ABSTRACTS OF PAPERS PRESENTED AT THE TENTH ANNUAL MEETING OF THE SOCIETY OF NEMATOLOGISTS, OTTAWA, CANADA, AUGUST 22–25, 1971

ABAWI, G. S., H. D. VANETTEN, AND W. F. MAI. *Phaseollin production induced by Pratylenchus penetrans in Phaseolus vulgaris.*

Phaseollin, a bean phytoalexin, was isolated from aseptically-grown *Phaseolus vulgaris* ('Red Kidney') seedlings infected with axenized *Pratylenchus penetrans.* Bean seeds were surface sterilized for 10 min in 1:2:2 v:v:v 5.25% sodium hypochlorite, 95% ethanol, and tap water. Three seeds were aseptically planted in damp white sand contained in sterilized glass tubes (5 × 30 cm). One week later, about 10,000 nematodes grown under monoxenic conditions on alfalfa callus were added to each tube. Alfalfa callus without nematodes was added to control seedlings. After 5 days at 21 C, hypocotyls and roots of seedlings inoculated with *P. penetrans* exhibited numerous small elongated brown or black lesions which were not observed on noninoculated seedlings. Equal weights of tissues from inoculated and noninoculated seedlings were extracted for phaseollin. Hypocotyl and root tissues were placed in 95% ethanol (1:4, w/v) and ground for 2 min in a Waring Blendor. The filtrate obtained by filtering through several layers of cheesecloth was centrifuged at 12,000 *g* for 20 min at 4 C. An equivalent volume of ethanol was added to the supernatant, and the ethanol was removed by evaporation under reduced pressure at 40 C. The aqueous fraction was partitioned twice with four volumes of petroleum ether (85% hexane). The petroleum ether fraction was taken to dryness and the residue taken up in 95% ethanol. The processed extracts were streaked on silica-gel-coated plates (250 μ thick) for thin-layer chromatography along with a phaseollin standard and developed with pentane: ethyl ether: acetic acid (75:25:1). The area on the chromatograms corresponding to phaseollin (R_f 3.0 – 4.0) were eluted with 95% ethanol. The ultraviolet absorption spectrum of the elutant prepared from *P. penetrans*-infected bean tissue was characteristic of phaseollin: an λ_{max} at 279 nm, a shoulder at 284 – 286 nm, and a minor peak at 315 nm. Using the molar extinction coefficient ($\log \epsilon = 3.96$) reported for phaseollin in ethanol at 279 nm to determine concentration, 59 μg phaseollin was obtained per g of *P. penetrans*-infected tissue while no phaseollin was detected in the noninoculated controls. Incubation 16 hr in a solution containing 47 μg/ml of purified phaseollin (m.p. 177–178 C) had no apparent effect on the survival of *P. penetrans.*—*Department of Plant Pathology, Cornell University, Ithaca, NY 14850.*

ANDERSON, R. V., AND J. R. BYERS. *Fine structure of the esophageal glands of Tylenchorhynchus dubius.*

The dorsal and subventral glands and associated ducts and duct orifices of the plant parasitic nematode *Tylenchorhynchus dubius* were studied with the electron microscope. The dorsal gland duct joins the cylindrical esophageal lumen where it bifurcates, 1 to 3 μ posterior to the spear knobs. The gland orifice dimensions resemble those of the inner esophageal lining, having outer and inner diameters of 400 to 440 nm and 130 to 140 nm, respectively, and a cuticle thickness of 100 to 200 nm. The gland orifice is contiguous with a specialized quadriradiate lumen that is 800 to 1300 nm in diameter and extends about 900 to 1200 nm into the matrix of the ampulla. The radii near the orifice are distally bifurcated and closed, but open near their termination in

the ampulla. The subventral glands join the esophageal lumen posterior to the valvular apparatus of the metacorpus, midway at the subventral sides of the triradiate inner lining. The cuticular lining of the subventral orifices is twice as thick as the esophageal lining at the point of junction (90 nm), having an outside and inside diameter of 370 to 420 nm and 180 to 220 nm, respectively. The duct orifices extend about 1000 nm into the subventral ampullae, gradually opening and becoming trough-shaped ventrally. The cytoplasmic matrix of the ampullae and ducts in the esophagus is heterogeneous and contains secretory granules that are 100–400 nm in diameter. These granules are also present in the basal bulb anterior to the gland nuclei and probably represent, in stored form, products of protein synthesis. Concentrations of microtubules also are present throughout the ducts, while absent in the basal bulb glands, indicating that they may function in transport of gland secretions. The cytoplasm of the dorsal and subventral gland cells comprising the basal esophageal bulb is composed largely of closely-packed rough endoplasmic reticulum with dilated cisternae. Mitochondria are scattered throughout the cytoplasm, but Golgi complexes appear to be associated only with secretory granules anterior to the gland nuclei. There are no detectable differences in the subcellular structure between these glands. —*Entomology Research Institute, Canada Department of Agriculture, Ottawa, Ontario.*

