may severely reduce emergence of larvae from cysts of both species. Consequently, a process permitting extraction of all cysts from undried soil was adopted. Six hundred cc of soil was washed through a 24-mesh screen onto a 60-mesh screen. The residue on the 60-mesh screen was transferred to 50-ml centrifuge tubes and suspended in a sucrose solution of sp. gr. 1.23. After centrifugation for 5 min at approximately 1150 G, the supernatant material was decanted onto a 60-mesh screen. The material on the screen was rinsed briefly with water to remove the sugar solution and washed into a counting dish for inspection. When processing organic soils, the residue remaining after the initial wet-sieving was put into half its volume of water, agitated vigorously with a Vibromixer to break up soil aggregates, and washed onto a 60-mesh screen. This reduced the volume of material to be centrifuged without apparent damage to the contents of cysts. Solutions of appropriate density of inorganic salts, such as magnesium and zinc sulfates, can be substituted for the sugar solution. Sugar centrifugation was more sensitive in detecting low infestations and was a faster technique for recovery of all cysts, including heavy egg-laden cysts, than was searching through all the residue from simply wet-screening undried soil without the aid of sugar centrifugation. In addition, no special elutriation equipment was required. The method proved highly satisfactory when used in routine processing of soil samples from the field and sugarbeet grading stations and in processing the soil from greenhouse tests. —*Department of Plant Pathology, Cornell University, Ithaca, New York.*

FELDMESSER, JULIUS. *Control of root-knot nematodes on tomato with systemic chemicals.*

Effects of foliar applications of systemic chemicals on root-knot indexes and on numbers of new roots being invaded by nematodes were observed in *Meloidogyne incognita acrita*-infected roots of *in vitro*-grown tomato plants. A dish procedure, previously described, allowed continual observations of plant roots. In initial experiments, 2,3,6-trichlorobenzoic acid (2,3,6TBA), 2,3,5,6-tetrachlorobenzoic acid (2,3,5,6TBA), and 2-methoxy-3, 6-dichlorobenzoic acid (dicamba) were formulated and applied to

plants with established infections and to plants inoculated with larvae at the time of treatment. Rates used were 250, 500, 1000, and 2000 ppm (vol./plant wt). Over a 2-month period, the most promising results were observed on the plants treated and inoculated at the same time. Both 2,3,6TBA and 2,3,5,6TBA showed a tendency to repel nematode attacks on roots. Treated plants showed chlorosis and wilt and drop of treated leaves within 10 to 12 days after treatment but continued to show root and top growth. Dicamba was too phytotoxic to enable us to determine nematode control due directly to the chemical and independent of poor plant condition. In additional work, 2,3,6TBA, 2,3,5,6TBA, and maleic hydrazide (MH) were used on plants at the same rates as above. Half of the plants were inoculated with nematode larvae the same day, and the rest were inoculated 4 days after the chemical was applied. Both 2,3,6TBA and 2,3,5,6-TBA were most effective under the latter conditions. Over a 2-month period, these chemicals reduced root-knot indexes and the percentages of new roots showing invasion. Root-knot indexes (4.0 maximum) were reduced from 3.5 (untreated) to 2.3 and 1.5 by the 1000 and 2000 ppm treatments of each chemical. 2,3,6TBA reduced root-knot invasion of new roots by 69% while 2,3,5,6-TBA caused a reduction of 41%. Tomato plants treated with these compounds showed varying degrees of chlorosis, wilt, epinasty, and drop of treated leaves within 10 to 14 days after treatment. The plants, however, continued to show root and top growth. Plant debility may be a factor in lowering nematode infections, but continued growth, especially of roots, under test conditions suggests a close association between the treatments and reduced nematode infections. As in other reported work, MH proved to be lethal at higher rates or caused severe wilt, chlorosis, and cessation of growth at lower rates.—*Crops Research Division, Agricultural Research Service, U. S. Department of Agriculture, Beltsville, Maryland.*

FOX, J. A. and MARIE G. KEREKES. *Hatching response of some Heterodera species to sodium hypochlorite.*

Hypochlorite solutions have been reported to stimulate hatching of eggs of some *Heterodera* species. Because the Osborne cyst nematode (OCN), an undescribed species, was observed to be very responsive to sodium hypochlorite, studies were conducted to compare the effects of sodium hypochlorite on eggs of OCN, *H. schachtii, H. glycines, H. weissi,* and *H. carotae.* Eggs of each species were treated for 10 min with an approximate 0.5% sodium hypochlorite solution, rinsed with distilled water and fixed in 4% formaldehyde. Two sources of sodium hypochlorite (Fisher Scientific Co. Lot No. 780565, 4 to 6% and Easy Monday Bleach®, 5.25%) were tested. Each species responded differently to the two solutions. OCN was the most responsive with 83% and 97% hatch due to the Fisher and Easy Monday solutions, respectively. A 71% hatch due to the Fisher solution and 21% hatch due to the Easy Monday solution was recorded for *H. weissi* while a 6% hatch due to Fisher solution and 68% hatch due to the Easy Monday solution was recorded for *H. schachtii. H. glycines* and *H. carotae* with hatches ranging between 50% and 80% did not show strong differential responses to the two solutions. In subsequent tests with OCN, *H. weissi,* and *H. schachtii,* all three species had a hatch greater than 95% due to a 1.0% sodium hypochlorite solution (Fisher). The hatching phenomenon due to sodium hypochlorite appears to be passive involving the dissolution of the egg membranes and pressure exerted by the larvae as they tend to assume a straightened posture.—*Department of Plant*

Pathology and Physiology, Virginia Polytechnic Institute, Blacksburg, Virginia.

GRIFFIN, G. D. *Attractiveness of resistant and susceptible alfalfa to stem and root-knot nematodes.*

A study was made to determine if the alfalfa stem nematode, *Ditylenchus dipsaci*, and the northern root-knot nematode, *Meloidogyne hapla*, are attracted to resistant and susceptible alfalfa, *Medicago sativa*. To determine possible attraction of *D. dipsaci*, nematodes were introduced into Provo sand at distances of 12.5, 25, and 50 mm from planting sites of resistant and susceptible alfalfa seeds. After 7 days of growth at 20 C, the seedlings were stained and examined for nematode infection. When the nematodes were introduced 12.5 mm from the alfalfa seed, 100% of the susceptible and 72% of the resistant alfalfa seedlings were infected. There were 2.59 times more nematodes infecting susceptible seedlings than resistant seedlings. At 25 mm no differences in infection of resistant and susceptible seedlings were observed (nematodes infected 52% of resistant and 56% of susceptible seedlings). No resistant and only 8% of the susceptible seedlings were infected when nematodes were introduced 50 mm from the seed; this may be attributed to the emergence of seedlings from the soil before nematodes reached the area of infection (hypocotyl, epicotyl, or cotyledonary petioles), or to a limited range of the attraction factor(s). Two methods were used to study the possible attraction of root-knot nematode larvae to germinating alfalfa seed. In the first method, germinating seeds of resistant and susceptible alfalfa were placed on 0.125% water agar in small plastic sampling boxes (one seed per box). An egg mass of *M. hapla* was placed 12.5 mm from each seed. In the second method, an egg mass of *M. hapla* was placed between a germinating resistant and a germinating susceptible seed on 0.125% water agar; the seeds were 12.5 mm from the egg mass. Although larvae were attracted to and found near the roots of both resistant and susceptible seedlings, more were attracted to susceptible than resistant seedlings. In the first method, 85% of the larvae were attracted to susceptible seedlings and 75% to resistant seedlings. In the second method, 71% were attracted to the susceptible seedlings and 29% to the resistant seedlings.—*Crops Research Division, Agricultural Research Service, U. S. Department of Agriculture, Utah State University, Logan, Utah.*

HEALD, C. M. *Histopathology of 'Tifdwarf' bermudagrass infected with Meloidogyne graminis (Sledge and Golden) Whitehead.*

Roots of 'Tifdwarf' bermudagrass infected with *Meloidogyne graminis* were processed for histological sectioning and stained according to Johansen's staining procedure. These studies revealed that *M. graminis* caused extensive damage to the vascular and cortical root tissue of 'Tifdwarf' bermudagrass. Many larvae entered the roots just posterior to the root cap and migrated parallel to the vascular system of the root. The nematodes then penetrated the vascular system initiating the formation of four to five giant cells surrounded by a thickened cell wall. Frequently, two nematodes were observed feeding in close proximity, with the resulting formation of eight to ten giant cells. Most giant cells appeared to be located in the xylem with fewer in the phloem. These cells were usually oblong extending longitudinally in the vascular system. Cellular contents of giant cells were more granular and heavily stained than were non-infected cells. Giant cells contained several nuclei, and prominent nucleoli were usually concentrated toward the center of the cell. The oval-shaped

bodies of the females remained in the cortex, adjacent to the vascular tissue; the lip regions were always oriented toward the root tip. A gelatinous matrix at the posterior end of the nematode contained eggs and sometimes hatched larvae. Only slight swellings were noticed in the area where *M. graminis* fed.—*Crops Research Division, Agricultural Research Service, U. S. Department of Agriculture, Weslaco, Texas.*

HÖGGER, C. H. *Comparison of penetration of potato roots by Pratylenchus penetrans grown in tissue cultures and from field populations.*

