ABSTRACTS OF PAPERS PRESENTED AT THE EIGHTH ANNUAL MEETING OF THE SOCIETY OF NEMATOLOGISTS, SAN FRANCISCO, CALIFORNIA, AUGUST 10–13, 1969

BIRD, G. W. *Somatic musculature of Trichodorus porosus.*

Specimens of *Trichodorus porosus* were fixed in a mixture of 3% glutaraldehyde and 3% acrolein in cacodylate buffer (pH 7), post-fixed in 1% osmium tetroxide, dehydrated in a graduated ethanol series, and embedded in a mixture of Araldite 6005. Silver to gold sections were cut on a Porter Blum microtome, placed on coated grids, and stained with uranyl acetate and lead citrate. They were examined with a Zeiss 9-A electron microscope. The arrangement of the somatic musculature of *T. porosus* was meromyarian. It consisted of elongated cells, with myofibrils, mitochondria, sarcoplasmic reticulum, and nuclei. The muscle cells were platymyarian, since the myofibrils were adjacent to the hypodermis, while the sarcoplasmic portion of the cell extended into the pseudocoel. The myofibrils were composed of thick myofilaments (myosin filaments) and thin myofilaments (actin filaments) embedded in sarcoplasm. The myofibrils were separated by the arrangement of the myofilaments, and by thin sheets of sarcoplasm. In general, the thick:thin myofilament ratio was 1:12. The thick myofilaments were 200 Å in diameter, 300–400 Å apart, and arranged in a triangular pattern. The thin myofilaments were 50 Å in diameter and were arranged in a circular group of twelve, surrounding a thick myofilament. In transverse sections of the posterior portion of *T. porosus,* the myofilaments in the dorsal half of the nematode were oriented longitudinally, while myofilaments in the ventral part of the body were oriented in a transverse direction. Muscles also extended from just inside the dorsolateral part of the hypodermis to the dorsolateral part of the intestine, dividing the body into three sectors. The myofilaments of these muscles were oriented transversely to the nematode body axis.—*Department of Plant Pathology and Plant Genetics, University of Georgia, Athens, Georgia 30601.*

BUECHER, E. J., T. GOTTFRIED and E. L. HANSEN. *Partial characterization of yeast extract for culture of C. briggsae.*

Axenic cultures of the free-living nematode *Caenorhabditis briggsae* can be maintained in a chemically defined medium supplemented with proteinaceous materials. Extracts of chick embryo and liver have often been used. We now report that extracts of yeast support reproduction of *C. briggsae*, other free-living nematodes, and two species of *Neoaplectana.* Cultures yield high populations and adults of large size. Biologically active material was prepared in large quantities from baker's yeast which had been broken either in a colloid mill or a Waring blender in the presence of Superbrite glass beads. The preparation was centrifuged and the supernatant was sterilized by membrane filtration. Biological activity was enhanced by dialysis. After dialysis for 3 hr against 1 mM potassium phosphate buffer, pH 7, the extract was used as a complete medium or, after prolonged dialysis against 50 mM buffer, as a supplement. Ammonium sulfate precipitation was successfully applied, the active material being precipitated with 70% ammonium sulfate. It was stable to lyophilization. Fractiona-

tion was also carried out by centrifugation for 20 or 30 min. at 30,000 × *g* or 48,000 × *g*. The pellets contained protein and denatured ribosomes. They were redispersed in 50 mM buffer before sterile filtration and assay. The 48,000 × *g* pellet was more active than any supplement previously reported, and, at 250 μg/ml, supported maturation in 3.2 days, with activity in bioassay extending to 20 μg/ml. The supernatant of the 30,000 × *g* centrifugation was treated with protamine sulfate to remove nucleic acid. It then had a pronounced protein peak (280/260 = 1.8). It was similar to liver growth factor in being activated by specific treatments, showing the same relationship between presence of particles and biological activity.—*Supported by Grant A1–07359. Clinical Pharmacology Research Institute, 2030 Haste St., Berkeley, California 94704.*

CAVENESS, CLARENCE E. and FIELDS E. CAVENESS. *Hatching responses of Meloidogyne incognita acrita to electric shock.*

The influence of electric shock on the hatching of *Meloidogyne incognita acrita* egg masses from roots of Acala SJ-1 cotton (*Gossypium hirsutum*) were studied. Egg masses were individually placed in about 0.009 ml of tap water on a glass ruled with one millimeter marks, except for 60 VDC/mm where sufficient water was used so heating did not become a factor. Two electrodes were brought into contact with the water with a distance of 1 mm between them. Egg masses were exposed to electric potentials of 1, 10, 20, and 60 VDC/mm at 1, 1, 1, and 86 ma DC, respectively, for periods of 2 and 60 sec for each electric potential. Ten egg masses made up each replication and there were five replications of each treatment. Each replication was placed in a Baermann funnel at room temperature (24 ± 2 C) and the hatched larvae counted every five days over a 60-day period. Collected larvae were introduced into soil around young potted cotton to test their infectivity. Of the 8 treatments used, 6 gave a greater egg hatch than the control, 5 of these significantly greater. The grand means were: Control 411; 1 VDC/mm for 2 sec 1670 larvae, 60 sec 2137; 10 VDC/mm for 2 sec 948, 60 sec 499; 20 VDC/mm for 2 sec 661, 60 sec 1072; and 60 VDC/mm for 2 sec 364, 60 sec 24. The largest hatch of 520% (control 100%) was from 1 VDC/mm for 60 sec. The next greatest of 406% from 1 VDC/mm for 2 sec. The higher potential of 60 VDC/mm decreased egg hatch, being 89 and 6% for 2 and 60 sec, respectively. Egg hatches exposed to 1 and 10 VDC/mm were 99 and 98%, complete, respectively, at 15 days compared to 81% for the control. Egg hatches exposed to 20 VDC/mm for 60 sec and 60 VDC/mm for 2 sec were 97 and 96% complete, respectively, by the 25th day. On the 25th day hatch of eggs exposed to 20 VDC/mm for 2 sec was 78% of the control, and for those exposed to 60 VDC/mm for 60 sec, only 6%. Hatched larvae from all treatments were infective and reproduced on young cotton in a greenhouse, except for 60 VDC/mm for 60 sec where the numbers of larvae were probably insufficient for infection. The results presented demonstrate that electric shock induces hatching of *M. incognita acrita* eggs. Whether this is a direct effect on the maturation of larvae in eggs, hatching of second stage larvae, or both, or an effect on one of the two layers of the egg shell or the gelatinous matrix needs further study.—*Space Division, North American-Rockwell Corporation, Downey, California 94041 and International Institute of Tropical Agriculture, Ibadan, Nigeria.*

CAVENESS, FIELDS, E. and CLARENCE E. CAVENESS. *Nematode Electrocution.*

The effect of electric shock on the survival of *Panagrellus redivivus* adults and larvae, and *Meloidogyne incognita acrita* larvae, in water on a glass slide was studied. The nematodes were placed in tap water between two stainless steel electrodes, 2 mm apart, which were cemented to a glass slide. Electric AC and DC potentials of 1, 5, 10, 15, 20, 30, and 60 V/mm were applied for periods of 1 sec to 5 min at currents of 0.05 to 77 ma. Two series were run. In one series the nematodes were placed a Baermann funnel for 24 hr for recovery of active or revived individuals. In the other series *Panagrellus* were placed on a yeast-oatmeal culture, and *Meloidogyne* larvae on young cotton plants, to test for survival and reproduction. In all tests, exposure to electric shock continued until movement ceased or for 5 min, whichever occurred first. Each replication consisted of 10 nematodes, and each treatment was replicated 5 times. Incubation periods were 7–9 days on yeast-oatmeal cultures and 4 weeks on cotton. Temperature change of the water due to the passage of electric current was from no increase to a maximum rise of 16C, but never greater than 42C. To demonstrate that electric shock not heat was the lethal agent the duration of the electric shocks, in another test, were spaced one second per minute to allow the water to cool. At 30 VAC/mm, 28 shocks of one second over a period of 27 min were lethal with a temperature rise of 2C. At 60 VAC/mm, 11 shocks over a period of 10 minutes were lethal with a temperature rise of 3C. Recovery of root knot larvae from Baermann funnels and galled bioassay (cotton) roots demonstrated that the larvae survived only the 1 VAC/mm and 1 VDC/mm for a period of 5 min. All other treatments were lethal. Recovery of *P. redivivus* larvae from Baermann funnels and subsequent reproduction in yeast-oatmeal cultures showed larvae survived 5 min of exposure only at 1 VAC/mm and 1 VDC/mm. Minimum exposure time was 1 sec at 60 VDC/mm and 1–3 sec at 60 VAC/mm. Numerous larvae were recovered from Baermann funnels in all tests with *P. redivivus* adults, and up to ½ million adults and larvae from cultures, indicating survivial of larvae and eggs within the female. This was visually confirmed by noting active larvae within dead females even after increasing the AC or DC exposure to 600 V/mm. Adult males and females were recovered up through 10 volts treatment AC and DC. The results presented demonstrate that electric shock of AC or DC, as low as 5 volts per mm for larvae and 10 volts per mm for adults, can be lethal even if exposure is intermittent but over a longer period of time than continuous exposure.—*International Institute of Tropical Agriculture, Ibadan, Nigeria and Space Division, North American-Rockwell Corporation, Downey, California 94041.*

COOPER, ALAN F., JR. *Glycogen catabolism and ethanol production in Aphelenchus avenae.*

Fourth-stage larvae and adult *Aphelenchus avenae* were starved for 24, 48, 72, 120, 144, 168, and 192 hr at 28C in aerobic and anaerobic environments. The rate (% dry weight) of glycogen catabolism, for the various periods of time, was determined gravimetrically by extraction with either cold water or trichloroacetic acid. Concentrated nematode suspensions (200,000/ml) stored under aerobic conditions maintain a constant percentage of glycogen (8%) for all time periods, while those stored in anaerobic conditions for 0, 24, 48, 72, 96,

120, 144, 168, and 196 hr contained 8.0, 6.9, 5.6, 4.2, 3.1, 2.4, 1.8, 1.8, and 1.8% glycogen, respectively. Glycogen catabolism ceased after 144 hr and the nematodes maintained a constant glycogen content. When these nematodes are returned to an aerobic environment, they rapidly anabolize glycogen, at the expense of neutral lipids, to the original level maintained in nematodes stored under aerobic conditions. Glycogen metabolic end-product determinations of whole nematodes and their bathing solutions were conducted with thin-layer and gas chromatographic techniques. The only end-product found in substantial amounts amounts was ethanol. There was a direct correlation between ethanol production and glycogen catabolism, with 75–80% of the catabolized glycogen being converted into ethanol. No ethanol production was observed under aerobic conditions. Final identification of the ethanol was determined by preparing a 3, 5,—dinitrobenzoyl derivative and measuring its melting point and nuclear magnetic resonance spectrum.—*Supported by National Science Foundation Grant. No. GB 6569. Department of Nematology, University of California, Riverside, California 92502.*

DICKERSON, O. J., R. T. ROBBINS and J. K. GREIG. *Sweetpotato transplants protected from root-knot nematode infection by chemical treatment of transplant beds.*

Sweetpotatoes (*Ipomoea batatas*) 'Tanhoma' infected with *Meloidogyne incognita* were bedded in fine sand in polyethylene containers 30 $cm^2 \times 15$ cm deep. "Seed" roots were covered with 6 cm of sand. Chemicals were evenly spread over the surface and 2½ cm of water were applied as a fine mist during a period of over 1 hr. Chemicals and rates used in 1966 were Zinophos® 10-G (O-Diethyl O-2 parazinyl phosphorothioate) at 1.04 g/30 cm^2 and Temik® 5-G [2-methyl-2-(methylthio) propionaldehyde O-(methyl carbamoyl)oxime] at 1.04 and 2.08 g/30 cm^2. Chemicals and rates used in 1968 were Mocap® 10-G (O-ethyl-S, S-dipropyl phosphorodithioate) at 1.04 g/30 cm^2, Disyston® 10-G (O, O-diethyl S- [2-(ethylthio)ethyl] phosphorodithioate) at 1.04 g/30 cm^2, Temik 10-G at 1.04 g/30 cm^2 and Lannate® 5-G (S methyl N-[(methylcarbanoyl)oxy] thioacetimidate) at 2.08 g/30 cm^2.