BAINES, R. C., AND R. H. SMALL. *Soil porosity effects on penetration and maturation of Tylenchulus semipentrans in 'Homosassa' sweet orange roots.*

Three soils and a mixed potting medium with porosities(voids) ranging 29–51% [sandy loam 29%, coarse sand 34.8%, loamy sand (l.s.) 37.5%, 2:1 sand:peat (v:v) 51%], were planted with 8-cm-tall sweet orange seedlings in 15-cm square plastic pots, 15 cm deep. Ten replicate plants were established for 7 weeks in each soil and then were inoculated with 40,000 *Tylenchulus semipenetrans* second and third stage larvae poured on the surface of each pot in 250 ml of tap water. To test the effect of depth of placement of the inoculum, six glass rods 0.8 cm in diam. were inserted 5 cm deep in each pot at the time of planting of a set containing sandy loam soil. These rods were later removed and a suspension of the nematodes was poured into the holes, and then filled with soil. Plants in coarse sand and sand-peat received Hoagland's solution weekly and all of the pots were watered when the surface soil appeared dry. After draining, the percentage of voids occupied by water were: coarse sand 56%, sandy loam 68%, l.s. 72%, and sand-peat 95%.

The roots from the upper 6.5 cm and those from the remainder of the root system were harvested separately 2 months after inoculation. They were stored in 10% formalin and the number of mature females were determined by a staining (acid fuchsin in lactophenol), blender maceration, and sieving method. Percentages of larvae in the inoculum that reached maturity were: sandy loam 7.3%, sandy loam with holes 8.6%, l.s. 4.5%, coarse sand 0.2%, and sand-peat 0.01%. Of these 80.6%, 79.5%, 91.3%, 74.1%, and 89.6%, respectively, occurred in the upper 6.5 cm of the root system. Nearly equal fresh weight of roots occurred in the sandy loam, sand, and sand-peat soils, and in both the top and bottom half of the pots. Approximately 66% as many roots occurred in the l.s. as in the other three soils. The *p*H of the sandy loam, l.s. and coarse sand ranged from 6.9–7.1 and that of the sand-peat mixture was 4.0–4.5. Low infection in the sand-peat may be due to the low *p*H.

Many larvae may have been trapped in the water that occurred at the points of contact of large particles in the coarse sand and thus prevented from reaching the roots. 1.3% more of the larvae developed to maturity in the sandy loam with the holes than without holes.—*Department of Nematology, University of California, Riverside CA 92502.*

BHATTI, D. S., AND J. N. SASSER. *Histopathology of 'Kobe' lespedeza roots infected with Heterodera lespedezae.*

'Kobe' lespedeza (*Lespedeza striata*) plants infected with lespedeza cyst nematode (*Heterodera lespedezae*) were grown at day and night temperatures 26/22 C. Second-stage infective larvae penetrated the roots and were oriented alongside the stele within 24 hr. A feeding relationship was established within 48 hr and syncytia began to form within 72 hr. Syncytia formation was initiated in the pericycle and extended into the phloem and xylem. Syncytium development comprised enlargement of nuclei and nucleoli and cell wall dissolution resulting in a multinucleate condition. Nuclei and nucleoli generally assumed different shapes and were heavily stained. In roots infected less than 10 days, the syncytial spread was greater longitudinally than radially. The cell walls near the head of the nematode remained intact and progressively thickened. The same characteristic changes occurred rapidly in the adjoining cell also, resulting in an enlarged syncytium with numerous nuclei and nucleoli. Fifteen to 20 days after infection, syncytia had expanded radially more than longitudinally and tended to occupy most of the stele at points of infection. Syncytial growth intruded upon xylary elements. In advanced stages of syncytial development, several syncytia seemed to fuse, resulting in a giant-cell complex in which intact cells were scarce. Degeneration of syncytia began 30 days after infection and was characterized by fewer nuclei, nucleoli and a loose granular consistency of the cytoplasm. After 40 days syncytial degeneration included cytoplasmic breakdown, vacuolation, and absence of nuclei and nucleoli.—*Supported by USAID Fellowship. Department of Plant Pathology, North Carolina State University, Raleigh, NC 27607.*