Nematodes used as inoculum were from monoxenic alfalfa callus cultures, from winter vetch kept in the greenhouse, and from potato roots growing in a field. Eight-week-old potato seedlings growing in a sandy loam in 2.5 cm clay pots were inoculated with a suspension of 100 *Pratylenchus penetrans* in 1 ml of water. After 6 days the plants were removed from the pots, and the soil was washed from the roots. The roots of each plant were placed in 2.5 by 15 cm test tubes to which 10 ml of water were added. The test tubes were shaken for 4 days with a wrist-action mechanical shaker, and the nematodes that emerged from the roots in the water were counted. In four experiments conducted at different times, in which always the aseptic and one of the septic types of inoculum were compared, averages of 7 to 10% of the nematodes added as inoculum were recovered. This percentage is regarded as being infective. There were no statistically significant differences within any of these experiments. Extraction was almost complete, because two or less nematodes per root system were detected following staining of roots with acid fuchsin. Penetration and emergence of both kinds of populations, septic and aseptic, were, therefore, of the same magnitude. This indicates, that nematodes grown on callus in the laboratory are as infective as nematodes from greenhouse cultures or from the field.—*Department of Plant Pathology, Cornell University, Ithaca, New York.*

HUANG, C. S. and A. R. MAGGENTI. *Root-knot nematode induced mitotic aberrations and nuclear changes of developing giant cells in Vicia faba.*

Mitotic behavior and nuclear changes in the giant cells of *Vicia faba* caused by *Meloidogyne javanica* were studied, using Feulgen squash technique, paraffin sections and electron microscopy. *V. faba* was chosen for these studies because mitosis and the morphological characters of the 12 diploid chromosomes are relatively well described. The chromosome counts of 168 giant cells were: 122 with 24 chromosomes, 23 with 48, 11 with 96 and 12 with 192 respectively. The data indicate that the chromosome counts in metaphase giant cells are predictable by $N = 2^d \times 12$, where "N" represents chromosome count at any metaphase, "d" is the number of mitotic cycles and 12 the diploid chromosome number. It is concluded that the giant cell nuclei are derived from repeated mitosis of a single nucleus within the same cell. Giant cells with more than 192 chromosomes were observed but chromosome counts were not attempted. Structural aberrations of the giant cell chromosomes were not evident except for an occasional Feulgen positive thread connecting some late metaphase chromosomes. Chromosomes were grouped unevenly into anaphase poles even though the number of poles increased with the increase in chromosome number. Neighboring anaphase poles were sometimes juxtapositioned or fused. Chromosomes unable to complete their anaphase migrations remained at the equatorial position through

subsequent interphases. This abnormal behavior resulted in interphase nuclear masses of various sizes and shapes. Electron microscopy revealed that the nuclear masses, frequently interconnected by anastomosing nuclear materials, were ensheathed with a continuous nuclear envelope. Some nuclei were irregularly lobed and exhibited amoeboid appearance. Nuclear divisions in the same giant cells tended to be in gross synchronization. However, in two incidences, the giant cell was observed to have metaphase and anaphase chromosomes concurrently. Cell plate formation in any division was not detectable by the electron microscope.—*Department of Nematology, University of California, Davis, California.*

HUNG, CHIA-LING PI and W. R. JENKINS. *Comparative embryology of two species of Pratylenchus.*

The development of embryos was studied in bisexual *Pratylenchus penetrans* and monosexual *P. zeae*. Unsegmented eggs were usually deposited by females. For observation, these eggs were mounted in water under coverslips supported by glass rods and sealed with Vaseline. The cleavage pattern and formation of the larva were identical in *P. penetrans* and *P. zeae* and are described as one. The first cleavage took place a few hours after the egg was laid. The four-celled stage was formed 8 to 12 hr after first cleavage. Rotation of some cells occurred at this stage and up to cell differentiation after which it was not observed. Within the next 8 to 12 hr the first anterior cell divided followed by division of one daughter cell. The second anterior cell move toward the central dorsal position, while the central posterior cell moved toward the central ventral position. Each of these two cells and the posterior cell divided once, increasing the total number of cells to nine. Following this stage, the number of cells increased rapidly until a blastula was formed. Cell differentiation immediately followed which was evidenced by the formation of darker and larger inner endodermal cells and smaller ectodermal cells. The anterior end of ectodermal cells divided and formed a hyaline region which increased in size and elongated anteriorly. Movement of the embryo was noted at this stage. Six to 7 days after egg deposition, the first stage larva was coiled three to four times within the egg shell. The different esophageal parts were not differentiated completely in the first-stage larva. During the first molt, a short sclerotized tube was shed with the old cuticle, indicating that an undifferentiated stylet had been present at the anterior part of the first-stage larva. Movement was slow during molting. The spear apex was formed first, then the shaft, and finally the basal knobs. By the time the second-stage larva had completed development, it began rapid movement with repeated thrusts of the stylet to the molting cuticle which was shed. During hatching, the larva moved frequently and vigorously and the stylet was repeatedly thrust into the egg shell. Finally the shell was broken and the second-stage larva released. It took 10 days from the unsegmented egg to hatching at 23 C. Average size of 25 living eggs of *P. penetrans* was 60.6 μ by 24 μ. Two polar bodies were found outside the vitelline membrane near the center of the embryo during the early developmental stages and disappeared during later development. Average size of 25 living eggs of *P. zeae* was 62.4 μ by 22.2 μ. Only one polar body was observed at one end of the embryo in *P. zeae* and it remained present up to the time of hatching. The different number of polar bodies present in *P. penetrans* and *P. zeae* indicated different modes of reproduction.—*Department of Entomology and Economic Zoology, Rutgers University, New Brunswick, New Jersey.*

HUNG, CHIA-LING PI and W. R. JENKINS. *Criconemoides curvatum and the peach tree decline problem.*

Tens of thousands of peach trees have died in New Jersey during the past 5 to 10 years. Death has occurred either as a slow decline over a period of several years or within a year or two of planting in an old orchard site. Severe winter injury and canker caused by *Fusicocum amygdale* are common but are not consistent throughout the decline-affected areas of an orchard. Large numbers of *Criconemoides curvatum* were found in 79% of all orchards surveyed and *Pratylenchus penetrans* in 38%. Populations of *C. curvatum* increased as soil depth increased up to about 1 m. All replant areas were infested with moderate to high populations of nematodes but decline areas varied in infestation. To investigate the effect of *C. curvatum* on peach, greenhouse and laboratory experiments were carried out using sterile-cultured seedling peaches of several varieties inoculated with surface-sterilized worms. Feeding by these nematodes caused extensive lesions and pits on roots. Nematodes were observed with their anterior ends embedded several cell layers deep, a condition not previously associated with *Criconemoides*. Under non-sterile conditions, the pits and lesions were invaded by other microorganisms which caused a general discoloration and low vigor of the root system. Field treatment of infested areas with nematicidal chemicals has reduced the loss of trees in replanted orchards and resulted in a greater growth of trees when compared with untreated areas. It is proposed that peach tree decline is a complex involving nematodes and one or more cultural or other disease factors.—*Department of Entomology and Economic Zoology, Rutgers University, New Brunswick, New Jersey.*

JENSEN, H. J. and S. R. SIEMER. *Protection of fungus spores from certain biocides following ingestion by saprozoic nematodes.*

Colonies of the saprozoic (saprobic) nematode, *Mesodiplogaster* (*Diplogaster*) *lheritieri,* were combined with cultures of R5-6 *Fusarium oxysporum* f. *lycopersici* and with cultures of *Verticillium dahliae* (potato strain) to allow ingestion of fungus spores. Suspensions of nematodes obtained from these mixed cultures (nematode and fungus) were washed repeatedly in a 400 mesh strainer to remove most of the external fungus material. Aliquots of the nematode suspension were exposed to various concentrations of residual chlorine (0.25–50 ppm) on a timed basis (2, 15, 30, and 60 min). Following the chlorine treatments, sufficient sodium thiosulfate solution was added to neutralize the residual chlorine. Ingested spores were recovered by crushing the nematodes in distilled water (5 females per replication for *Verticillium* and 10 for *Fusarium*). These spore suspensions were placed in petri dishes, combined immediately with potato-dextrose agar containing streptomycin-sulfate and later read for fungus survival. Non-ingested spores were treated similarly, and served as controls. Non-ingested spores of *Verticillium* failed to germinate after a 2-min exposure in 2.5 ppm of residual chlorine while ingested spores tolerated a 5 ppm concentration for 16 hr, far beyond the 60-min duration time of the experiment. Similarly, non-ingested spores of *Fusarium* failed to germinate after a 2-min exposure in 2.5 ppm of residual chlorine, but ingested spores tolerated a 5 ppm concentration for 16 hr. Besides chlorine, certain fungicides (thiabendazole-TBZ, or 1-(butylcarbamoyl)-2-bendimidazole carbamic acid, methyl ester) gave analogous results. Our results indicate that

nematodes can ingest and protect spores of two important pathogenic fungi from fungicidal chemicals.—*Department of Botany and Plant Pathology, Oregon State University, Corvallis, Oregon.*

JOHNSON, A. W. and R. H. LITTRELL. *Effect of Meloidogyne incognita, M. hapla, and M. javanica on the severity of Fusarium wilt of chrysanthemum.*