In 1966, efficacy was measured as yield of roots graded as "Number-One." Treatments were applied April 19, transplants were removed and set in the field May 27 and roots were harvested October 12. Yields of "Number-One" roots were increased 40% by Zinophos and 36% by the lower rate and 2% by the higher rate of Temik. Zinophos delayed emergence and caused stunting of transplants, which was quickly outgrown in the field.

In 1968, efficacy was measured as total yield of marketable size roots and recovery of *M. incognita* larvae from fibrous roots taken at harvest. Treatments were applied March 28, transplants were set in the field May 16 and roots were harvested October 10. Total yields from treatments were increased over controls 38% by Mocap, 23% by Disyston, 19% by Temik, and 16% by Lannate. The number of larvae recovered was significantly less for all treatments than for the control. Actual numbers of larvae recovered by a mist system over a period of 7 days was 4,025/g dry wt from the control and 854, 160, 34, and 0/g dry wt from Disyston, Lannate, Mocap, and Temik treatments, respectively. Mocap severely stunted the first harvest of transplants but this effect had diminished by the second harvest. The plants in the field soon appeared similar to the control.—*Departments*

of Plant Pathology and Horticulture, Kansas State University, Manhattan, Kansas, 66502.

DISANZO, C. P. *Some observations on the effect of carbofuran on three plant-parasitic nematodes.*

Soil was treated with 10 ppm of carbofuran (2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate). Tomato seedlings, 'Heinz 1350', were transplanted into pots containing treated and untreated soil and inoculated with a population of second-stage *Meloidogyne incognita* larvae. Ten days after inoculation, tomato plants were removed from the pots, and the root systems were observed for presence of galls. Roots of plants grown in carbofuran-treated soil were free from swellings compared to the heavily galled root systems of plants grown in untreated soil. Higher numbers of nematodes, however, were recovered from the treated than from the untreated soil. Root systems of cucumber plants inoculated with nematodes recovered from the carbofuran-treated soil developed swellings. In other tests, three surface-sterilized tomato seeds were placed aseptically in each of several petri dishes containing 10 ppm carbofuran in nutrient agar medium. Second-stage larvae of *M. incognita* or specimens of *Pratylenchus penetrans* were transferred into these dishes at a distance of 4 cm from the germinating seedlings. Nematodes deposited into dishes containing untreated medium moved toward the roots of tomato seedlings, penetrated, and established infection. Both species moved at random when deposited in media containing carbofuran; only occasionally did an individual reach a root and feeding was never observed. Specimens of *M. incognita* and *P. penetrans* were also placed directly upon the developing roots of tomato seedlings. These nematodes moved near the roots growing in the untreated medium, and they were seen to penetrate them causing lesions or swellings. Nematodes inoculated into treated medium moved more rapidly than those inoculated into untreated medium, were not seen feeding, and those that touched the roots were apparently repulsed. One single specimen of *P. penetrans* was seen attempting root penetration several times, but each time moved away from the roots. In another test, aluminum trays, $5 \times 5 \times 40$ cm, were each filled with treated soil. A corn seedling was planted at the center of each tray, and specimens of *Tylenchorhynchus claytoni* were inoculated at different distances from the center. Ten days after treatment, soil samples were collected every 2 cm away from the corn plant and processed for nematode recovery. The greatest number of nematodes were found at the point of inoculation. Nematodes were recovered from the area immediately adjacent to the roots of all the corn plants grown in untreated soil even when the nematodes were inoculated at a distance of 12 cm from the plant. Nematodes inoculated into treated soil were found only near the point of inoculation.—*Niagara Chemical Division, FMC Corporation, Middleport, New York 14105.*

ENDO, B. Y. and J. A. VEECH. *Comparative enzyme histochemistry in root-knot resistant and susceptible soybeans.*

A comparison was made of the histochemical and morphological responses of susceptible ('Lee') and resistant ('Delmar') soybeans to infection by *Meloidogyne incognita acrita.* The activity of certain oxidoreductive, hydrolytic and oxidative enzymes of the susceptible variety were significantly increased, primarily within the syncytium.

Initially, galling response to infection was similar in both susceptible and resistant plants, i.e., slight galling was observed prior to the microscopic detection of syncytia. With some exceptions, differences between the susceptible and resistant responses were not apparent until several days after inoculation. During the first few days both varieties showed a similar, slight increase of host enzyme activity at the nematode feeding site. Host enzyme activity in susceptible plants continued to increase with time, a response not observed in resistant plants. By the time syncytial induction occurred in the susceptible host, the most common resistant-host response was cell necrosis. Often, after inducing necrosis, the nematode migrated to non-necrotic, resistant-host cells and commenced feeding; these cells subsequently became necrotic. Typically, the resistant host response is characterized by extensive necrosis, whereas the susceptible response is characterized by increased enzyme activity at the nematode feeding site. Since enzyme activity is not stimulated significantly at the feeding site of the nematode in resistant plants, extensive syncytia did not develop, preventing nematode maturation. Conversely, in the susceptible host, enzyme activity is greatly increased, syncytia develop, and the nematode completes its life cycle. The previously-noted exceptions to the above description of resistant-host response include occasional observations of the intense stimulation of enzyme activity, the development of extensive syncytia and concomitant nematode maturation. We interpret those exceptions to mean that a few nematodes are successful in stimulating resistant-host metabolism to produce syncytia. This interpretation is consistent with observations of other investigators that a few nematodes will complete their life cycle on a resistant host. However, the incidence of this is greatly reduced in the resistant variety ('Delmar') compared with the susceptible variety ('Lee').—*Crops Research Division, Agricultural Research Service, U.S. Department of Agriculture, Beltsville, Maryland 20705.*

EPPS, JAMES M. *Survival of the soybean cyst nematode in the digestive systems of birds.*

During the past few years, the soybean cyst nematode, *Heterodera glycines,* has spread to many new areas, and has been identified from 11 states. These include some of the major soybean producing states in the nation. A study was made to determine if the soybean cyst nematode could survive passage through the digestive system of birds fed under laboratory conditions, and if cysts were found in birds trapped from naturally infested fields. Infective larvae were recovered from cysts in the excrement of birds 24 and 48 hr after they were fed cysts. Birds that were forced-fed eggs and larvae dicharge infective larvae in the excrement. Birds which consumed cysts mixed with feed discharged in the excrement numerous cysts containing infective larvae. Starlings were trapped and killed from an infested field and the contents of their digestive tracts were sieved to determine if the birds contained cysts. The results showed that 8.6% of the birds contained at least one cyst each and as many as three were found in one. The soybean cyst nematode can and does survive passage through the digestive tract of birds. Therefore, birds may spread the soybean cyst nematode to new locations, and this could account for many of the infestations.—*Crops Research Division, Agricultural Research Service, U.S. Department of Agriculture, Jackson, Tennessee 38301.*

EVANS, A. A. F. and I. J. THOMASON. *Effect of 1–2,dibromoethane on biology of Aphelenchus avenae.*

Since most laboratory studies with alkyl halide nematicides have determined dosages lethal to 50–95% of population, other ways in which such nematicides affect the various life stages of nematodes have remained largely unknown. Using exposures of 1–2, dibromoethane (EDB) at 5.3×10^{-4} M (1000 ppm) for different time intervals, we studied the effects of nematicide on i) mobility of nematodes; ii) susceptibility of different stages; and iii) fecundity of adult females. When treated for periods of 0.5–24 hr, the behavior of a mixed population of *Aphelenchus avenae* suggested reversible intoxication (narcosis) up to about 8 hr after which irreversible intoxication (leading rapidly to death) took place. Similar tests with *Meloidogyne javanica* and *Tylenchulus semipenetrans* indicated different susceptibility among species.

Effects on individual second, third, and fourth-stage larvae and adult nematodes were studied by culturing them in isolation after exposure to EDB. The ability to complete the life cycle and lay eggs was the criterion of survival. Moulting larvae were more susceptible than nonmoulting larvae. Moulting fourth-stage larvae were selected and treated during the moult, at completion of the moult, and 6 days after the moult. The frequency of distribution of egg production was compared with untreated nematodes. A two hr exposure of moulting nematodes caused most to die without laying eggs; a 4 hr exposure at the end of moulting caused a delay in the onset of egg laying while a 6 hr exposure at 6 days decreased both rate and total numbers of eggs produced. The effect of temperature on toxicity was also investigated.—*Supported in part by USDA Grant No. 12-14-100-9162(34).* *Department of Nematology, University of California, Riverside, California 92502.*

FASSULIOTIS, GEORGE and GINA P. SKUCAS. *The effect of a pyrrolizidine alkaloid ester and plants containing pyrrolizidine on Meloidogyne incognita acrita.*

The effect of a pyrrolizidine alkaloid ester, monocrotaline, on the root-knot nematode *Meloidogyne incognita acrita* was investigated. This alkaloid, extracted from *Crotalaria spectabilis,* is highly toxic to vertebrates. Root-knot nematode larvae, exposed to various concentrations of monocrotaline solutions in distilled water, showed definite alterations in motion immediately upon transfer. This was still evident after a 3-hr exposure. The degree of altered movement in the form of jerking motions was related to the concentration: 1.5% > .5% > .01% > .001% > distilled water. No difference, however, in larval tracks after transfer of larvae to water agar could be detected. Infectivity tests conducted with 'Homestead' tomato plants grown in plastic growth pouches showed significant reduction in infectivity after larvae were exposed to 1% and 1.5% monocrotaline solutions for 24 hr. However, no differences in the root knot index or in the number of females/gm root were found on 'Homestead' tomato roots inoculated with larvae exposed for 24 hr to concentrations of .001, .01, 0.1 and 1.0% monocrotaline solution. No relationship was found between the pyrrolizidine-containing plants and root-knot nematode resistance. *Crotalaria mitchelli, C. spectabilis,* and *C. striata, Cytisus linifolius* and *C. scoparius* were resistant. *Cynoglossum amabile, C. glochidiatum, C. hictum, C. microglochum, C. montarrum, C. officinale, Echium vulgare, E. wildpretti,* and *Silene* sp. were susceptible. Resistance of Crotalaria to root-

knot nematode is apparently not due to the pyrrolizidine alkaloid ester, monocrotaline. —*Crops Research Division, Agricultural Research Service, U.S. Department of Agriculture, Charleston, South Carolina 29407.*