BHATTI, D. S., H. HIRSCHMANN, AND J. N. SASSER. *Postembryogenesis and effect of temperature on development of Heterodera lespedezae in 'Kobe' lespedeza.*

Morphological changes during postembryonic development of the lespedeza cyst nematode, *H. lespedezae*, were studied. The cuticular markings changed from transverse striae in second-stage infective larvae to longitudinal markings in fourth-stage larvae and the typical rugose zig-zag pattern in adult females. The subventral esophageal glands progressively atrophied following larval penetration of the roots, whereas the dorsal gland enlarged with the onset of feeding. Larvae at each stage had a distinct stylet and fed actively; anus and rectum were well defined. The reproductive system gradually increased from the four-celled primordium of infective larvae to the paired, elongated and highly convoluted gonads of the female. Granular particles, possibly associated with breakdown and reabsorption of the inner cuticular layers of the old cuticle, appeared between the molted and the newly-forming cuticles. To determine the influence of temperature on the length of the life cycle, *H. lespedezae* was reared on *L. striata* in a phytotron at the following day/night temperatures: 18/14 C, 18/18 C, 22/18 C, 26/22 C, 26/26 C, and 30/26 C. In 'Kobe' lespedeza, the optimum temperature regime appeared to be day/night 26/22 C, at which the life cycle was completed in 26 days. At higher (30/26 C) and lower (18/14 C) temperatures 36 and 51 days were required,

respectively. Time required the life cycle appeared to be governed by a direct effect of temperature on the nematode rather than by an indirect effect upon the host plant. Certain developmental phases were correlated with color changes of the adult female body. Matrix production began in white females, oviposition commenced during the yellow phase and ceased when females turned brown. Afterwards hatching occurred inside the cysts and second-stage larvae emerged through natural cyst openings.—*Supported by USAID Fellowship. Department of Plant Pathology, N. C. State Univ., Raleigh, NC 27607.*

BOLLERUP, G. J., AND A. H. BURR. *Chemical nature of the eyespot pigments of some free-living marine nematodes and of the chromatrope of Mermis nigrescens.*

Other investigators have identified both melanin and hemoglobin in marine nematode eyespots and hemoglobin in the chromatrope of *Mermis subnigrescens*. By solubility tests, histochemistry and microspectrophotometry we detected other pigments in addition to these. The solubility characteristics in a variety of acids, alkalies, organic solvents and detergents were determined on squashed heads (to ensure reagent penetration). The histochemistry was performed on fresh frozen tissue sections. The absorption spectra were determined with a recording microspectrophotometer on whole mounts and frozen sections. The presence of melanin was confirmed in the paired eyespots of two species of *Enoplus*, however the pigment in granules distributed posteriorly along the esophagus from the paired spots was identified as hemosiderin. Small amounts of hemosiderin are also present in the eyespots. The absorption spectrum of the hemosiderin granules revealed the presence of porphyrins. This suggests that this pigment may be derived from the hemoglobin which was diffusely distributed in the esophageal muscle and hypodermal chords. This hemoglobin was found to have Soret, β and α absorption peaks at 415, 535 and 570 nm, respectively. The eyespot pigment of *Oncholaimium vesicarium* resembled melanin in its absorption spectrum and its insolubility in organic solvents, but did not exhibit the usual histochemical reactions of a fully developed melanin. The pigment of this species may therefore consist of a phaeomelanin or an incompletely polymerized eumelanin. The latter possibility is also indicated by the fibrous texture of the eyespot pigment in electron micrographs. Hemosiderin was detected in the esophageal granules posterior to the eyespot and hemoglobin was diffusely distributed in the esophageal muscle of this species. The pigments of *Oncholaimus skawensis* and *Chromadorina germanica* have different characteristics than those of *Oncholaimium vesicarium* and consist of neither melanin nor hemoglobin but may be carotenoid. Confirming the finding of Ellenby on *Mermis subnigrescens*, we found that the chromatrope of the similar species *Mermis nigrescens* contains a water soluble hemoglobin.—*Supported by National Research Council of Canada Grant No. A4418. Department of Biological Sciences, Simon Fraser University, Burnaby 2, B. C., Canada.*