The effects of three species of root-knot nematodes on the severity of Fusarium wilt of chrysanthemum (*Chrysanthemum morifolium*) were studied in greenhouse inoculation experiments. Chrysanthemum cultivars 'Yellow Delaware' (wilt susceptible) and 'White Iceberg' (wilt resistant) were used. Inoculum of *Fusarium oxysporum* was obtained by growing the fungus in Hoagland's No. 2 nutrient solution with 2% glucose in flasks for 4 days on a wrist-action shaker. The methods of fungal inoculation used were: dipping the root system in the inoculum, and injecting the inoculum with a syringe into the rhizosphere of plant roots 3 weeks after inoculation with nematodes. Inocula for *Meloidogyne incognita, M. hapla,* and *M. javanica* were 10 well-developed egg masses of each species. These were hand-picked from heavily galled tomato roots. The following treatments were employed: (i) fungus alone, (ii) nematode species alone, (iii) fungus-nematode species simultaneously, and (iv) addition of fungus 3 weeks after nematode inoculation. Nematodes increased the severity of wilt symptoms in 'Yellow Delaware' but did not break resistance of 'White Iceberg.' Wilt symptoms appeared earlier and were more severe in plants inoculated simultaneously with *M. javanica* and *Fusarium* than with *M. incognita* or *M. hapla* and *Fusarium.* Disease severity was less when *Fusarium* was inoculated 3 weeks after the nematodes were added. Root systems were examined 125 days after inoculation; all nematode species completed their life cycles but did not appreciably affect the growth of the plants.—*Cooperative Investigations, Crops Research Division, Agricultural Research Service, U.S. Department of Agriculture, Beltsville, Maryland; and the University of Georgia College of Agriculture Experiment Stations, Coastal Plain Station, Tifton, Georgia.*

JOHNSON, P. W. *Electron microscopic observations on the cuticle of Hemicycliophora arenaria.*

The nature of the double cuticle of mature females of *Hemicycliophora arenaria* was studied with the electron microscope. Measurements presented herein are averages. The outer cuticle or sheath appeared to be composed of a single, apparently porous layer, 0.49 μ in thickness. The inner cuticle was 0.97 μ in thickness and was composed of five distinct layers which could be grouped into three zones. The outermost zone was a trilaminate structure of two osmiophilic components separated by an electron transparent layer. These layers from outside to inside were 55A, 88A, and 145A in thickness. The next innermost zone was a thick matrix layer which could be subdivided into two regions, an outer one, 0.56 μ in thickness, with a superficial appearance of striations, and an inner fibrous or filamentous region 0.16 μ in thickness. The cuticle seemed structurally weak within this inner region and was frequently separated during processing. The third and innermost zone appeared as a porous layer, 60 mμ in thickness, having micro pores with a periodicity of 22 mμ in cross sectional views and 16 mμ in longitudinal views. Adjacent to this layer was the hypodermis which was separated from the somatic musculature by a basal lamella. Both the hypodermis and basal

lamella were bounded by membranes. Mitochondria and other intracellular structures were absent in the interchordal hypodermis but present in chordal areas.—*Supported by National Science Foundation Grant No. GB 6569. Department of Nematology, University of California, Riverside, California.*

LAUGHLIN, C. W. and A. S. WILLIAMS. *The influence of temperature on the rate of development and sexual differentiation of Hypsoperine graminis Sledge and Golden.*

The influence of temperature on the rate of development and sexual differentiation of *Hypsoperine graminis* was studied on 'Tifgreen' bermudagrass, *Cynodon* sp. The rate of development was determined in soil held in constant water temperature tanks at 15.6, 21.1, 26.7, and 32.2 ± 1 C. Rooted sprigs of 'Tifgreen' bermudagrass were planted in 100 cc polypropylene centrifuge tubes, inoculated with 100 larvae per plant and these tubes were embedded in waterproof sand-filled containers in each temperature water bath. The progress of nematode development at each temperature was observed at various time intervals by examining roots stained with acid fuchsin in ethanol and glacial acetic acid (1 : 1) and cleared in a saturated chloral hydrate solution. At 26.7 and 32.2 C saccate females exuding matrices were apparent 14 days after inoculation. Females at these temperatures were laying eggs 21 days after inoculation and larvae were present in the matrix at 25 days. At 15.6 and 21.1 C females were beginning to exude matrices 21 days after inoculation. Eggs and larvae were evident in 28 days at 21.1 C. Eggs were observed at 15.6 C in 28 days but larvae were not apparent until 35 days after inoculation. Males predominated in cultures incubated at 32.2 C, with few males observed at the lower soil temperatures. In order to establish more critically the influence of temperature on sex ratios, additional plants incubated at 26.6 C were removed from soil 48 hr after inoculation, the roots were washed free of soil and placed individually in beakers of tap water. Four of these beakers with plants were placed at each temperature. Starting at 14 days after inoculation and continuing at 3-day intervals for 15 additional days, males were removed and counted from each beaker. Males were emerging from roots 20 days after inoculation. At 32.2 C the frequency of males was greater than 80%, an average of 24 out of 29 total nematodes developing per replication. At 26.7 C males occurred on the average of less than 1 out of 30 developing nematodes, a frequency of less than 3%. Males were not observed at the two lower temperatures. Thus, sex differentiation in *H. graminis* is influenced by temperature.—*Department of Plant Pathology and Physiology, Virginia Polytechnic Institute, Blacksburg, Virginia.*

LEHMAN, PAUL S. *Hatching responses of Heterodera glycines to hydrogen ion concentrations and inorganic ions.*

Experiments were designed to determine the effects of hydrogen ion concentrations [H^+] and inorganic ions on hatching ("hatching and emergence") of the soybean cyst nematode, *Heterodera glycines*. Experiments were conducted at 25 ± 1.0 C, and test solutions were changed every 48 hr. Phosphate or phthalate (5mM) were found to be suitable buffers and were used in these experiments. The hatching response of yellow cysts without egg masses was determined at pH 2.5, 3.5, 4.5, 5.5, 6.5, and 7.5. Maximal stimulation of hatching occurred at pH 3.5, whereas hatching was depressed at pH 5.5. Response to [H^+] also varied with cyst maturity and differed between cysts and

egg masses. The influence of inorganic ions on hatching was determined in a factorial experiment in which the cations NH_4^+, Ca^{++} were used in combination with each of the anions NO_3^-, SO_4^{--}, and Cl^-. Each of the nine compounds was tested at 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4} M concentrations. Brown cysts without egg masses were used, and the pH of the test solutions was kept at pH 5.6 ± 0.1 with 5 mM phthalate buffer. The cations Mg^{++}, NH_4^+, were inhibitory ($P < 1\%$) when compared to Ca^{++}, and among the anions, NO_3^- was more inhibitory ($P < 1\%$) than Cl^- and SO_4^{--}. Relative to the buffer control or distilled H_2O, NH_4NO_3 greatly inhibited hatching and $CaSO_4$ stimulated hatching. Interactions of inorganic ions and $[H^+]$ were investigated using buffered solutions of $CaCl_2$ and NH_4NO_3 (10^{-2} M) at pH 3.2, 4.0, 4.8, 5.6, and 6.4. The prime influence of $[H^+]$ was at pH 3.2 and 4.0, whereas the influence of $[H^+]$ was secondary to that of inorganic ions at pH 4.8, 5.6, and 6.4. The results presented demonstrate the necessity of controlling pH in hatching experiments and suggest the influence that inorganic ions applied as fertilizers may have on the inoculum potential of the soybean cyst nematode. *—Supported in part by USDA Grant Number 12-14-100-8069(34). North Carolina State University, Raleigh, North Carolina.*

MANKAU, R. and SITANATH DAS. *The influence of chitin amendments on Meloidogyne incognita.*

The specific effects of organic amendments such as cellulose, starch, chitin, and dextrose on nematode population dynamics in root-knot nematode infested soil were studied in laboratory, greenhouse, and microplot experiments. Chitin was mixed with soil at from 1000 to 10,000 ppm; it was particularly effective in reducing the recovery of *Meloidogyne incognita* larvae from amended soil, and in reducing or eliminating gall formation by the nematode on roots of seedlings planted in treated soil. In amended soils, incubated at room temperature in the laboratory for periods of up to 90 days, measurements of CO_2 evolution and microbial populations indicated that most of the chitin was degraded within 15 days. Bacteria and actinomycetes, especially chitinoclastic forms, were markedly stimulated. When chitin was added at high rates (10,000 ppm) in laboratory incubated soil, total nematodes increased to over 10 times the population in non-amended soil after 5 days and declined gradually over 90 days to approximately the level of control soil. Bacterial and fungal-feeding species accounted for the increased population due to an increased food base, but the latter forms declined rapidly after 30 days and were lower than controls at 60 and 90 days apparently because of high actinomycete populations and rapidly declining fungal activity. Root-knot nematode larvae were completely eliminated from the soil. The soil became phytotoxic to tomato seedlings during peak microbial activity, but in a sandy loam in outdoor microplots phytotoxicity did not develop and tomatoes grown in soil amended with chitin had ⅓ the root-knot nematode damage found in non-amended controls. Soil samples from the treatments were assayed with tomato seedlings after 7 months, and chitin-amended samples had an average of 0.75 galls per seedling versus 101.5 galls per seedling in non-amended soil. Purified chitin appeared more effective against root-knot nematode development than crude material. Microbial activity associated with chitin degradation created soil conditions adverse to *M. incognita*. Toxin production may be implicated in the suppression of root-knot nematodes, but an indication of apparent direct pathological effects on larvae was also observed. The other organic amendments also in-

creased microphagous nematode populations. Cellulose, starch, and dextrose produced high counts of mycophagous nematodes. Soil populations and subsequent infectivity of root-knot nematode larvae were also markedly reduced by cellulose but not to the degree observed with chitin. Starch and dextrose had little effect on infectivity of root-knot nematode larvae.—*Supported by USDA Research Contract 12-14-100-8282 (34). University of California, Riverside, California.*