GORDON, R. *The relationship between the nematode Hammerschmidtiella diesingi and the neuroendocrine system of its insect host, Blatta orientalis.*

The possibility of neuroendocrine relationship between the thelastomatid parasite *Hammerschmidtiella diesingi* and its insect host, *Blatta orientalis* was investigated. Removal of the insect's median neuro-secretory cells (m.n.c.) effected a significant reduction in total numbers of adult nematodes 2 weeks after m.n.c. cautery. A similar reduction was obtained, when the retrocerebral complex (corpora cardiaca and corpora allata) of the cockroach was extirpated, whilst simultaneous re-implantation of the corpora allata into these "retrocerebralectomized" insects failed to prevent this reduction. This suggests that the m.n.c.—corpus cardiacum complex influences the host-parasite relationship; the corpora allata do not appear to be implicated, although a possible intervention by these glands cannot be entirely excluded. In all experiments, separate counts of adult male and female nematodes gave inconsistent results. In some cases, the reduction in total numbers of adult nematodes, effected by the operations, was not correlated with either sex. In other cases, however, there was a significant decrease in numbers of either male or female nematodes. It is suggested that in *H. diesingi,* sex is not genetically predetermined. In an attempt to elucidate the precise nature of the neuroendocrine intervention in the host-parasite relationship, it was shown that m.n.c. cautery did not affect the total activity of the insect's midgut amylases, lipases and esterases, measured 4 weeks after m.n.c. cautery. Although the total activity of midgut proteases was unaffected by 2-weeks m.n.c. cautery, separate assays of midgut wall homogenates and midgut lumen contents gave inconclusive results. Hence, no conclusions can be drawn as to the possible role of the m.n.c. in controlling secretion of digestive enzymes. It is uncertain, therefore, whether the environment of the nematode in the m.n.c.-cauterized insects had been altered by a change in enzymatic secretory activity of the midgut epithelial cells. The fecal output of the cockroaches, measured over a 3-week experimental period, was unaffected by m.n.c. removal. Hence, it is concluded that food intake is independent of m.n.c. activity in this insect, which would preclude it as a possible factor in the neuroendocrine aspect(s) of the host-parasite relationship. The volumes of the corpora cardiaca and corpora allata were unaffected by m.n.c. cautery; likewise, there was no apparent change in the histology of the corpora allata and "glandular" region of the corpora cardiaca, although the function of the corpora cardiaca as a neurohemal organ was greatly impaired. No significant effect was produced on the concentrations of fecal uric acid, protein-nitrogen and non-protein nitrogen by the cautery operation. This would indicate, with respect to the above substances, that the environment of the parasite was similarly unaffected by the cautery operation.—*Pestology Centre, Department of Biological Sciences, Simon Fraser University, B.C., Canada.*

GRIFFIN, G. D. and W. W. WAITE. *Pathogenicity of Meloidogyne hapla to sainfoin.*

Little is known about the host-parasite relationship between plant-parasitic nematodes and sainfoin (*Onobrychis viciaefolia*).

Since there is some interest in sainfoin as a forage crop, a study was made to determine the resistance or susceptibility of this plant to the northern root-knot nematode, *Meloidogyne hapla*. Three-week-old plants of three varieties of sainfoin ('Eski,' 'Agusta,' and 'Vica'), and 'Lahontan,' a susceptible alalfa variety used as a check, were each inoculated with 1000 *M. hapla* larvae. After 4 weeks growth at 22 ± 4 C, all plants were harvested and root-knot galling determined. Mean root gall ratings (0 = none; 1 = very light; 2 = light; 3 = moderate; 4 = severe; and 5 = very severe) were 'Lahontan,' 4.90; 'Eski,' 5.00; 'Agusta,' 4.98; and 'Vica,' 5.00. To study the effect of temperature on the pathogenicity of *M. hapla* to sainfoin, 3-week-old plants of the previously mentioned varieties were inoculated with 1000 *M. hapla* larvae and grown at 15, 20, 25, and 30 C. There were no differences in the root weight of inoculated and control plants of sainfoin and alfalfa at any given temperature after 4 weeks. However, *M. hapla* caused a reduction in top growth of sainfoin at all temperatures; and a reduction in top growth of 'Lahontan' at 20, 25, and 30 C. The root-knot gall ratings on 'Lahontan' were very light at 15 C; and severe to very severe at 20, 25, and 30 C. The root-knot gall ratings on all sainfoin varieties were severe to very severe at 15, 20, and 25 C; and light to moderate at 30 C. Roots of 'Lahontan' and 'Eski' inoculated with 100 *M. hapla* larvae were stained and nematode infection and development was determined at 15, 20, 25, and 30 C after 28 days. Maximum infection of both 'Lahontan' and 'Eski' occurred at 25 C. Nematode development increased with an increase in temperature from 15 to 25 C but was suppressed at 30 C in both varieties. There were no differences between varieties in relation to the percent of inoculum developing into adult females ('Eski' = 36%; 'Lahontan' = 34%) at 25 C. Observations made 4 days after inoculations of 4-week-old plants at 22 ± 4 C, showed that the preferred area of infection of 'Eski' plants were the root tips; infection here caused spindle or sickle shaped galls that were a maximum of 9 × 1 mm in size. However, larvae were able to infect and gall roots above the tip. Galls were found up to 30 mm from the root tip, but were smaller, more oval, and approximately 1–2 mm diameter. Root-knot galls were found only on the root tips of 'Lahontan' plants after 4 days, and the galls were very small (0.30 × .50 mm).—*Crops Research Division, Agricultural Research, U.S. Department of Agriculture, Utah State University, Logan, Utah 84321.*

HARLAN, D. P. and G. R. PADGETT. *Tests of the nematode DD-136 and an associated bacterium for control of white-fringed beetle larvae, Graphognathus peregrinus.*

The nematode DD-136 and an associated bacterium was field tested to determine its value as a parasite of white-fringed beetle, *Graphognathus peregrinus* (Buchanan), larvae. Nematodes were applied with a Hypro® piston type pump sprayer mounted on a jeep. They were applied at 10,000, 20,000 and 40,000 infective stage juveniles/0.093 square meter, with three replications each. One year after the nematodes were applied the host population in the 40,000 nematodes/0.093 square meter plots was 38% lower than in the check plots. The nematode has potential use as a biological control agent of the white-fringed beetle.—*Entomolgy Research Division, Agricultural Research Service, U.S. Department of Agriculture, Gulfport, Mississippi 39501.*

HAWN, E. J. *A new technique for studying bacterium-nematode interactions in alfalfa.*

A new technique has been developed using twin-crowned alfalfa plants to determine if *Ditylenchus dipsaci* (Kühn) predisposes wilt-resistant alfalfa to infection by *Corynebacterium insidiosum* (McCull.) Jensen. The plants were grown until the upper tap roots were roughly the diameter of a lead pencil. They were then dug and washed clean of soil. Each plant was carefully split through the crown and upper 5 cm of root with a sharp scalpel and then transplanted into a 15-cm plastic pot of steam-sterilized soil. A plexiglass strip was fitted across the diameter of the pot and pressed into the soil between the two crown sections to prevent mixing of inoculum. When both crowns resumed shoot growth, they were inoculated with *C. insidiosum* and *D. dipsaci* according to a plan designed to demonstrate the effect of different periods of nematode infection on the subsequent reaction of wilt-resistant alfalfa to bacterial wilt.—*Research Station, Canada Department of Agriculture, Lethbridge, Alberta, Canada.*

HEALD, C. M., and A. W. JOHNSON. *Survival and infectivity of nematodes after passing through an overhead sprinkler irrigation system.*

Survival and infectivity of plant-parasitic nematodes discharged through an overhead sprinkler irrigation system were studied. Water was pumped from an irrigation pond which was partially recharged by runoff water from container-grown woody ornamentals. Two replicates of sixty No. 10 cans were placed randomly within an area approximately 128 by 55 meters, covered with 6-mil black plastic on which were placed various species of ornamentals. This area was watered for one hour each time and was sampled on four dates. At the end of each watering period the sample (approximately 2.5 ml) from each container was collected and counts were made. A total of eleven *Meloidogyne* larvae, four *Pratylenchus* and one *Hoplolaimus* adult were collected with a chance of recovery being 540 to 1 computed on the basis of collection container area in each irrigation plot. Nematode counts from surface water returning to the pond totaled 44 *Criconemoides*, 1 *Helicotylenchus*, 2 *Pratylenchus*, 575 *Meloidogyne*, 16 *Tylenchorhynchus*, 4 *Hoplolaimus*, and 10 *Trichodorus*. To determine if nematodes passing through the irrigation system were infective we placed ten plants each of *Lycopersicon esculentum* 'Rutgers', *Ilex crenata* 'Helleri', and *Ilex crenata* 'Compacta' under the irrigation system. Nematode-free plants were container-grown in steamed soil. The containers were placed on inverted No. 10 cans to avoid contamination from splashing water. Four months later, roots were carefully freed from soil and examined for root-knot nematode galls. Soil from test plants was processed for nematodes by the centrifugal-flotation method. No galls were observed on the roots and no plant-parasitic nematodes were found in the soil. We hypothesized that although nematodes were collected from irrigation water, they were not capable of infecting nematode-free plants, and that this incapability was probably caused by the pressure exerted on the animals at the pump or at the sprinkler orifice. Additional research is being conducted to prove or disprove this hypothesis.—*Crops Research Division, Agricultural Research Service, Weslaco, Texas 78596 and Crops Research Division, Agricultural Research Service, U.S. Department of Agriculture, University of Georgia College of Agriculture Experiment Stations, Coastal Plain Experiment Station, Tifton, Georgia 31794, respectively.*

HIRUMI, H., D. J. RASKI and N. O. JONES. *Ultrastructure of somatic muscles of Trichodorus christiei and Longidorus elongatus: Comparative cytology of two plant-parasitic nematodes.*