MAUR, K. M. and V. G. PERRY. *Life cycle studies and pathogenicity of Hypsoperine graminis Sledge and Golden on roots of St. Augustine grass and bermudagrass.*

Life cycle studies on *Hypsoperine graminis* were conducted in the greenhouse at approximately 28 C, and relative humidity of 30 to 60%. Sixty 10-cm plastic pots of fumigated field soil were planted with rooted cuttings of St. Augustine grass, collected from Winter Haven, Florida. Each pot was inoculated with 200 second-stage larvae. At intervals of 2 to 3 days the roots of plants in one or sometimes 2 pots were examined, stained with acid fuchsin in lactophenol, and dissected to prepare temporary and permanent slides of all larval stages. Measurements were taken of egg masses, eggs, second-stage larvae and males fixed in 3% formalin. Life cycle studies indicated that the parasite has five developmental stages separated by four molts. The life cycle was completed within 32 to 35 days after penetration. Egg production started as early as 26 days following entrance, and continued for 7 to 10 days. Eggs were found inside the body of the female and outside the body in a gelatinous matrix. The number of eggs produced per female varied from 181 to 1000. The larvae underwent their first molt inside the egg and emerged as preparasitic second-stage larvae capable of entering host plants within 24 to 48 hr. In cases of heavy infection the regions of cell multiplication, cell elongation, and cell differentiation were invaded. The migration of nematodes inside roots was both inter- and intracellular. Within 3 to 6 days after entering the roots, larvae became sedentary feeders on cells inside the endodermis. The second and third molts took place within 16 to 19 days after entrance into host roots. The larvae destined to become males stopped feeding after the second molt and underwent molting inside the third-stage cuticle to become eelworm-shaped, approximately 22 days after entry. The fourth molt then occurred. Pathogenicity tests using common St. Augustine grass from Winter Haven, Florida and bermudagrass (variety T 328) from Gainesville, Florida were conducted in the greenhouse at 28 C. Ten replicates of paired treatments in 10-cm plastic pots containing fumigated field soil, were used in each experiment. Hand-picked second-stage larvae were used as inoculum. The average fresh weight of the roots of St. Augustine grass which had been inoculated with 1000 larvae of *H. graminis* for 120 days was 42% less than uninoculated controls; and the average fresh weight of the roots of bermudagrass which had been inoculated with 1000 larvae for 120 days was 36% less than uninoculated controls. These data were significant at the 1% level. Two other pathogenicity tests with St. Augustine grass conducted similar to the above but using 500 second-stage larvae for periods of 60 and 80 days were significant at the 1% level also. A similar test of bermudagrass using 500 second-stage larvae for a period of 80 days was significant at the 5% level. In all cases chlorosis and wilt symptoms appeared in host plants, and galls were produced on infected roots.—*Department of Entomology, University of Florida, Gainesville, Florida.*

MAYOL, P. S. and G. B. BERGESON. *The role of secondary invaders in premature breakdown of plant roots infected with Meloidogyne incognita.*

The microfloras normally present as secondary invaders of roots infected with a root-knot nematode were studied in regard to their contribution to growth reduction, nutritional classification, and dependence on the nematode for entry into roots. Two experiments were designed so that 'Rutgers' tomato seedlings were grown in sandy loam soil under septic and aseptic conditions with or without surface-sterilized *Meloidogyne incognita*. The experiments were terminated as soon as contamination was detected. In the first experiment (duration 7 weeks), only the foliage of plants grown under septic conditions with *M. incognita* (5000/plant) was significantly reduced (41%). There was no difference in dry root weights among the treatments. In the second experiment (duration 12 weeks), again only plants grown under septic conditions with *M. incognita* (6000/plant) were significantly reduced. Dry foliage weight was reduced by 75% and dry root weight by 48%. The root surface area was about 30% that of control plants. Root-knot nematode infected plants grown under aseptic conditions had massive galls but they remained firm and did not undergo decay or disintegration. Their root surface area was comparable to those of control plants. The number of root-knot females in aseptic plants was about 25% greater than in septic plants. Twenty-four galls from each treatment (septic *vs.* aseptic) were surface sterilized, macerated, and plated. Galls from septic treatments averaged 281 bacterial colonies *vs.* 0.12 colonies per gall from near aseptic treatments. Bacteria were the predominant microflora isolated from necrotic galls, although *Fusarium* spp. and *Rhizoctonia solani* occasionally were found. Bacteria from these and other experiments were classified into 10 nutritional groups according to Lockhead and Chase and as modified by Stevenson and Rouatt. Forty-three per cent of the bacterial isolates belonged to the nutritional group most often associated with root rots. Of 15 bacterial isolates introduced into steam sterilized soil with or without *M. incognita*, two isolates were recovered from host roots when nematodes were present. None was recovered from roots in the absence of the nematode.—*Department of Botany and Plant Pathology, Purdue University, Lafayette, Indiana.*

MCCLURE, MICHAEL A. *Uptake and incorporation of carbon-14 by Aphelenchoides dactylocercus.*

Uptake and incorporation of carbon-14 by *Aphelenchoides dactylocercus* was studied under axenic conditions. Surface sterilized nematodes incubated in solutions containing antibiotics and ^{14}C-labeled sodium acetate or glucose increased in specific radioactivity over a 640 min period. A decline in total activity was noted when the incubation period was extended to 1280 min. After 1280 min incubation in 100 ml distilled water containing 0.1 mc ^{14}C-labeled glucose, 68% of the total activity was incorporated by the nematodes and 7% remained in the incubation solution. The remaining 25%, unaccounted for, was presumably lost as $^{14}CO_2$ and other volatile metabolites. Nematode uptake of ^{14}C-sodium acetate in distilled water was compared with uptake in Fenwick's salt solution. Specific activities of whole nematodes from both treatments differed little after 1280 min incubation. Similarly, total lipids extracted from nematodes incubated in either distilled water or Fenwick's solution contained like amounts of radioactivity, with the greatest portion being

found in the phospholipid fraction. Separation of lipids by thin layer chromatography and radioassay of the resulting fractions indicated the following percentages of radioactivity per fraction: total lipids, 100% (2.1×10^6 dpm); phospholipids plus monoglycerides, 90.9%; free fatty acids, 4.0%; triglycerides, 3.5%; sterol esters, 0.7%; sterols, 0.5%; 1,2-diglycerides, 0.2%; and 1,3-diglycerides, 0.3%. Nematodes incubated with ^{14}C-glucose incorporated significantly more radioisotope than did nematodes incubated in ^{14}C-acetate. This difference could be accounted for in part by the high specific activities of amino acids and sugars following incubation with ^{14}C-labeled glucose. Lipids and amino acids isolated from ^{14}C-acetate labeled nematodes contained nearly equal amounts of radioactivity while the specific activity of sugars was relatively low. These findings, in broad terms, are in general agreement with what is known regarding the origins of lipids, carbohydrates and amino acids in higher animals. The incubation method of introducing radioactive substances into stylet-bearing nematodes should find broad application in the study of nematode nutrition and metabolism.—*Department of Entomology and Economic Zoology, Rutgers University, New Brunswick, New Jersey.*

MINTON, NORMAN A. and CURTIS R. JACKSON. *Effects of lesion nematodes on invasion of peanut pods by certain fungi.*

Peanut plants (*Arachis hypogaea*) were grown in field plots in 1965 and 1966, and in microplots in 1967, to determine if relationships exist among lesion nematodes (*Pratylenchus brachyurus*) and certain fungi in peanut pods and pegs. In 1965 and 1966, experiments were in adjacent plots of Tifton loamy sand having a natural infestation of lesion nematodes. Finely ground peanut shells containing *Aspergillus flavus* and other fungi were incorporated into the soil to increase fungal inocula. Treatments both years were a control and a nematicide, 1,2-dibromo-3-chloropropane (DBCP). DBCP was injected 20 cm deep in the row at a rate of 11 g active ingredient per 10 m of row. The 'Argentine' cultivar of peanuts was grown in 1965 and 1966. In 1967, three peanut cultivars, 'Argentine,' 'Early Runner,' and 'Florigiant,' were grown in methyl bromide treated Tifton loamy sand in microplots (60 cm diameter drainage tiles set on end). Treatments were a suspension of *A. flavus* alone, or a suspension of *A. flavus* and *P. brachyurus*. At harvest, numbers of nematodes in shells and pegs were determined, and shells and kernels were assayed for fungi. In 1965, assays were made at four different developmental stages (92, 107, 126, and 133 days after planting) to determine the progressive stages of invasion by nematodes and fungi. Numbers of nematodes in shells and pegs from control and treated field plots increased between 92 days and 126 days. Numbers of nematodes at 133 days continued to increase in shells and pegs from control plants, but declined considerably in shells and pegs from plants grown in soil treated with DBCP. The greatest increase in both treatments occurred between the 107- and 126-day samples. Fewer nematodes were present on all sampling dates in the shells and pegs from the nematicide-treated plots than from the non-treated. However, numbers of *A. flavus*, *A. niger*, and total fungi recovered from shells or kernels sampled on the four dates were not statistically different at the 5% level among sampling dates or between treatments. In 1966, all plants were harvested and nematode and fungi assays made 120 days after planting. Mean numbers of nematodes in shells from the nematicide treatment again were statistically smaller than from the control while differences in numbers of

fungi were still negligible. In 1967, relatively high numbers of nematodes were recovered from shells of all cultivars from the nematode-inoculated microplots, whereas none was found in the non-inoculated microplots. Differences among cultivars were not significant at the 5% level. Kernels from all cultivars contained fungi at maturity. The numbers of *A. flavus* and *A. niger* from kernels of all cultivars were not significantly different between soil infestation treatments. But, total numbers of all fungi were significantly greater from kernels of plants inoculated with *P. brachyurus* and *A. flavus*. 'Argentine' kernels contained significantly less *A. niger* and total fungi than the other two cultivars.—*Cooperative Investigations, Crops Research Division, Agricultural Research Service, U. S. Department of Agriculture, Beltsville, Maryland; and the University of Georgia College of Agriculture Experiment Stations, Coastal Plain Station, Tifton, Georgia.*