Although coelomyarian muscle ultrastructure of *Ascaris, Deontostoma,* and some other species has been studied extensively, there is little information on the utrastructure of platymyarian or shallow coelomyarian muscles. An attempt was made to clarify the ultrastructure of somatic muscles in a platymyarian nematode, *Trichodorus christiei,* and in a shallow coelomyarian nematode, *Longidorus elongatus.* Adult nematodes were dissected and fixed in 3% glutaraldehyde, then post-fixed in 1% osmium tetroxide. Fixed materials were embedded in 1.5% agar dehydrated in a graded ethanol series. The agar blocks were embedded in an epoxy resin. Thin sections were prepared and stained with uranyl acetate followed by lead citrate. In *T. christiei* nematode, the platymyarian muscle cells varied in number from one to four in each cross section quadrant of the midbody. The contractile portion of the muscle fiber was enclosed by basement membrane, while the sarcoplasm was uncovered. The contractile region consisted of thin and thick myofilaments. Electron-dense material formed uniform Z bars invaginated perpendicularly from the peripheral plasma membrane into the contractile portion. These Z bars partially divided the myofilaments longitudinally forming two to six wide zones. Each of these zones was composed of I, A, and H bands that were not as distinct as those of coelomyarian nematodes. The thin myofilaments were assumed to end in the Z bars. The arrangement of the ultrastructure indicates that the platymyarian muscles are able to perform simple, sluggish movements. In *Longidorus elongatus* an average of ten muscle fibers appeared in each cross section quadrant of the midbody. Each muscle cell was completely enclosed by a basement membrane. The contractile region, consisting of thin and thick myofilaments, appeared in the outer portion of the fiber while the sarcoplasm was located in its inner portion. The Z bars extended from the plasma membrane, irregularly dividing the contractile region. No regular arrangements of I, A, and H bands were distinguishable. Aggregations of thin myofilaments appeared around the Z bars. The myofilaments of this nematode run in different directions within a cell, although presumably forming obliquely striated muscle fibers. The Z bars of these muscle fibers differed in shape from those of either the platymyarian or the high coelomyarian muscle fibers. The arrangement of the shallow coelomyarian muscles and of their myofilaments in the cells indicates that the coelomyarian nematodes can move in a more complicated and more lively way than the platymyarian nematodes, but less so than the highly developed coelomyarian species. It has been reported that differences in muscle striation patterns may be clues to phylogenetic relationships within the Nemata. The findings in ultrastructure of the platymyarian and the shallow coelomyarian muscles support this suggestion and indicate that other differences in ultrastructure of somatic muscles may also be useful in the study of phylogenetic relationships of nematodes. The findings in the ultrastructure also agree with the previous hypothesis which suggested the evolution of meromyarian to polymyarian muscles.—*Supported in part by U.S. Public Health Service Research Grant No. AI-07687.* *Boyce Thompson Institute for Plant Research, Yonkers, N.Y. 10701, and Department of Nematology, University of California, Davis, California 95616.*

HIRUMI, H. and C. L. HUNG. *Utrastructure of intestinal epithelium in a plant-parasitic nematode, Trichodorus christiei.*

Ultrastructure of the intestinal epithelium has been studied rather extensively in several animal-parasitic nematodes but only to a limited extent in plant-parasitic nematodes. Since the intestine is one of the suspected sites of virus penetration and proliferation in a nematode vector, an attempt was made to study the detail of the intestinal ultrastructure of *Trichodorus christiei,* a vector of tobacco rattle virus. Adult nematodes were dissected and fixed in cold 3% glutaraldehyde in 0.1M sodium cacodylate buffer, then post-fixed in 1% osmium tetroxide in cold 0.14M veronal-acetate buffer with 5% sucrose. Fixed materials were embedded in 1.5% agar and dehydrated in a cold, graded ethanol series. The agar blocks were embedded in an epoxy resin, Epon 812. Thin sections were prepared and stained with uranyl acetate followed by lead citrate. From the stomatal opening to the posterior portion of the esophagus the digestive tract was lined internally with a triradiate cuticular wall which probably makes it impossible for the virus to penetrate into the nematode cells, except through the cephalic gland openings. The cuticular wall ended at the anterior portion of the intestine and the triradiate esophageal lumen became irregular in cross-section. Many large granules varying in size and resembling lipid granules and/or secretory granules appeared in the cytoplasm of the epithelium. Cytoplasmic matrix observed in this region was sparse. In the middle portion of the intestine, the lumen became wider. The internal plasma membrane of the epithelium formed small irregular microvilli. These microvilli gradually increased in number and size posteriorly. In this region, the large granules decreased in number, while the cytoplasmic matrix was denser, containing general cytoplasmic organelles. Usually at this region, the digestive tract was located in one side of the body cavity. Although microvilli have been reported in several animal-parasitic nematodes it was demonstrated in this study that microvilli also exist in a small plant-parasitic nematode. It has been considered that the intestinal epithelium of nematodes is presumably acting in food absorption as well as storage. The finding that the intestine of *T. christiei* consists of at least two major regions, the granular region and the microvillous region, supports this suggestion.—*Supported in part by U.S. Public Health Service Research Grant No. AI-07687. Boyce Thompson Institute for Plant Research, Yonkers, N.Y. 10701.*

HWANG, SHUH-WEI and R. M. SAYRE. *A method for the freezing and recovery of viable Ditylenchus dipsaci larvae.*

The fourth larval stage of *Ditylenchus dipsaci* that aggregate into masses under drying conditions was the only stage of the nematode successfully preserved at subzero temperatures.

Active *D. dipsaci* larvae of all stages were extracted from alfalfa callus cultures by the Baermann funnel technique, rinsed twice in tap water, and resuspended in 10% aqueous (v/v) dimethyl sulphoxide (DMSO). A 0.2 ml suspension of the nematodes containing from 150 to 500 larvae and adults, were pipetted into 1.5 ml ampoules, heat sealed, and subjected to a temperature of –20C for 10 or 15 min. After initial freezing treatment, all ampoules were stored 24 hr in liquid nitrogen (–196 C). Nematodes were thawed at 38 C, and 2 ampoules

of the nematodes in each treatment were added to 4 ml of Heller's solution, and incubated 24 hr at 20 C. Only actively moving nematodes were considered to be viable and counted. In three separate experiments from 16 to 24% of the nematode populations survived temperatures of −196 C for 24 hr. The ability of thawed nematodes to penetrate and develop in alfalfa seedlings was also determined and compared to nematodes treated in DMSO and in water only. The nematodes were suspended in a 2% solution of hydroxyethyl cellulose and from 7 to 20 viable nematodes were pipetted onto the cotyledons of alfalfa seedlings germinating on moist blotter paper. The motile nematodes that survived the freezing treatments were indistinguishable from the other nematodes in their ability to penetrate and reproduce on alfalfa seedlings. The results of this test would indicate that partial dehydration of *D. dipsaci* is not a necessary prerequisite for the survival of the nematode populations at subzero temperatures.—*American Type Culture Collection, 12301 Parklawn Drive, Rockville, Maryland, and the Crops Research Division, Agricultural Research Service, U. S. Department of Agriculture, Beltsville, Maryland 20705.*

JENSEN, H. J. and J. O. STEVENS. *Survival of Mycoplasma gallisepticum after ingestion by saprozoic nematodes.*

Colonies of a saprozoic nematode, *Pristionchus lheritieri,* were combined with 3-day-old colonies of *Mycoplasma gallisepticum* (avian serotype A) isolates 1056 and 801 grown on M_2 medium (turkey meat infusion agar). Suspensions of nematodes obtained from these mixed cultures (nematodes and either mycoplasma isolate) were washed repeatedly in a 400-mesh strainer to remove most external contaminants. Then the nematode carriers were surface sterilized in 20 ppm free chlorine for 20 min. This concentration was far in excess of the 1 ppm tolerated by the mycoplasma. Following chlorination the nematode carriers were rinsed repeatedly in distilled water to eliminate chlorine. A portion of each nematode sample was added directly to M_2 agar plates and to tubes of M_2 broth containing tetrazolium red. The remainder of each nematode sample was ground in a Ten Broech tissue grinder and added to tubes of M_2 broth containing tetrazolium red or to M_2 agar plates. The broth tubes were incubated at 37 C for 7 days then subcultured another 7 days at the same temperature. The nematodes were allowed to crawl on the plates 24 hr before incubation in a candle-jar at 37 C for 3–4 days. Controls (nematodes that did not have contact with mycoplasma, or with rinse water after chlorination) were treated similarly. The best model appeared to be *P. lheritieri* and the 1056 isolate as only a trace of the 801 isolate was reisolated from the nematodes. In addition to *P. lheritieri* other nematodes including *Panagrolaimus subelongatus* and a species of *Rhabditis* ingested and defecated viable mycoplasma. The best medium was the M_2 agar plates as broth tests were somewhat erratic with only 1/3 testing positive. Both types of controls were negative. Passage of mycoplasma through the nematodes seemed to alter some metabolic process evidenced by the inability to reduce tetrazolium red until the 2nd subculture. Also colony growth on plates was somewhat atypical as the typical nipples were reduced or absent.—*Department of Botany and Plant Pathology and Department of Veterinary Medicine, Oregon State University, Corvallis, Oregon 97331.*

JOHNSON, A. W. *Pathogenicity and population development of Criconemoides ornatum, Tylenchorhynchus martini, and Belonolaimus longicaudatus singly and combined on six bermudagrasses.*

Pathogenicity and population development of three species of plant-parasitic nematodes were studied in greenhouse inoculation experiments with bermudagrasses. *Criconemoides ornatum, Tylenchorhynchus martini,* and *Belonolaimus longicaudatus,* alone and in all combinations, were used with six bermudagrass varieties on selections representing a broad range of genotypes. Initial population densities for *C. ornatum, T. martini,* and *B. longicaudatus,* whether used alone or in combination with others, were calibrated at about 500, 900, and 200, respectively, per kg of soil. Final population densities were determined by the use of appropriate assay techniques 155 days after inoculation. All varieties and selections were suitable hosts for each nematode species but final population densities were influenced by the host variety and the presence of other nematodes species in the combination treatments. Populations of *C. ornatum* were adversely affected by the presence of either one or both of the other nematode species on all bermudagrass varieties but more so by *T. martini* than by *B. longicaudatus.* The only significant suppressions noted in *B. longicaudatus* population densities were on 'Common' bermudagrass caused by the 2- and 3-species combination treatments. *C. ornatum, B. longicaudatus,* and both species combined suppressed *T. martini* populations on 'Tufcote' and 'Tiffine'; however, no species adversely affected *T. martini* on 'Tifdwarf'. In most cases, the population dynamics of each species were influenced by other species sharing the same host plant.—*Cooperative Investigations, Crops Research Division, Agricultural Research Service, U.S. Department of Agriculture and the University of Georgia College of Agriculture Experiment Station, Georgia Coastal Plain Experiment Station, Tifton, Georgia 31794.*

JORGENSON, E. C. *Tolerance of sugarbeet to the sugarbeet nematode, Heterodera schachtii* Schmidt.