MUSE, BARBARA D. and A. S. WILLIAMS. *A comparison of pectolytic and cellulolytic enzymes in two populations of Ditylenchus dipsaci.*

It has been reported that garden pea (*Pisum sativum* var. Wando) responds differentially to the Raleigh, N. C. (RNC) and Waynesville, N. C. (WNC) populations of *Ditylenchus dipsaci*. RNC causes conspicuous gall formation on pea seedling shoots, whereas WNC produces a necrotic reaction and death of the apical meristem. Pectolytic and cellulolytic enzyme activity were studied *in vitro* with nematodes of the two populations and *in vivo* with RNC-infected, WNC-infected, and control (uninoculated) Wando pea plants. Polygalacturonase, polymethylgalacturonase, and cellulase (C_x) activity were determined by measuring the reduction in viscosity of 1.0% sodium polypectate, 1.0% pectin N.F., and 1.0% carboxymethylcellulose, respectively. Viscometric measurements were made at 30 C with Ostwald-Fenske viscosity pipettes. The dinitrosalicylic acid test was used to assay for reducing groups liberated in reaction mixtures. Nematodes for all studies were obtained from 6 to 8 week old cultures propagated on alfalfa callus tissue. Nematodes used in *in vitro* tests were surface-sterilized according to the Peacock method. Nematode extracts were prepared by grinding frozen nematodes with 0.3 M NaCl (1:12 v/v). Enzyme activity was tested at pH 5.0, 6.0, and 7.0. While both populations formed polymethylgalacturonase and polygalacturonase, RNC nematode extracts were more active in decreasing the viscosity of pectin, whereas WNC extracts were more active towards sodium polypectate. Cellulase activity was similar for both populations. The liberation of reducing groups occurred with a reduction in substrate viscosity. For the *in vivo* studies, plants were harvested 4 days after the inoculation of germinating seeds. Extracts from control and infected tissues were prepared by grinding frozen tissue with 0.5 M NaCl (1:3 w/v). Enzyme activity was tested over a pH range of 4.0 to 9.0. While polygalacturonase and polymethylgalacturonase activity were present in control and infected tissue extracts, the activity was greatest in WNC tissue extracts. Cellulase activity was similar in control and RNC tissue extracts and greatest in WNC tissue extracts. The liberation of reducing groups did not always accompany a reduction in viscosity. Pectolytic and cellulolytic enzymes were found to be present in the uninoculated control plants. The levels of pectolytic enzyme activity differed between the two nematode populations, whereas cellulolytic activity did not. These differences were not reflected in the host-nematode interactions. —*Department of Plant Pathology and Phys-*

iology, Virginia Polytechnic Institute, Blacksburg, Virginia.

MYERS, R. F. *Physical limitations of liquid media used in culturing Aphelenchoides sp.*

Although axenic cultivation of plant-parasitic nematodes is potentially important, little research has been attempted. Techniques are deceptively simple. Nematodes are placed into medium and after a suitable period of time, maintenance, survival, growth, development, length of life cycle, reproduction, or other parameters are determined. Since a partially successful liquid medium was available in which populations of *Aphelenchoides* sp. increased 10 times over inoculation levels, the effects of the physical factors, gaseous exchange, specific gravity, osmotic pressure, and viscosity, were examined. Calculations indicated that the radius of fluid surrounding *Aphelenchoides* sp. should not exceed a maximum of about 1 mm if optimal oxygen concentration was to be maintained. Reproduction was not affected, however, when nematodes were reared in fluids up to at least 9 mm deep. Tentative results indicated that a medium should not exceed a viscosity of 3.5 centipoise, 12 atmospheres osmotic pressure, and a specific gravity of 1.045. Experience gained during culturing suggested that populations be limited to 10,000 nematodes/ml at harvest. Since the life cycle appeared to be about 25 days in medium, but less than 1 week when *Aphelenchoides* sp. was reared on the fungus *Pyrenochaeta terrestris,* the liquid medium may have been too dilute. However, low solubilities of certain constituents preclude further concentration of liquid medium.—*Supported by USDA Grant 12-14-100-9124(34).* *Department of Entomology and Economic Zoology, Rutgers University, New Brunswick, New Jersey.*

NICKLE, W. R. *Observations on Hexatylus viviparus and Neotylenchus abulbosus.*

The type species of the family Neotylenchidae, *Neotylenchus abulbosus* Steiner, 1931, has not been collected or studied since its discovery in association with diseased strawberry plants in California. Studies of syntype specimens of *N. abulbosus* and *Hexatylus viviparus* T. Goodey, 1926, revealed that they were conspecific. *N. abulbosus,* the type species of the genus *Neotylenchus,* then becomes a junior synonym of the type species of the older genus *Hexatylus. Neotylenchus* becomes unavailable. The taxa Neotylenchidae and Neotylenchinae retain their status with the type species now being *Hexatylus viviparus,* which carries *N. abulbosus* as a synonym. The esophagus of *Hexatylus viviparus* was found to have dorsally overlapping esophageal glands instead of a fusion of the esophagus with the intestine as reported in the original description. Steiner's specimens of *N. abulbosus* also had dorsally overlapping esophageal glands and did not have the definitely set-off posterior bulb, that was expected. The stylet knobs of the original *N. abulbosus* specimens were not outwardly projecting curved processes and, in fact, were similar to *H. viviparus* and slightly bifid. Also, it is significant that the stylet in the Steiner material, was surrounded by strengthening rings exactly like those characteristic of *H. viviparus.* There was a prominent junction of the esophageal and intestinal lumena, just anterior to the nerve ring in both *N. abulbosus* and *H. viviparus.* The author considers that members of the genus *Hexatylus* should be limited to those nematodes having the strengthening rings surrounding the stylet, bifid stylet knobs, the prominent junction of the esophageal and intestinal lumena, and overlapping glands. *H. viviparus, H. mulveyi,* and *H. vigissi* are con-

sidered valid species of the genus, *Hexatylus*. —*Crops Research Division, Agricultural Research Service, U. S. Department of Agriculture, Beltsville, Maryland.*

O'BANNON, J. H. and A. T. TOMERLIN. *Movement of Radopholus similis on a weed host (Solanum nigrum).*

Field observations have indicated that the rate of movement of the burrowing nematode (*Radopholus similis*) in citrus groves is approximately 15 m per year. In Florida, buffer zones have been established between non-infested and infested areas to check the movement of the nematodes. The soil in buffer zones is treated with nematicides and herbicides to keep the buffers free of nematodes and weeds. Occasionally weed hosts grow in the buffer zone. This apparently offers a means of spreading the burrowing nematode. We conducted this experiment to study the movement of *R. similis* from an infected citrus source to a weed host (*Solanum nigrum*) and subsequently to non-infected citrus. In replicated tests, 1-year-old rough lemon (*Citrus limon*) seedlings, previously infected with *R. similis*, were transplanted at one end of troughs filled with Eustis sand. A clean rough lemon seedling, free of infection, was planted at the opposite end of each trough. The intervening space of approximately 230 cm served as the buffer zone and was seeded to black nightshade (*S. nigrum*). The nightshade plants were grown for 11 months, the duration of the study. Soil and root samples were collected at monthly intervals at depths of 0–15 cm and 15–30 cm to define the advancing front of the nematode. *R. similis* migrated readily on *S. nigrum*. A continuous spread of the nematode on the weed host was noted. The mean temperature during this study was 20.5 C, with a minimum of 13.5 and a maximum of 25.5 C. These conditions were favorable for infection and reproduction of *R. similis* on *S. nigrum*. Periodic sampling for *R. similis* showed that successive generations of this nematode had migrated a distance of approximately 216 cm in 44 weeks, an average of 21.2 cm per month. Numbers of nematodes extracted from either soil or root samples at the margin were nearly equal. There was no significant difference in rate of movement through *S. nigrum* at either depth sampled. This study has shown that a host other than citrus can serve adequately for the migration of *R. similis* through successive generations.—*Crops Research Division, Agricultural Research Service, U. S. Department of Agriculture, Orlando, Florida.*

OLTHOF, TH. H. A. and A. A. REYES. *Effect of Pratylenchus penetrans on Verticillium wilt of pepper.*

Greenhouse studies were made of the effects of the root-lesion nematode, *Pratylenchus penetrans*, alone (N); the fungus *Verticillium dahliae* alone (F); and a combination of these two organisms (N + F) on pepper (*Capsicum frutescens*) var. 'Vinedale.' The nematode was introduced at 15,000 specimens per 10-cm clay pot, containing 600 g of autoclaved Fox sandy loam. The fungus was added 3 weeks later at the rate of ⅛ of a culture that had completely covered a PDA medium in a 60 × 15 mm petri dish. After 14 weeks of growth, N, F, and N + F had significantly reduced the heights of the plants by 14.7, 17.9, and 49.6%; the top weights by 44.5, 40.1, and 87.6% and root weights by 42.9, 32.7, and 73.5%, respectively. The number of *P. penetrans* recovered from the soil and roots of treatments N and N + F were not significantly different; they were 1580 and 1380/500 g of soil and 144 and 133/root system. respectively. These data indicate that the damage caused by *P. penetrans* and *V. dah-*

liae on pepper are additive.—*Research Station, Canada Department of Agriculture, Vineland Station, Ontario, Canada.*