The intensity of attack, rate of maturation, and sexual differentiation of *Heterodera schachtii* was studied on tolerant and susceptible sugarbeet seedlings. Seedlings, aged 2, 4 and 6 weeks, of tolerant and susceptible varieties of sugarbeet, were transplanted into fumigated soil reinfested with 150 *H. schachtii* larvae per cc of soil. Seed also were planted. An identical series of plants of each age and variety was established in sterilized soil. Plants of each age of each variety were dug from each series 5 days after transplanting, and at 5-day intervals thereafter for a period of 25 days. Each plant was washed and its fresh root weight recorded. A 4-cm length of main root from each plant, beginning 4 cm below the crown, with the attached lateral roots, was weighed, fixed, stained and stage of development, sex, and nematode location in the root determined. There was no difference in the intensity of attack upon tolerant and susceptible seedlings after 5 days. However, a greater number of nematodes attacked susceptible plants at 10, 15, 20 and 25 days. Third-stage males were present 5 days after transplanting in the lateral roots of tolerant and susceptible seedlings which were 28 and 42 days old at the time of initial exposure. Third-stage males did not appear until 15 days following planting of seed. This delay does not indicate the rate

of maturation was faster on older plants because larval invasion necessarily was delayed until roots were produced. Therefore, maturation of the nematode was not influenced by seedling age of tolerant or susceptible varieties. Fourth-stage females appeared in 14, 28 and 42-day-old transplanted seedlings 10 days after initial exposure. Adult males were first noted on the 15th day, and adult females on the 20th day in tolerant and susceptible roots. Sex ratio was not altered by seedling age or by sugarbeet tolerance or susceptibility to the nematode. Nematode infected roots of the susceptible seedlings weighed less than those of non-infected plants. However, total weight accumulation in infected roots of tolerant seedlings slightly exceeded that of the uninfected seedlings. The roots from infected tolerant plants weighed nearly twice as much as roots from susceptible plants on 28 and 42-day-old transplanted seedlings. The differences were not as great on plants grown from seed and on 14-day-old seedlings.—*Crops Research Division, Agricultural Research Service, US Department of Agriculture, Utah State University, Logan, Utah 84321.*

KRALL, E. *Atypical migrations of larvae and evolution of parasitic interactions in grass-infesting species of the subfamily Anguininae (Nematoda:Tylenchida).*

There are three major ecological groups of species belonging to the subfamily Anguininae parasitizing aerial parts of grasses: (i) species localized within the root neck, (ii) those forming galls on leaves, stems and panicles, and (iii) those forming seed galls. It is evident that the single species in the first group, *Paranguina agropyri* which causes obvious enlargement of the root neck in couch grass, *Agropyron repens,* should be regarded as one of the most primitive representatives of the subfamily in evolutionary respect. Parasitic interactions between host plants and nematode species or races, of the second and particularly the third group, are of a more complicated nature because they are associated with the upward migration of parasite larvae. In most cases, such a migration seems to be of passive character only. In leaf-infesting species, larvae enter the young leaves, while they are still within the sheaths. Seed infesting species attack growing points of young plants and are elevated to the embryo by the lengthening of the culm. Although *P. agropyri* is a typical parasite of basal parts of grasses and cereals, we have observed that larvae of this species may be elevated to some extent by the intercalary growth of culms. This rarely occurs in couch grass but is more common in cereals and especially in winter rye (*Secale cereale*). Although gall-like swellings of basal parts occur in very young stages of this host, they are not characteristic for parasitic interactions of later growth stages. A limited number of nematode larvae may be responsible for growth cessation of several first internodes in seedlings by attacking young meristematic tissues. The larvae inhabit small slit-like cavities, 3–5 mm in length, located mostly in the lower 1–2 cm of the culm. Due to the rapid intercalary growth of rye at the stage of shooting, larvae may be "elevated," and therefore, slit-like cavities become apparent in one or several subsequent internodes. They are always located in the region immediately above the corresponding nodes of the culm. Up to 5 internodes may be directly damaged by the presence of nematodes. In 72.0% of the seedlings, nematodes occurred at 3 cm, in 21.4% at 5 cm, and in 6.6% at 10 cm above the tillering node of culms. In some cases,

adult specimens and eggs were established in such "elevated" positions. It is known that the wheat nematode, *Anguina tritici,* which is a typical seed-gall former, may occasionally produce leaf galls on wheat and mature within them. Atypical upward migrations of the quack grass nematode larvae in the culms of rye clearly indicate the way of evolution of parasitic interactions between representatives of this nematode group and cereals, and, particularly, throw more light on the origin of species adapted to the parasitism in generative organs of flower plants.—*Institute of Zoology and Botany, Academy of Sciences of the Estonian S.S.R., Tartu, U.S.S.R.*

LAPP, N. A. and A. C. TRIANTAPHYLLOU. *Relative DNA content of some members of Heteroderidae.*

Relative DNA content of hypodermal nuclei of larvae was determined photometrically in 18 populations of various species of *Heterodera, Meloidogyne,* and *Meloidodera.* The major objective was to test the hypothesis of polyploidy in the family Heteroderidae, and to clarify the relationships of the chromosomal complements of the three genera. Second-stage larvae were sectioned in a cryostat, Feulgen-stained and mounted permanently on slides. DNA determinations of selected ventral-chord nuclei were made according to the two-wavelength method with a Leitz MPV microscope photometer equipped with a Xenon arc lamp, an interference-graded line filter, and a Photovolt 520-M photometer. DNA content appears to be proportional to the chromosome number within *Heterodera* and *Meloidogyne.* This suggests that species of these genera with chromosome numbers equivalent to 3n or 4n are truly triploid or tetraploid, and that the increased chromosome numbers have not resulted through chromosomal fragmentation or other chromosomal rearrangements. Furthermore, DNA content of *Heterodera* species with $2n = 18$ chromosomes, is much higher than that of *Meloidogyne* species with $2n = 36$ chromosomes. If the *Meloidogyne* karyotype has evolved from a karyotype similar to that *Heterodera,* this must have happened through a mechanism of chromosomal number increase other than polyploidization. Some adjustment of the total amount of DNA per nucleus probably occurred subsequently. If the opposite pathway of karyotype evolution is assumed, the *Heterodera* karyotype may have derived from *Meloidogyne* karyotype through chromosomal fusions with subsequent increase of total DNA. On the basis of DNA content, *Meloidodera* karyotype is intermediate between that of *Heterodera* and *Meloidogyne.*—*Supported by National Science Foundation Grant (GB-7214).* *Department of Plant Pathology and Department of Genetics, North Carolina State University, at Raleigh, North Carolina 27607.*

MACDONALD, D. H. *Effect of mineral nutrition on number of Pratylenchus penetrans entering plant roots.*

Nematode-free seedlings or rooted cuttings of winter vetch (*Vicia villosa*) were grown in acid-washed quartz sand in a growth chamber or greenhouse at 22 or 24 C and 12 or 16 hr days, respectively. Nutrient solutions differing only in Ca^{++}, K^{+} and Mg^{++} content from Hoagland and Arnon's No. 2 solution were supplied to the plants. After at least 30 days of nutrient treatment, the plants were transplanted to 10-cm plastic pots filled with a sandy-loam soil infested with *Pratylenchus penetrans* or to which *P. penetrans*-infected root pieces had been added. The plants were harvested a mini-

mum of 7 days later and the roots were incubated in tap water for at least 20 days. Nematodes emerging during this period were counted. Minimum penetration of roots by this nematode occurred when the plants had previously been supplied with a solution having the same Ca^{++} plus Mg^{++} to K^{+} ratio as Arnon's No. 2 solution. Roots of plants supplied with solutions either high in Ca^{++} and Mg^{+} and low in K^{+} or vice versa, contained more nematodes than did those receiving the balanced solution. Differences in the numbers of nematodes recovered could not be explained by differences in the size of the root systems.—*Department of Plant Pathology, University of Minnesota, St. Paul, Minnesota 55101.*

MARKS, C. F. *Effects of EDB on the respiration of Pelodera sp. and Aphelenchus avenae.*

The respiration rates of nematodes exposed to sublethal concentrations of EDB (1,2-dibromoethane) were determined with a Warburg constant volume respirometer. When suspended in a 0.53×10^{-2} M solution of EDB, third-stage larvae of a *Pelodera* sp. reached a respiration rate of about 22 μl O_2/mg dry wt/hour, an increase of 130% over that of the controls. Maximum respiration rate occurred immediately after exposure to EDB. However, after 7 hr the respiration rate had fallen below that of the controls. In contrast, *Aphelenchus avenae* showed no significant changes in respiration when exposed to EDB at concentrations up to 0.53×10^{-2} M. However, a concentration of 1.06×10^{-2} M depressed the rate of respiration of *A. avenae*. Although the normal respiration rate of *Pelodera* decreased with temperature the degree of response to EDB remained constant. There was no correlation between the time required to reach maximum internal concentration of EDB and the respiration response of *Pelodera*. Since EDB readily penetrates into both *A. avenae* and *Pelodera* the differences in respiration with the nematicide could be due to differences in metabolism of these nematodes.—*Research Station, Canada Department of Agriculture, Vineland Station, Ontario, Canada.*

MILLER, L. I. *Correlation of pairs of morphometric characters of eleven isolates of Heterodera glycines.*

Correlation of pairs of morphometric characters were made of 11 isolates of *Heterodera* glycines reared in the greenhouse (24–29 C) to determine whether the use of ratios was justified in the characterization of the isolates. One hundred cysts containing 180–240 eggs/cyst were introduced into methyl bromide-fumigated soil in 15-cm pots, and a single 'Lee' soybean plant was grown in each pot. Soil from the pots was examined from males 6 weeks after planting and for cysts, eggs, and second stage larvae 8 weeks after planting. Measurements were made of selected morphometric characters of 100 cysts, 360 eggs, 100 males, and 108 second stage larvae of each isolate. The measurements of the characters were compared as to correlative relationship (significant values $P < 1\%$). (i) CYSTS.—Breadth (BR) with neck length (NK) and NK with length without neck (LWON) were not mutually related; NK to length with neck (LWN) were significantly correlated in 8 of the 11 isolates, but the highest correlation coefficient (r) for the Virginia (Va) 4 isolate was only 0.54. LWN with BR was significantly correlated in all isolates, the lowest r was 0.32 for Missouri (Mo.) 1 and highest r of 0.71 for Tennessee (Tenn.) 1. LWON with BR was also

significantly correlated in all isolates, the lowest *r* was 0.38 for Illinois (Ill.) 1 and the highest *r* of 0.74 for Va. 3. (ii) EGGS.—L and BR were not mutually related for the combined data of the 11 isolates and only Va. 4 exhibited a significant *r* of 0.62. (iii) MALES.—No reciprocal relation was found for the 11 isolates or for the combined data of all the isolates in comparisons of L with: BR, stylet length(S), and spicule length(SP); BR with: S, tail length (T), and SP; S with: T, and SP. (iv) LARVAE.—BR with: S, posterior part of stylet (PS), head to excretory pore (EP), T, tail terminus length(TT), and breadth of tail at anterior portion(BT) were not mutually related. The same characters compared with L exhibited significant *r* values for several or all of the isolates, but the reciprocal relation between them was least for L with: BR, S, PS, BT and greatest for L with: EP (max. *r*, Ill. 1 = 0.84), T(max. r, Ill. 1 = 0.77), and TT(max. *r*, Tenn. 1 = 0.68). S with: PS, EP, T, TT, and BT exhibited significant *r* values for several or all of the isolates, and the relation between them was least for S with BT; a medium relationship of S with: EP(max. *r*, Ill. 1 = 0.52), T (max. *r*, Tenn. 1 = 0.40), TT(max. *r*, Arkansas 1 = 0.47); and the highest relation for S was with PS(max. *r*, Ark. 1 = 0.72). A reciprocal relation occurs between T and TT for all isolates (min. *r*, Va. 3 = 0.62 and max. *r*, Va. 1 = 0.80) but none of the isolates showed significant *r* values for T with BT. TT and BT were not mutually related. Since only a few of the pairs of morphometric characters measured had a high degree of correlation for all of the isolates, the use of ratios to characterize isolates of *H. glycines* is limited.—*Department of Plant Pathology and Physiology, Virginia Polytechnic Institute, Blacksburg, Virginia 24061.*

MINTON, NORMAN A. and D. K. BELL. *Criconemoides ornatus pathogenic on peanuts.*

Pathogenicity studies with *Criconemoides ornatus* on peanuts (*Arachis hypogaea*) were conducted in microplots consisting of 16 stainless steel containers 0.8 m in diameter and 0.6 m deep. Containers were located in a shadehouse and contained methyl bromide fumigated Tifton sandy loam. Eight containers were seeded with 20 'Argentine' and eight with 20 'Starr' peanuts. Soil in four containers of each cultivar was infested with *C. ornatus* and four of each served as checks. Nematodes collected from soil in which peanuts were growing were washed for 30 min in 0.001% 8-hydroxyquinolin sulfate and returned to tap water. Seeds were planted one per hole, and approximately 75 nematodes were placed in each hole. On week later, 200,000 nematodes were dispersed into 4-cm deep trenches 4 cm from the plants on both sides of the peanut row in each container that received inoculum and covered with soil. After 130 days plants were harvested, and the soil was assayed for nematodes. Percent dead plants, fresh pod weight, and root and pod discoloration were recorded. Twenty-five pods from each replication were assayed for fungi. An average of 6.6 nematodes per cc of soil, or approximately 5,000,000 per microplot based on the upper 15 cm layer of soil, were recovered from the inoculated containers, indicating that reproduction had occurred. No nematodes were recovered from the checks. The same kinds and infestation levels of contaminating fungi were isolated from pods from both treatments. Differences in percentages of dead plants were not significant between treatments. Pod yields from nematode inoculated plants were slightly less than half that of the check plants. Root and pod discoloration indices

were significantly greater for inoculated than for non-inoculated plants. Discoloration was due to lesions in which partially embedded nematodes were often found. Lesions were present on roots of all ages. Many lateral root primordia and young roots were killed. The two cultivars responded similarly to the nematode.—*Cooperative Investigations, Crops Research Division, Agricultural Research Service, U.S. Department of Agriculture, and the University of Georgia College of Agriculture Experiment Stations, Georgia Coastal Plain Experiment Station, Tifton, Georgia 31794.*

MUSE, BARBARA D. *Histopathology of susceptible and resistant reactions in 'Wando' pea seedlings to 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 eventual death of the apical meristem. These observations posed an opportunity to study the histopathological aspects of resistance and susceptibility in a single host-nematode complex. Pea seedling shoots inoculated with either the RNC or WNC populations of *D. dipsaci* were processed for sectioning and stained with safranin and fast green. Uninoculated plants served as controls. Larvae and adults of both nematode populations penetrated the epidermal layers of the cotyledonary petiole and the stem within 6 hr after inoculation. Penetration occurred both directly and through stomata. Small intercellular and intracellular cavities were formed by both RNC and WNC nematode movement. Affected cell contained granular and plasmolyzed cytoplasm and enlarged nuclei and nucleoli. Differential host responses were visible macroscopically 36 hr after inoculation. Hypertrophy and hyperplasia, correlated with gall formation in RNC-infected plants, were observed in the cortex of the stem. Dark-staining cells, correlated with the necrotic reaction in WNC-infected plants, were observed in the lateral buds, shoot tip, and cells lining cavities in the stem. By 168 hr after inoculation, cavities in RNC-infected plants contained cell fragments and clusters of intact cells, extending throughout the stem and in parenchymatous leaf mesophyll. In WNC-infected plants necrotic areas, up to three cell layers in thickness, lined cavity areas. When these cavities were located adjacent to the epidermis, the epidermal cells buckled away from the cortex and became convoluted. Eventually, the outermost necrotic areas of the epidermis and cortex partially sloughed off. Pith and vascular tissue appeared unaffected by RNC and WNC infection. Neither secondary wall thickening nor cork formation was observed. Cellular effects often preceded the direct contact of RNC and WNC nematodes with cells, suggesting the diffusion of a nematode secretion.—*Department of Plant Pathology and Physiology, Virginia Polytechnic Institute, Blacksburg, Virginia 24061.*

O'BANNON, J. H. and A. T. TOMERLIN. *Population studies on two species of Pratylenchus on citrus.*

In greenhouse experiments, 6-month-old Rough lemon (*Citrus limon*) seedlings were individually inoculated with 20 *Pratylenchus coffeae* or with 20 *P. brachyurus* in January 1968. Root samples were collected at 6 week intervals to assay population responses for 16 months. A second series of experi-

ments were initiated to study population density in relation to time of inoculation. Six-month-old Rough lemon seedlings were individually inoculated with 20 *P. coffeae* or with 20 *P. brachyurus;* January, June, and October 1968. Seedlings were harvested at 6 week intervals for 18 weeks and the nematodes extracted from the roots. Multiplication of *P. coffeae* after an initial lag was rapid and large numbers were extracted. Peak populations were reached at 36 weeks with 10,000 *P. coffeae* per g of root extracted. Conversely, *P. brachyurus* populations dropped below inoculation levels during the first 36 weeks to a level of 10 *P. brachyurus* per g of root. Peak populations of *P. brachyurus* were reached at 58 weeks with nearly 100 *P. brachyurus* per g of root extracted. Influence on population multiplication in relation to date of inoculation was linear with *P. coffeae* in the 3 inoculation periods with summer temperatures favoring maximum development. *P. brachyurus* populations decreased during high summer temperatures and increased during the fall and winter. An indirect correlation was found between the vigor of the host and numbers of *P. coffeae* found. Shoot growth of infected seedlings was reduced by 22%. No measurable reduction in growth of seedlings infected with *P. brachyurus* (compared with the non-infected seedlings) occurred in these studies to date.—*Crops Research Division, Agricultural Research Service, U.S. Department of Agriculture, Orlando, Florida 32803.*

PEREZ-MENDEZ, G., E. BUECHER and E. L. HANSEN. *Relation of precipitation to activation of growth factor for Caenorhabditis briggsae.*

The portion of activated growth factor biologically effective for culture of *C. briggsae* was found to be the precipitate formed in the activation procedures. The growth factor (GF), a proteinaceous material isolated from liver homogenate, was added to chemically defined basal medium (CbMM) at 10–250 μg/ml. At these low supplement levels it must undergo an activation treatment in order to support growth and reproduction. The activation procedures, controlled heating at 37 C or 53 C, adjustment of the pH of the medium to 5.5 (the approximate isoelectric point of GF), addition of Ficoll,® or freezing the complete medium, precipitated about half of the supplement. Growth factor was precipitated and activated also by the addition of specific particles, precipitated gamma-globulin or polystyrene latex beads; neither supported growth in CbMM alone.

Precipitation was measured by increase in absorbance at 550 mμ after activation. Media containing activated and precipitated growth factor were centrifuged and the resuspended precipitates and supernatants were tested for biological activity. The resuspended precipitates were active; the supernatants were inactive unless subjected to a further activation process which then precipitated some of the protein remaining in solution. Several other proteins, including bovine serum albumin, γ-globulin, fetuin, serum fraction IV-4, and β-lactoglobulin, were subjected to the activation procedures and assayed for GF activity at 250 μg/ml. They were not able to support growth of *C. briggsae* either at this level or at higher levels up to 5 mg/ml.

Growth factor, a high molecular weight, partially purified globulin with a marked tendency to aggregate and precipitate, appears to be unique. Microscopic examination at 900 $\times$ magnification revealed irregular masses of precipitated protein composed of loose aggregates of particles about one-half micron in diameter. Similar particles

were observed in the intestine of the nematode. In nature, *C. briggsae* feeds on bacteria; the particles of precipitated growth factor are within the size range of bacteria. It is suggested that particles may have a special physiological role, perhaps in stimulation of ingestion or regulation of the passage of material along the intestine. Precipitation of the protein would thus serve a two-fold purpose; first, to greatly concentrate the supplement for utilization by the nematode, and second, to bring it into the bacterial size range. Previous reports had postulated that changes in molecular configuration were responsible for activation of growth factor. Inasmuch as these studies were carried out only on the residual soluble portion of growth factor, they now appear to be irrelevant. The prime effect of several diverse methods of activation seems to be simply the precipitation of growth factor.—*Supported by Grant A1-07359 TMP. Clinical Pharmacology Research Institute, 2030 Haste St., Berkeley, California 94704.*

POINAR, G. O., JR. *On the occurrence of an undescribed nematode in the genital system of a tropical dynastid beetle, Oryctes monoceros.*

A nematode representing a new genus and a new species was recovered from the bursa copulatrix and aedeagus of the dynastid beetle, *Oryctes monoceros*, in West Africa. The nematodes possess morphological characters found in representatives of both the Rhabditidae and Angiostomatidae, as well as unique features. They breed in the bursa copulatrix of female beetles and are transferred from infected to non-infected beetles during copulation. Non-infected male beetles pick up the nematodes in the canals of their intromittent organ and introduce them into female beetles during subsequent matings. The nematodes can maintain themselves for long periods within the aedeagus of the beetle, yet live for only a short period outside the insect's body. There is no indication that a dauer stage is formed or that the nematode can develop in the insect after the latter has died. In fact, the nematode has never been found outside the genital system of the insect. Histological sections showed that the nematodes occasionally penetrated through the inner glandular wall of the bursa copulatrix and came to rest between this layer and the outer muscle fields. The possible pathogenic effect of this nematode on its host and the evolutionary significance of this relationship are discussed.—*Supported by the Joint United Nations Special Fund and South Pacific Commission Project for Research on the Control of the Rhinoceros Beetle. Department of Entomology and Parasitology, University of California, Berkeley, California 94720.*