OSBORNE, W. W., C. HARRIS, L. M. HARRISON, W. F. BROWN, R. L. SHAW, and H. S. ADAMS. *The efficacy of certain chemical soil treatments for the control of Meloidogyne incognita acrita in tobacco.*

Nematicide trials were conducted on a sandy loam soil which was naturally infested with the cotton root-knot nematode, *Meloidogyne incognita acrita.* The tobacco variety, McNair 20, was transplanted on May 19, two weeks after chemicals were applied. Methods of chemical application were: method A, 60 cm band, incorporated 20 cm deep; method B, 30 cm band, incorporated 20 cm deep; and method C, 60 cm band incorporated 5 cm deep. Chemicals, rates of application of 10% granular materials, and methods of their application were: Mocap® (O-ethyl-S,S-dipropyl phosphorodithioate) applied at the rate of 56 Kg per ha using methods A, B, and C; Mocap 45 Kg per ha using method A; Furadan® (2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbonate) applied at the rate of 56 Kg per ha using methods A and B; Dasanit® (0,0-diethyl 0-(P-(methyl sulfinyl phenyl) phosphoroithioate) applied at the rate of 56 Kg per ha using method A. When compared with the untreated check, the following increase in dollars per hectare was obtained with the designated treatment: Mocap at 56 Kg per ha using method A, $1,994; Mocap at 45 Kg using method A, $1,749; Mocap at 56 Kg per ha using method B, $1,685; Mocap at 56 Kg per ha using method C, $1,512; Furadan at 56 Kg per ha using method B, $1,141; Dasanit at 56 Kg per ha using method A, $1,087. A closely correlated inverse relationship existed between value per hectare and root-knot indices in the various treatments.—*Virginia Polytechnic Institute, Blacksburg, Virginia.*

PAULSON, R. E. and J. M. WEBSTER. *Ultrastructural response of plant cells to gall-forming nematodes.*

Studies were made of the changes in ultrastructure of the root tissue of susceptible varieties of tomato plants exposed to an infection of *Meloidogyne incognita.* Sequential sampling of host tissue over a period of several weeks demonstrated the formation and development of giant cells and associated host cell response. Giant cell development was characterized by changes in the cell walls, cytoplasm, and organelles. The first indications of giant cell development in tomato, were observed 2 days after inoculation with larvae, and consisted of increased electron density of cytoplasm and decreased size of the large central vacuole. After about ten days, some regions of the giant cell walls became excessively thickened, while other parts remained relatively unchanged. In old giant cells, projections of wall material extended far into the cytoplasm. Microtubules occurred adjacent to the walls in all stages of giant cell development. As the giant cell developed, the nuclei became swollen and irregularly shaped, nucleoli became enlarged, and clumps of chromatin accumulated near the distinct nuclear membrane. Plastids did not change significantly, whereas mitochondria appeared to form vesicles during the later stages of giant cell development. Increased numbers of dictyosomes appeared to produce smooth and coated vesicles. During the later stages of giant cell development smooth and rough endoplasmic reticulum was extensively produced in the form of vesicles and cisternae. In 4–5 week-old galls cells adjacent to the giant cells often divided rapidly to produce a mass of small thick-walled cells resembling xylem vessels. Cells

further removed from the giant cells became swollen but did not appear to be otherwise modified. Neither galls nor giant cells have been observed in resistant varieties of tomato infected with *Meloidogyne* larvae.—*Simon Fraser University, Burnaby, British Columbia, Canada.*

PHILLIPS, D. V. and K. R. BARKER. *Responses in growth and yield of soybeans to several population levels and combinations of certain nematodes.*

Growth and yield of 'Lee' soybeans were compared in small plots treated with nematicides and in non-treated plots naturally infested with different nematode species combinations at various population levels. The nematicides used were Nemagon® (1,2-dibromo-3-chloropropane), Furadan® (2,3-dihydro-2,2-dimethyl benzo furenyl-7 N-methyl carbonate), and Temik® (2-methyl-2-(methylthio) proionaldehyde-O-(methyl carbamoyl) oxine). Growth and yield were significantly less in non-treated plots than in treated plots at four of five locations. At three locations on sandy loam soils control varied, depending on the composition of the nematode populations and on the type of nematicide. In test I, non-treated plots yielded 15% less than the highest yielding treatment (Temik) where the initial populations of *Pratylenchus brachyurus, P. zeae, Tylenchorhynchus claytoni* were low and where *Meloidogyne incognita* was moderate. In test II, non-treated plots yielded 44% less than the highest yielding treatment (Furadan) where the initial populations of *M. incognita, P. brachyurus, P. zeae, Belonolaimus longicaudatus* were low and *Helicotylenchus dihystera* and *Trichodorus christiei* were moderate. In test III, non-treated plots yielded 78% less than the highest yielding treatment (Nemagon) where the initial population of *T. christiei* was low, *H. dihystera* was moderate, and *Heterodera glycines* was high. The average yield of non-treated plots at these three locations was 32%, 36%, and 37% less than the average yield of plots treated with Nemagon, Furadan, and Temik, respectively. At two locations on loamy fine sand, results were as follows: in test IV, non-treated plots yielded 3% less than the highest yielding treatment (Nemagon) where the initial populations of *T. claytoni, H. dihystera, T. christiei* were low and *H. glycines* was moderate. In test V, non-treated plots yielded 16% less than the highest yielding treatment (Nemagon) where the initial populations of *P. brachyurus, P. zeae, T. claytoni, T. christiei* were low, and *H. dihystera* and *B. longicaudatus* were high. The average yield of non-treated plots at these two locations was equal to the average yield of plots treated with Furadan and Temik, but 10% less than the average yield of plots treated with Nemagon. In addition to *M. incognita* and *H. glycines,* an analysis of yield and nematode population data indicates that *Pratylenchus* spp., *B. longicaudatus,* and *T. claytoni* are contributing to substantial yield reductions in soybeans in North Carolina.—*North Carolina State University, Raleigh, North Carolina.*

RADEWALD, J. D., A. O. PAULUS, F. SHIBUYA, J. W. OSGOOD, and K. MAYBERRY. *Responses to preplant soil fumigation for the control of a Longidorus sp. in head lettuce in southern California.*

A species of *Longidorus* was found in soil around the roots of stunted lettuce seedlings in the Imperial Valley of California in the fall of 1967. Seedlings also appeared wilted and some were chlorotic. The tips of the tap roots were swollen as much as two times normal diameter, and some necrosis of tissue was present. When tap root tips were swollen, elongation ceased, and excessive

lateral root production occurred. Many lateral roots showed similar symptoms. The same *Longidorus* species was also found in an adjoining fallow field and a preplant soil fumigation trial was established. One,3-dichloropropene was applied at the rate of 49.2 liters per acre. Plots were 218 m in length and four beds wide, replicated four times. Ten days after lettuce emergence only a few (.02%) of the plants from the fumigated plots exhibited the root symptoms previously described. Seventy-one per cent of the plants in the nonfumigated plots were injured. The average number of nematodes per 500 ml of soil two weeks after emergence in the fumigated and nonfumigated plots was 11 and 220, respectively. Lettuce in the fumigated plots matured three weeks prior to that in the nonfumigated plots. Average head weight at harvest was 90 g greater in the fumigated plots. A more uniform maturity was also evident in the fumigated plots. At harvest, 90% of the tap roots in the fumigated plots were normal. Eighty percent of the tap roots of the plants in the nonfumigated checks were forked. Preliminary pathogenicity trials under controlled greenhouse conditions with this *Longidorus* sp. have produced symptoms on lettuce seedlings similar to those on seedlings obtained from the field.—*Agricultural Extension Service, University of California, Riverside, California.*

RIEDEL, R. M. *The influence of onion seedling age on the development of symptoms caused by Ditylenchus dipsaci.*

Seeds of onion (*Allium cepa*) var. 'Aristocrat' were placed on moist filter paper at 24 C in petri dishes. At 24-hr intervals for 4 days, 15 seeds or seedlings were transplanted from the dishes to muck soil in each of five 7.5-cm clay pots and watered with 5 ml of tap water containing 1700 *Ditylenchus dipsaci* from monoxenic culture. A sixth pot of seedlings served as an uninoculated control. The pots were placed immediately in a growth chamber programmed to provide conditions of 21 C day and 15.5 C night temperatures; a 14-hr photoperiod; 2000 ft-c light intensity at bench height; and 78% day and 68% night relative humidity levels. Seedling susceptibility to the nematode was assessed by determining numbers of seedlings emerged and numbers of emerged seedlings with bloat symptoms 30 days after they had been transplanted. For the seedlings transplanted at 24 or 48 hr, emergence was 20 to 25% and all emerged seedlings were bloated. Emergence of seedlings transplanted at 0, 72, or 96 hr was 55 to 65%; of the emerged seedlings 80 to 100% were bloated. Emergence of seedlings in the check pots was 70 to 80%. No bloating of seedlings in check pots was observed. The basis for the fluctuations in susceptibility is not known. At the time seedlings were planted in soil, comparable seedlings were also placed in FAA and then subjected TBA dehydration and paraffin embedding. In sections cut 12 μ thick, lignin was localized with Azure B and cutin in pyridine-extracted sections with Sudan IV. Lignin was found only in the proximal end of the piliferous zone beginning at 58 hr. Except for the root tip, cutin was present on all parts of the seedlings from the beginning of the test. There were no changes in mechanical properties of the host's cell wall that could be attributed to lignification or cutinization. Therefore, these processes could not be correlated with differences in susceptibility. In another test, 1700 *D. dipsaci* in 5 ml of water were added to each of 35, 7.5-cm pots of moist, fallow muck soil and maintained in the same growth chamber. Immediately after infestation of the pots and thereafter at 24-hr intervals for 6 days, the nematodes were extracted from 5 pots by the

pie-pan modification of the Baermann funnel technique. Numbers of nematodes extracted at each sampling time did not differ significantly. This suggests that the observed fluctuation in severity of symptoms developed by seedlings inoculated with *D. dipsaci* was not the result of short-term changes in nematode numbers.—*Plant Pathology Department, Cornell University, Ithaca, New York.*

ROMAN, J. and HEDWIG HIRSCHMANN. *Development, morphology, and cytology of certain species of Pratylenchus.*

The following species were cultured monoxenically on alfalfa callus or on suitable host plants in the greenhouse: *Pratylenchus penetrans, P. coffeae, P. vulnus, P. pratensis, P. brachyurus, P. zeae, P. scribneri, P. neglectus,* and *P. crenatus.* The cleavage pattern was followed accurately to the eight-cell stage. Eggs were usually laid unsegmented. The first and second cleavages were perpendicular to the longitudinal axis of the egg and resulted in four blastomeres arranged at first in tandem but shifted positions later. The third cleavage, which started in the anterior blastomere and ended in the posterior, brought the egg to the eight-cell stage. Subsequent divisions proceeded rapidly and could not be followed precisely. The first molt took place within the egg and the second-stage larva hatched by piercing the egg shell with the stylet. Prior to the second, third, and fourth molts active motion of the larvae ceased. The esophagus became faint, stylet shaft and knobs disappeared, and the cuticle separated from the anterior end carrying with it the conical part of the old stylet, cephalic framework and linings of amphidial ducts. The new conical section of the stylet, shaft and knobs, and cephalic framework were formed shortly afterwards. The esophagus became visible again and the new cuticle separated completely from the old cuticle. Two distinct types of gonad development were encountered during postembryogenesis: The monodelphic type observed only in *P. scribneri* and the amphidelphic type observed in all other species. In the amphidelphic type two gonads developed originally, but the posterior gonad degenerated during the fourth molt. Some females of *P. zeae* retained the posterior gonad even in the adult stage. It was concluded that all species studied, except *P. scribneri,* are potentially amphidelphic. Female larvae in *Pratylenchus* could be distinguished from male larvae early in the second molt by the presence of four specialized ventral chord nuclei opposite the genital primordium. Morphological studies revealed the existence of a high degree of variability with regard to most taxonomic characters such as number of annules in the lip region, number and arrangement of incisures in the lateral field, shape of stylet knobs and tail terminus. Vulva percent and stylet length were the least variable among 20 morphometric characters analyzed statistically. Bisexual as well as monosexual species possessed a spermatotheca. The distal part of the uterus was composed of 12 cells arranged in three rows of four cells each. *P. penetrans, P. vulnus,* and *P. coffeae* had a chromosome complement of $n = 5$, 6, and 7 respectively, and reproduced by cross-fertilization. In *P. brachyurus, P. zeae,* and *P. scribneri* $2n = 21$ to 32 and, reproduction was by mitotic parthenogenesis. One population of *P. scribneri,* however, had a chromosome complement of $n = 6$ and reproduced by meiotic parthenogenesis.—*Department of Plant Pathology, North Carolina State University, Raleigh, North Carolina.*

ROWSE, THURMAN W. *Embryology of Aphelenchoides dactylocercus.*

As part of a study of the biology of *Aphelenchoides dactylocercus,* an investigation of this nematodes' embryology was carried out. The hanging drop technique was utilized to observe time between cell divisions and other aspects of development. Eggs to be photographed were mounted under a cover glass supported by glass rods on a glass microscope slide. Petroleum jelly was used as a seal to prevent drying. The elapsed time from egg laying to hatch averaged 69.5 hr. When deposited, the eggs were rounded at the ends with a smooth unmarked surface. The first cell division was equatorial and perpendicular to the long axis of the egg. The second division was similarly transverse to the long axis and resulted in two cells of equal size and a larger third cell. A similar division of the smaller terminal cell gave a four-cell stage. One of the daughter cells of this division moved and rotated so that it appeared to lie over the two terminal cells. The fourth cell division occurred in the large cell formed in the initial mitotic division and was followed by immediate division of the small terminal cell at the opposite end of the egg. These divisions resulted in a six-cell stage. The end of the egg in which mitotic activity had been concentrated up to this point became the anterior portion of the nematode. Many cell divisions occurred in quick succession in this area following the formation of the six-cell stage. Simultaneous divisions then took place throughout the cell mass until approximately 36 hr after egg laying. During the next 12 hr, cell division appeared to be most active at the periphery of the cell mass. A nematode shape was evident approximately 48 hr after egg laying and movement was seen 4–6 hr later. Prior to hatching, a stylet was formed and was used to assist in rupture of the egg shell. Movement of the nematode caused the egg shell to become distended and assisted in its rupture. The nematode emerged from the shell immediately after its rupture.—*Department of Entomology and Economic Zoology, Rutgers University, New Brunswick, New Jersey.*

SAYRE, R. M. *A method for rearing tardigrades.*

Since January 1967, the tardigrade species, *Hypsibius myrops,* has been maintained in laboratory culture with *Panagrellus redivivus* as its prey. Autoclaved *Sphagnum* was a suitable inert support material on which tardigrades could move and find physical support to feed on nematodes. The bottom of a 60 × 100 mm culture dish was covered to a depth of 2 cm with moistened *Sphagnum.* One hundred tardigrades were added to each culture dish. The prey, *P. redivivus,* was previously cultured on pre-cooked oatmeal, a few grains of dried viable yeast and water. The nematodes were extracted on a Baermann funnel and rinsed twice. About every 3 days, several thousand larvae and adults of *P. redivivus* were added to each culture dish and feeding and development of the tardigrades was observed. In feeding, the tardigrade did not need to grasp the nematode with any of its legs. Using only mouthparts, tardigrades were capable of holding nematodes, puncturing the cuticle with their oral stylets, and then feeding on the body contents. This species of tardigrades molts four times and may lay from 1 to 18 eggs during its life cycle. From the original inoculum, as many as 5900 tardigrades were recovered from a culture dish 3 months later. Tardigrades and moss were removed from culture dishes every week, put on a 9 cm filter disc in a Büchner funnel, and rinsed with approximately 500 ml of

deionized water. The filter that retained the tardigrades and the moss was backwashed into a clean culture dish. By this procedure staling products that were injurious to the tardigrade were removed. Other species of tardigrades might be cultured by this method, which provides readily available populations of predatory tardigrades that can be evaluated for their possible use as biological control agents of plant nematodes.—*Crops Research Division, Agricultural Research Service, U. S. Department of Agriculture, Beltsville, Maryland.*

SOUTHARDS, C. J. *The effect of temperature and storage media on survival of Meloidogyne incognita and Pratylenchus brachyurus.*

Second-stage larvae of *Meloidogyne incognita* and mixed stages of *Pratylenchus brachyurus* were placed in plastic petri dishes containing either tap water, sterile tap water, sandy loam, or sterile sandy loam. The dishes were stored in refrigerator-incubators maintained at temperatures of 4, 10, 15, 20, and 25 C. At the end of storage periods of 5, 10, 15, 20, 30, 60, and 120 days, nematode survival, based on infectivity, was determined for each medium and temperature by bioassay and staining *in situ* as follows: Three-week-old 'Rutgers' tomato seedlings were transplanted into the petri dishes used for storage, or into 6 cm plastic pots to which the contents of the storage dishes were transferred. The plants were placed under artificial light in the laboratory at room temperature for 10 days. Roots of the plants were washed, placed in vials, and then fixed for 4 or more hr in 10 ml of a 1 : 1 solution of glacial acetic acid and 95% ethanol to which 3 drops of 1% acid fuchsin-lactophenol had been added. The roots were then cleared in a saturated solution of chloral hydrate for 6 to 12 hr, drained, glycerine was added, and they were mounted on slides. In general, *P. brachyurus* survived longer periods of storage than *M. incognita*; however, infectivity of both species decreased rapidly after 12 to 15 days of storage. Survival and subsequent infectivity was not affected significantly by surface sterilization of the nematodes or by sterilization of the media. For periods of storage up to 30 days, there was no difference in survival of nematodes in soil versus those stored in water, except at 4 C storage temperature. Less than 2% of *M. incognita* larvae were infective following storage in water at 4 C, but 10% of the larvae were infective when stored in soil at 4 C. Infectivity of *M. incognita* was greatest following storage at temperatures of 10 and 15 C, with an average of 55 to 60% of the larvae remaining infective. Infectivity of *P. brachyurus* averaged 55% after 10 days storage at 4 C, and decreased inversely with temperature to 36% after storage at 25 C. Infectivity after 30 days storage was 30 to 50% less than at the beginning of storage at all temperatures. Less than 2% of *M. incognita* larvae were infective after 60 days storage; however, approximately 13% of *P. brachyurus* individuals remained infective after 60 days.—*The University of Tennessee Agricultural Experiment Station, Knoxville, Tennessee.*