PRASAD, N. and R. MANKAU. *Studies on a sporozoan endoparasite of nematodes.*

A minute parasite was observed in a glasshouse population of *Meloidogyne javanica* which originated in southern California. Mature nematode females within galls and larvae in the soil were heavily infected. Parasitized nematodes were fixed in a formol-calcium mixture and osmium tetroxide, embedded in plastic, section by ultramicrotome, and examined by electron microscopy. The platelet-like spores which adhere to the nematode cuticle are dome-shaped in profile and measure about $1.5\ \mu \times 2.75\ \mu$. The parasite resembles *Duboscqia penetrans* Thorne, 1939, but the absence of a polar filament does not permit its inclusion in the *Microsporidia.* In sectioned *Meloidogyne* females, the parasite was observed developing in localized areas. Reproduction was us-

ually by repeated fission or budding to form characteristic amoebulae or plasmodia in the host tissue. No eggs were produced by infected females, which developed to mature size, but the host became filled with spores of the parasite. Root-knot males were never found infected. Laboratory host range studies were conducted by placing nematodes for varying time periods in distilled water containing the adhesive parasite spores. Larvae of *M. javanica* and *M. incognita,* and adults and larvae of *Pratylenchus scribneri* became heavily infected, while larvae of *M. arenaria, M. hapla, Tylenchulus semipenetrans,* and *Aphelenchoides* sp. were only slightly infected. Larvae of *Heterodera schachtii,* adults and larvae of *P. vulnus, P. brachyurus, Tylenchorhynchus claytoni* and *Ditylenchus dipsaci,* were not attacked. The adhesive nature of the spore appears biochemically specific to certain species of nematodes. Distribution of the non-motile spores in soil is apparently limited to movement by parasitized hosts or by soil water movement. Glasshouse experiments showed 50% reduction in an infected population of *P. scribneri* within 55 days; however, *M. javanica* maintained high populations in the presence of the parasite in the soil. Resistance of the spores to desiccation was demonstrated when a parasite-free population of *M. javanica* became infected when added to soil containing the parasite which had been air-dried for 6 months.—*Supported in part by USDA Research Contract 12-14-100-8282(34). University of California, Riverside, California 92502.*

REYNOLDS, H. W. and W. W. CARTER. *The response of Meloidogyne incognita acrita in resistant and susceptible alfalfa.*

A study was made to compare the penetration, development and migration of the cotton root-knot nematode, *Meloidogyne incognita acrita,* in resistant and susceptible alfalfa varieties. Three varieties of alfalfa, 'African', 'Moapa' and 'Sonora', which are resistant to *M. incognita acrita,* and one susceptible variety, 'Lahontan', were used in these studies. To determine the penetration and subsequent development of *M. incognita acrita* in resistant and susceptible roots, 4 plants of each alfalfa variety were harvested at 1, 2, 3, 4, 5, 6, 7, 10, 14, 18 and 21 days after inoculation of the seedlings with 100 larvae. An average of 30 larvae was observed in either resistant or susceptible varieties. After 3 to 4 days a sharp decrease in the number of larvae was observed in resistant roots until at 7 days these roots contained less than 5 larvae per seedling with no nematode development. Ten days after inoculation, no larvae were observed in any of the resistant roots. Larvae became sedentary and developed normally in susceptible roots. Egg production began as early as 18 days after host penetration. To compare the migration of larvae out of resistant and susceptible alfalfas, 2 plants of resistant 'African', and 2 of the susceptible 'Lahontan', were inoculated with 100 larvae per plant and harvested at 3, 4, 5, 6 and 7 day intervals. The plants were placed in a dish containing sterile distilled water at 25 C. The roots were examined microscopically 5 times daily for a 72-hr period and all larvae emerging from the roots were counted and removed from the dish. Resistant roots harvested at 3, 4, 5, 6 and 7 days yielded 22, 29, 19, 15 and 5 larvae, respectively. Susceptible roots yielded 5, 5, 2, 2 and 1 larvae for the same harvest intervals. Whenever *M. incognita acrita* larvae invaded the resistant alfalfas, there was no apparent effect on either the plant tissue or nematode. The most commonly reported reaction of resistant plants

to nematode invasion is hypersensitivity which results in the death of the nematode. In this study the larvae apparently failed to initiate any type of response by the plant that might result in either giant cells or necrotic cells. Since the larvae fail to establish a nutritive relationship with the plant and are not entombed by necrotic tissue, they soon leave the root.—*Crops Research Division, Agricultural Research Service, U.S. Department of Agriculture, Phoenix, Arizona 85040.*

RUEHLE, JOHN L. and DONALD H. MARX. *Parasitism of pine mycorrhizae by lance nematodes.*

In greenhouse tests, considerable variance was encountered in repeated studies of the effect of the lance nematode, *Hoplolaimus galeatus,* on the growth of pine seedlings. Mycorrhizal fungi rapidly colonized the steamed soil with varying degrees of mycorrhizal synthesis, possibly accounting for the variance in nematode parasitism and seedling growth. To investigate this possibility, mycorrhizal and nonmycorrhizal roots on intact seedlings were inoculated with *H. galeatus. Thelephora terrestris* was used to form ectotrophic mycorrhizae in open pot culture on roots of shortleaf pine (*Pinus echinata*) in a growth room supplied with especially filtered air to keep out fungus spores. *Pisolithus tinctorius* was used in aseptic jars on roots of loblolly pine (*Pinus taeda*). Nematode inoculations on these plants were made both in individual root cylinders (1 × 2 cm) with 100 nematodes per cylinder and in pots in the growth room with 6,000 nematodes per pot.

After 9 days of incubation in root cylinders, or 43 days of incubation in pots, the roots were removed and examined. Some roots were prepared for histological examinations and others were fixed, bleached, and cleared for whole mount observations.

Regardless of pine host, mycorrhizal fungus, or method used to form mycorrhizae, both male and female nematodes penetrated the fungus mantle of mycorrhizae. Some nematodes entered and migrated through the cortex; others penetrated perpendicular to the root axis and remained partially embedded. Some nematodes occurred singly at points of entry and others fed in groups. Common entrance points were at natural breaks caused by short root initials erupting in the surface of the lateral roots. Nematodes were commonly found in the cortex of mycorrhizae and of lateral roots supporting mycorrhizae. On nonmycorrhizal seedlings, however, nematodes were more commonly confined to the cortex of the lateral roots and were rarely found in nonmycorrhizal short roots. No generalized necrosis was found in invaded mycorrhizae, and neither hypertrophy nor hyperplasia was associated with nematode feeding. Toxified cells were found 1 or 2 cells in advance of certain feeding sites of nematodes in the cortex of both lateral roots and mycorrhizae. In some cases, cells devoid of contents were found in areas adjacent to the head of a feeding nematode.

Although lance nematodes do not parasitize fungi, they readily parasitize plant cells morphologically altered by fungus symbionts. The mycorrhizal fungi did not offer resistance to nematode attack. The possibility exists that nematode parasitism may alter the resistance of mycorrhizal roots to attack by fungus pathogens.—*Forestry Sciences Laboratory, Southeastern Forest Experiment Station, Forest Service, U.S. Department of Agriculture, Carlton Street, Athens, Georgia 30601.*

SCHMITT, DONALD P. *Population patterns of some stylet-bearing nematodes in a native Iowa prairie.*

The Kalsow Prairie is a 160-acre unplowed rolling native prairie in Iowa. The clay loam to silty clay loam soil has pH values of 5.0–6.5 and organic matter contents of 8.5–12.9%. Ten soil samples were taken from each of 15 sites during four sampling periods at two-month intervals from February through September, 1968. Total nematode counts were highest during February at all except the two sites located at pothole boundaries. Adults and juveniles of *Helicotylenchus pseudorobustus, H. digonicus, H. exallus, H. platyurus, H. hydrophilus, Aorolaimus torpidus, Tetylenchus* spp., an undescribed *Tylenchorhynchus* sp. found in the pothole regions, *Aphelenchoides* spp., an undescribed *Trichodorus* sp., *Xiphinema americanum,* and *X. chambersi* were observed infrequently or not at all during February, indicating they overwintered largely as eggs. *Tylenchorhynchus maximus T. silvaticus, Tylenchus* spp., and some members of the Dorylaimoidea mainly accounted for the large numbers of nematodes found in the February sampling, but *Tylenchorhynchus nudus, Helicotylenchus leiocephalus, Aglenchus costatus, Aphelenchus* spp., were also found to overwinter as adults and juveniles. Total nematode populations declined in the early summer at most sites and increased at all sites in the late summer. Population patterns of the same species varied with the habitat even though the habitats were in close proximity. For example, the numbers of individuals of species found only in potholes and pothole boundaries (*Tylenchorhynchus* sp., *Tetylenchus* spp., and *Xiphinema chambersi*) were high in potholes and low in pothole boundaries during one sampling period, while the opposite was true at another sampling period. *Helicotylenchus hydrophilus,* also found only in the potholes and their boundaries, exhibited different patterns at each pothole site and pothole boundary site. An increase in soil pH was correlated with a greater number of *Tylenchorhynchus maximus* ($r = 0.58$) and *T. nudus* ($r = 0.58$). Prevalence of the latter species was negatively correlated with organic matter ($r = -0.66$). The largest populations of *Helicotylenchus pseudorobustus* were found in soils with low sand content which resulted in a high negative correlation between numbers of this species and per cent sand ($r = -0.90$).—*Department of Botany and Pathology, Iowa State University, Ames, Iowa 50010.*

SIDDIQUI, I. A. and D. P. TAYLOR. *Histopathogenesis of galls induced by Meloidogyne naasi in wheat and oat roots.*

Histopathological and cytological developments of galls induced by *M. naasi* in roots of 'Pawnee' wheat and 'Wintok' oat were studied, using paraffin-embedded serial sections. Three seedlings of each host were grown under controlled conditions and inoculated with *M. naasi* larvae. Roots were harvested and fixed each day for the first 6 days and every 3 days thereafter until the 30th day and terminated on the 40th day after inoculation. Large numbers of larvae penetrated root tips of both hosts within 24 hr, causing cortical hypertrophy. Larvae migrated both inter- and intracellularly and giant cells were formed in the stele around the head of each nematode in 4 to 5 days. Initiation and development of giant cells and related abnormal changes in wheat roots were of the highly susceptible type. No necrotic host response was observed in infected wheat roots. In oat roots, however, the host response to infection was variable: (i) necrosis of inner

cortical and endodermal cells adjacent to the nematode lip region was often associated with failure of larvae to enter the stele and continue their development; (ii) when larvae entered the stele and initiated giant cells, the cells adjacent to giant cells showed hypersensitive necrotic reactions; (iii) the same degree of abnormal changes occurred as observed in wheat. Normally 2 to 3 days after penetration larvae became sedentary with their heads embedded in the stele and the rest of their bodies extending into cortex with no definite orientation. Protophloem and protoxylem cells close to the parasite's lips enlarged. The nuclei and nucleoli hypertrophied and the cytoplasm became granular and dense. Serial sections of giant cells indicated the presence of 2 to 8 agglomerated multinucleolate nuclei. The giant cells were thick walled and projections into the cell cytoplasm were not observed. Rapid proliferation of pericycle cells coincided with termination of cell differentiation and growth of infected root tips. Cortical cells displayed a general hypertrophy beyond the area immediately around the parasite. The initial multinucleate condition in giant cells appeared to result from the incorporation of nuclei of adjacent cells by dissolution of cell walls because synchronous nuclear mitoses were not observed until 9 days after inoculation. Decline in giant cell development and related abnormal changes was noticed 12 days following inoculation. After 21 days, giant cells became highly vacuolated and their cytoplasm, nuclear and nucleolar material showed signs of degeneration. Observations after 40 days revealed a complete degeneration of cell contents in many giant cells but their thick walls remained. Abnormal xylem completely surrounded the degenerated or partially degenerated giant cells and an extensive necrosis of hyperplastic parenchyma and endodermis occurred.—*Department of Plant Pathology, University of Illinois, Urbana, Illinois 61801.*

SIDDIQUI, I. A. and D. P. TAYLOR. *Embryonic and postembryonic development of Meloidogyne naasi and comparative development on wheat and oats.*

Embryonic and postembryonic development of *M. naasi* was followed sequentially and the approximate duration of each stage was determined. Embryogenesis was studied at 22–26 C using water mounts of eggs under coverslips sealed with Vaseline. Postembryonic development was studied on seedlings of 'Pawnee' wheat. A comparative study of development of this nematode was made on 'Pawnee' wheat and 'Wintok' oats. The infected seedlings were grown at 26 C day temperature and 20 C night temperature. Eggs measured $96 \times 40\ \mu$ ($n = 50$). The first and second cleavages were perpendicular to the long axis of the egg. The first division resulted in two equal blastomeres, S_1 and P_1. Twelve hours later, the S_1 blastomere divided into a terminal cell (A) and the center cell (B). The third cleavage was transverse but oblique, dividing the P_1 blastomere into two cells S_2 and P_2 8 to 11 hr after the second division. The A and B blastomeres simultaneously divided in a longitudinal plane forming 4 cells at the anterior pole. Soon after, the P_2 blastomere divided in the same plane as A and B, thus giving rise to a 7-cell stage. From this point on it was difficult to follow the individual blastomeres, because of rapid succession of cell divisions and considerable rotation of these cells. The blastula and gastrula stages were observed at 60 to 72 hr and 84 to 96 hr, respectively, after the first cleavage. The embryo displayed the first sign of movement 7 to 9 days after the first cleavage. The first molt

occurred inside the egg 8½ to 11 days after the first cleavage and lasted from 3 to 5 days. At ambient temperature, *M. naasi* eggs required 15 to 17 days to develop into second stage larvae. No eggs hatched in the preparations used for studying embryogenesis. Larvae became sedentary with their heads embedded in the stele 2 to 3 days after penetration of host roots. Sex differentiation first became apparent on the 12th day, and the second molt was first observed on the 18th day. All three molts followed each other between the 18th and 24th day. Adult females secreted the gelatinous matrix between the 27th and 30th day, and started laying eggs between the 30th and 35th day. Under the controlled conditions of these experiments, the life cycle required 39 to 51 days. Mean number of larvae penetrating per unit root weight was 2.4 times higher in wheat than in oats. Necrosis and subsequent death of heavily infected root tips were commonly observed in oats and only occasionally in wheat seedlings. Three times as many larvae reached maturity and laid eggs in wheat as in oat roots. Egg production per seedling was considerably higher in wheat than in oats, however, the number of eggs per egg mass did not differ significantly. 'Pawnee' wheat therefore was susceptible and 'Wintok' oat relatively resistant to *M. naasi.*—*Department of Plant Pathology, University of Illinois, Urbana, Illinois 61801.*

STEELE, ARNOLD E. *Occurrence of a diurnal rhythm in hatching and emergence of larvae from cysts of the sugarbeet nematode, Heterodera schachtii.*

Two devices were designed to automatically collect larvae hatched from cysts of the sugarbeet nematode, *Heterodera schachtii* Schmidt, at consecutive intervals of 1 or 4 hr of each daily period. Tests indicated that during periods of maximum activity, accumulated daily hatch was proportional to time. However significantly different hatching rates were measured during periods spaced 12 hr apart suggesting that undetermined factors produced a diurnal rhythm in the hatching of the sugarbeet nematode. These factors are thought to be environmental rather than of biological origin. The devices may have additional applications in nematode research.—*U.S.D.A. Agricultural Research Service, P. O. Box 5098, Salinas, California 93901.*

STOKES, D. E. *Andropogon rhizomatus parasitized by a strain of Tylenchulus semipenetrans not parasitic to four citrus rootstocks.*

A colony of citrus nematode, *Tylenchulus semipenetrans,* collected from and reared on a grass, *Andropogon rhizomatus,* failed to parasitize four citrus rootstock species; whereas, in concurrent inoculations the nematodes readily parasitized the grass host. Citrus rootstocks used were *Citrus limon, C. sinensis, C. aurantium* and *Poncirus trifoliata.* Nematodes on the grass were not morphologically differentiated from those on citrus. Grass root sections revealed epidermal, hypodermal, and cortical cells invaded by female citrus nematodes before selection of permanent feeding sites. Feeding by the nematode was limited to the cortical region of the root. Cortical cells fed upon by the nematodes differed from apparently healthy cells in the same region. Failure of this colony of *T. semipenetrans* to infect citrus in repeated tests suggests that it is a distinct strain of *T. semipenetrans,* designated as the "grass strain" of citrus nematode.—*Division of Plant Industry, Florida Department of Agriculture, Gainesville, Florida 32601.*

TIMM, R. W. *Evolution of the Desmoscolecida.*

A collection of 69 species of the order Desmoscolecida, including six new genera and 45 new species, provided sufficient representation for a reasonable interpretation of evolutionary progressions within the order. The immediate ancestor was probably a nematode with vesiculate amphids on the head; large, simple, unadorned annules; and paired subdorsal and subventral rows of tubular setae. Although pseudosegmentation is commonly considered to be a characteristic of the Desmoscolecida, there are actually only two genera out of 14 which have detachable concretion rings (*Desmoscolex* and *Tricoma*), and these genera are among the most advanced. Two independent kinds of evolution have occurred within the Desmoscolecida: (i) reduction of somatic setae, with only subdorsal rows present (*Eudesmoscolex*), with only subventral rows present (*Prodesmoscolex*), or with setae completely lacking (*Paratricoma*); (ii) development of annular adornments—scales in *Paratricoma* and two new genera, warts in *Pareudesmoscolex,* short spines in a new genus and long spines in *Greeffiella, Desmoscolex* probably evolved, not by amalgamation of adjacent rings of *Tricoma*, but by enlargement and concretization of setae-bearing annules. A new genus may be a transitional form. *Quadricoma* very likely developed from *Desmoscolex* by elimination of the inter-ring annules; many transitional species are known. *Tricoma* could have resulted from a new genus by expansion of the narrow, granular concretion bands in the center of the large, uniform, simple annules. *Greeffiella* probably developed from a new genus with short spines on the posterior of each annule by further outgrowth of the spines.—*Department of Nematology, University of California, Davis 95616.*

TOWNSHEND, J. L. and L. R. WEBBER. *Movement of Pratylenchus penetrans in three Ontario soils.*

The influence of bulk density and moisture tension (suction) on the movement of *Pratylenchus penetrans* was studied in Fox loamy sand, Vineland silt loam, and Jeddo clay loam. Certain conclusions and facts became evident when movement and soil moisture content were plotted as a function of suction. Nematode movement was greatest as moisture began to drain freely and was related to moisture tension and not to moisture content. Movement was inversely proportional to bulk density. Moreover as the bulk density of a soil increased, greater suctions were required to initiate free drainage and consequently there was a corresponding shift in the peak of maximum movement. Maximum distance that *P. penetrans* moved in 7 days was 2 cm when each soil was of low bulk density and moisture tension was optimal. However, soil type did influence movement when the soils were of common bulk density; adults and fourth-stage larvae moved significantly further in Fox sand (2.1 cm) than in Jeddo (1.4 cm) and Vineland (0.5 cm) loams.—*Research Station, Canada Department of Agriculture, Vineland Station and Department of Soil Science, Ontario Agricultural College, University of Guelph, Ontario, Canada.*

VEECH, J. A. and B. Y. ENDO. *Alterations in enzyme localization in soybeans infected with Meloidogyne incognita acrita.*

The sites of activity of alkaline phosphatase, acid phosphatase, non-specific esterase, peroxidase, adenosine triphospha-

tase, and cytochrome oxidase were demonstrated histochemically in fresh sections of 'Lee' soybeans infected with *Meloidogyne incognita acrita*, a species of root-knot nematode. Each of the enzymes was found to be more active at the feeding site of the nematode than in adjacent cells of the same tissue. During the early stages of infection increased enzyme activity was localized in several cells in the proximity of the lip region of the nematode. However, when definite syncytia were observed, the increased enzyme activity was confined primarily within the boundaries of the syncytia. An increase in enzyme activity preceded the induction of syncytia. However, after syncytia were formed further increases in activity paralleled syncytial development and nematode maturation. Based upon previous studies, and the enzymes demonstrated here, it appears that a general increase in host metabolism is associated with the induction and development of syncytia. Further, this increased metabolic response is more peculiar to susceptible than resistant host plants. —*Crops Research Division, Agricultural Research Service, U.S. Department of Agriculture, Beltsville, Maryland 20705.*

WEBSTER, J. M. and S. CRAIG. *The direct effect of plant and insect hormones on Cephalobus sp.*

It is known that the host-parasite relationship of i) plant parasitic nematodes can be modified by the addition of the plant growth hormone kinetin and ii) insect parasitic nematodes can be influenced by the insect moulting hormone ecdysone. In order to determine whether or not these growth hormones affected the nematode directly, a free-living parthenogenic nematode, *Cepalobus* sp., was cultured on a bacillus culture on potato dextrose agar to which had been added different concentrations of hormones (gibberellic acid at 0.1, 1.0, 5.0, 10.0 and 20.0 mg/liter, kinetin at 0.1, 1.0, 5.0 and 10.0 mg/liter and ecdysone at 0.001, 0.005, 0.01, 0.05 and 0.1 mg/liter) were added to the agar medium and then bacteria were cultured on this medium for 48 hr at 28 C prior to the inoculation of each culture plate with a single gravid female nematode. The time taken for the different larval stages to develop was recorded. The female *Cephalobus* sp. produced eggs on the unsupplemented agar; the second-stage larvae (P generation) hatched and developed through to the second-stage larvae of the F_1 generation in 171 hr. The rate of development of nematodes from the P generation of second-stage larvae to the F_1 generation increased with increasing concentrations of both kinetin (127 hr with 10.0 mg/liter) and ecdysone (121 hr at 0.05 mg/liter). The development of the nematode was inhibited at 0.1 mg/liter of ecdysone and 20 mg/liter of gibberellic acid. Except in those treatments where nematode development was inhibited by high concentrations of hormones the nematodes continued to develop and produce populations of morphologically normal nematodes.—*Pestology Centre, Department of Biological Sciences, Simon Fraser University, Vancouver, Canada.*