SPECHT, C. H. and J. T. WALKER. *Survival of Pratylenchus penetrans under increased carbon dioxide within soil cylinders.*

To ascertain the influence of anaerobic conditions and high CO_2 concentrations on survival of *Pratylenchus penetrans* in soil, a chamber was constructed whereby nematodes were exposed to gaseous atmospheres for extended time. Nematodes (6000–10,000) were added to sterilized and screened soil. A 100 cc soil sample then was placed in each of eight 3 × 29 cm

Plexiglas® cylinders. Nalgene tubes (9 mm in diam), perforated with 1.25 mm diam openings, passed through the center of the cylinders. Carbon dioxide, or compressed air (control), was bubbled through water and introduced into the perforated tubes via a manifold. A 22 gauge hypodermic needle was inserted between the manifold and each perforated tube. The flow rate was adjusted to approximately one atmospheric exchange in the cylinders every 2 min. After exposure, three 25 cc soil samples were removed from each cylinder and assayed for nematodes. The number of nematodes recovered from the CO_2 cylinders were 70 and 83% less than the controls after 1 and 2 weeks, respectively. Our results indicate that the lesion nematode is capable of surviving extremely high concentrations of CO_2 for at least 2 weeks. This device, or modifications thereof, seems particularly suited for studying the effect of other gases on nematode populations in soil.—*Kitchawan Research Laboratory of the Brooklyn Botanic Garden, Ossiming, New York.*

THISTLETHWAYTE, B. *Hatch of eggs of Pratylenchus penetrans in various salt solutions.*

About 1200 eggs of *Pratylenchus penetrans*, plus a small amount of plant material derived from the alfalfa callus tissue on which the nematodes had been cultured monoxenically, were pipetted from a stirred suspension in deionized water onto nylon screens of diameter 2.5 cm and mesh 15 μ. Excess water was drained, and each screen was placed in 9.0 ml test solution in a plastic dish. Screens were supported about 3 mm below the surface of the solution. Dishes were kept in the dark at 25 C. At 2-day intervals, each screen was raised, allowed to drain, then transferred to fresh solution in a second dish. Juveniles migrating through the screen during the preceding 2 days were counted. Test solutions were 0.05, 0.10, 0.15, 0.20, 0.30, and 0.60 M NaCl; 0.05, 0.15, 0.30, and 0.60 M KCl, KNO_3, or $NaNO_3$; and deionized water. Any water loss by evaporation was replenished every 6 to 10 hr. Selected dishes were weighed before and after addition of deionized water; maximum variation in concentrations of test solutions was between +10% and –5% of those specified. For changes made every 2 days between the 12th and 24th days, deionized water was used in place of the salt solutions. There were 4 replicates of each treatment. After 12 days, a mean total of 321.5 juveniles had hatched in deionized water; 334.5, 283.0, 207.2, 113.5, 30.5, and 13.5 had hatched in 0.05, 0.10, 0.15, 0.20, 0.30, and 0.60 M NaCl, respectively. Differences greater than 35.0 and 46.0 were significant at $p = 0.05$ and $p = 0.01$, respectively, according to Duncan's Multiple Range test. Hatch in solutions of the other 3 salts after 12 days showed a similar trend. Mean total hatch for eggs transferred to deionized water for 12 days after being kept in 0.05, 0.10, 0.15, 0.20, 0.30, and 0.60 M NaCl for 12 days was 348.0, 308.5, 270.2, 222.5, 207.8, and 122.5, respectively. Mean total hatch for eggs kept in deionized water for 24 days was 330.2. Differences greater than 42.5 and 53.3 were significant at $p = 0.05$ and $p = 0.01$, respectively. A similar resumption of hatching also occurred after transfer of eggs from solutions of the other 3 salts to deionized water. The data indicated a high proportion of eggs of *P. penetrans* were able to survive high osmotic potentials (e.g., about 25 atm for the 0.60 M solutions) for 12 days, but provided no information on whether the effects of osmotic potential were on embryonic development or hatching.—*Department of Plant Pathology, Cornell University, Ithaca, New York.*

TJEPKEMA, J. P. *Parasitism of aporcelaimid nematodes by mermithid nematodes.*

Mermithid parasites occurred in 36% of 36 female and immature *Aporcelaimellus obscurus* found in 470 cc of soil collected June 1967, in a wooded area near the Purdue University campus. Six mermithids were found free from their hosts in the same soil. Six *A. obscurus* contained one parasite each, three contained two parasites each, one contained four parasites, and three contained an undetermined number of parasites. A single female nematode, very similar to *Aporcelaimus pachydermus,* collected August 1967, from a cornfield near Hartsburg, Illinois, contained mermithid parasites similar to the parasites found in *A. obscurus.* There were at least two and probably three parasites in the female *Aporcelaimus.* The parasites moved about inside their hosts, which were also active. The parasites of *A. obscurus* were usually reflexed once within their host. In the *Aporcelaimus* female the parasites were reflexed three or more times and were also twisted around themselves. The parasites occupied most of the body cavity of their hosts. In *A. obscurus* all of the internal organs were intact including the reproductive system, but the intestine and the ovaries and sometimes the esophagus were flattened and distorted. The internal organs of the *Aporcelaimus* female were similarly affected by its parasites except that the ovaries were not discernible. However, the spermathecae were visible and contained sperm. All of the parasites within the hosts appeared to be encased within an unshed, loose cuticle. Most of the parasites which were studied within the hosts, and all of those which were dissected out of their hosts, contained reproductive systems without eggs. Apparently the parasites are fully developed except for completing the last molt before leaving the host. One of the mermithids found in the soil appeared to be shedding its last larval cuticle; the others were all mature and contained at least a few eggs. Eggs within gravid parasites were too large to be swallowed by their host; therefore the hosts were probably infected by larval parasites which penetrated their body walls or entered through natural openings.—*Department of Entomology, Purdue University, Lafayette, Indiana.*

TOMERLIN, JR., ARTHUR H. and GROVER C. SMART, JR. *The influence of organic soil amendments on nematodes and other soil organisms.*

Previous investigators have shown that when certain organic materials were incorporated into the soil, numbers of plant parasitic nematodes decreased while non-plant parasitic nematodes and certain other microorganisms increased. An Arredondo fine sand infested with plant parasitic and non-plant parasitic nematodes was used to study this phenomenon quantitatively. Alfalfa meal, cottonseed meal, and rice straw at rates equivalent to 2.2, 4.5, 9.0, and 17.9 metric tons per ha (1, 2, 4, and 8 short tons per acre) were mixed with 20 kg of the soil and placed in redwood boxes. Each treatment was replicated five times and planted to *Phaseolus vulgaris* 'Contender.' Not all rates or all materials were used in any one of the three completely randomized experiments. The following data were recorded weekly: populations of plant parasitic and non-plant parasitic nematodes; relative numbers of fungi and bacteria; soil pH; nitrates; ammonium acetate (pH 4.8) extractable Ca, K, Mg, and P; bean seed germination; plant height and green weight of bean pods. Laboratory experiments were conducted with the amended soil to obtain data on CO_2-C evolution, nitrate accumula-

tion, numbers of nematodes, and relative numbers of bacteria and fungi. All data were statistically analyzed using an analysis of variance with an "F" test. Populations of plant parasitic nematodes, primarily *Belonolaimus longicaudatus*, were effectively controlled by the 9.0 and 17.9 metric tons per ha amendment rates for at least sufficient time for plant establishment. Certain free-living nematodes, primarily rhabditids, initially increased in the amended soils. Possible predaceous nematodes (diplogasters, dorylaims, and aphelenchoids) fluctuated greatly and were few in number in all treatments. The relative numbers of bacteria and fungi (zymogenous organisms) increased greatly in the initial phases of the experiments and then decreased. Predaceous or parasitic fungi were not observed affecting nematodes in these experiments. Carbon dioxide production and nitrate accumulation in the amended soils showed only a relationship to the C:N ratio and biodegradibility of the added organic materials. Soil pH and amounts of Ca, K, Mg, and P in the soil were not significantly different among treatments. These data indicate that the plant parasitic nematodes were not controlled by predaceous nematodes, fungi, or the other factors measured. Control was due to other factor(s), one of which might have been the production or liberation of one or more chemicals by microbial degradation of the organic amendments.—*Department of Entomology and Nematology, University of Florida, Gainesville, Florida.*

VIGLIERCHIO, D. R., A. R. MAGGENTI, and R. N. JOHNSON. *Axenic ovarial explants from the marine nematode Deontostoma californicum on culture media.*

The gonads of *Deontostoma californicum* were isolated from the organismal environment by dissection. In an attempt to approximate the *in vivo* condition and to maintain *in vitro* architecture and function ten media recommended for the culture of insect cells or organs were selected. The media were prepared in two series: one was prepared according to published recommendations; the second series substituted filtered seawater for inorganic salts and distilled water. Media were prepared as both liquid and gel (agar) with and without antibiotics. Whole animals and eggs were placed on the same culture media series as the ovarial explants. Ovarial explant reactions to media were assigned to three categories: those supporting the entire gonad, the ovary, or ova-containing oviduct. Seawater preparations of Samia and Grace culture media were outstanding for support of the entire explant. Eggs underwent their greatest development in seawater without antibiotics. In relation to whole animals prolonged survival took place only in those media (A-1, C-G, 26c, 199, Mosquito, and Media B) in which the inorganic salts and distilled water were replaced by filtered seawater. The results demonstrated that those conditions suitable for adults on culture media are not necessarily suitable for eggs, larvae, or tissue explants.—*Department of Nematology, University of California, Davis, California.*