BRODIE, B. B., AND J. M. GOOD. *Relative efficiency of selected volatile and nonvolatile nematicides for control of Meloidogyne incognita on tobacco.*

Several volatile and nonvolatile nematicides were evaluated in 5-yr field trials (Tifton sandy loam) for best dosage level, method of application, and efficiency against *Meloidogyne incognita* on tobacco. Treatments were applied to single-row plots (50 ft long and 4 ft apart) and arranged in a randomized complete block design replicated 7 times. Increase in tobacco yield was used

to measure efficiency of *M. incognita* control. Nonvolatile nematicides were 10–26% more effective in increasing tobacco yield than were volatile soil fumigants, except 1,3-dichloropropene, 1,2-dichloropropane (DD). Among the nonvolatile nematicides, 2,3-dihydro-2,2-dimethyl-7-benzafuramyl methylcarbamate (carbofuran) and 0,0-diethyl 0-p-(methylsulfinyl) phenyl phosphorothioate (Dasanit®) were 5–25% less effective than were 2-methyl-2 (methylthio) propionaldehyde 0-(methylcarbamoyl) oxime(aldicarb); 0,ethyl S,S-dipropyl phosphorodithioate (Mocap®); or ethyl 4-(methylthio) *m*-tolyl isopropyl phosphoroamidate (Nemacur®). Likewise, 80% DD + 20% methylisothiocynate (Vorlex®); 80% 1,3-dichloropropene + 15% chloropicrin + 5% propargyl bromide (Telone PBC®); and tetrachlorothiophene were 7–10% less effective than was DD. Yield increase was up to 2.5 times greater with a dosage of 84 liters/ha of DD or a mixture containing 95.2% DD + 4.8% methane sulfonic acid (SD-14647) than at dosages of 47 or 64 liters/ha. Efficient dosage levels of nonvolatile nematicides varied. The most efficient dosage of aldicarb and of Mocap (10–48% greater yield) was 6.7 kg active ingredient/ha. Carbofuran was 34% more effective at 4.2 kg/ha than at 6.7 kg/ha. All dosages (4.2, 6.7, and 11.2 kg/ha) of Nemacur were equally effective (40% yield increase) whereas Dasanit required 10 kg/ha active ingredient to obtain a significant yield increase. Aldicarb and Dasanit performed best when incorporated in the top 15–20 cm of soil. Greatest response from Nemacur and Mocap was obtained when these compounds were incorporated in the top 5–10 cm of soil. In-furrow application of carbofuran (injected 15–20 cm deep and bedded) was superior to soil incorporation.—*Plant Science Research Divsion, Agricultural Research Service, U. S. Department of Agriculture, Beltsville, MD 20705. Senior author at Department of Plant Pathology, Cornell University, Ithaca, NY 14850.*

BYERS, J. R., AND R. V. ANDERSON. *Ultrastructure of the stomatal region of Tylenchorhynchus dubius.*

The ultrastructure of the body wall, stoma and stylet of *Tylenchorhynchus dubius* was studied in both transverse and longitudinal sections. The body wall consists of a six-layered cuticle, about 1 μ thick, and an underlying hypodermis which, except at the chords, is very thin. In the lip region the cuticle is thinner due largely to the absence of an outer matrix and an inner striated matrix layer. The thin interchordal hypodermis contains many hemidesmosomes which represent specialized sites of adhesion between it and either the basal layer of the cuticle or the basement membrane of the muscles. The hemidesmosomes are particularly numerous where muscles attach to the body wall. The labial framework consists of a central tube composed predominantly of electron-dense cuticle and six radiating blades of a less electron-dense cuticle that is similar in structure to the internal cortex layer of the body wall cuticle. The anterior stomatal opening leads through a small peristomal cavity into the vestibule within the central tube. The anterior portion of the vestibule extension has a thick (1 μ) cuticular lining which, except for an osmiophilic zone at the inner surface, is composed of finely granular material with a radiating columnar substructure. Posteriorly the cuticle of the vestibule extension diminishes to about 150 nm in thickness near its point of attachment to the stylet shaft. The cuticular lining does not fuse directly with the stylet shaft but abuts an interposed electron-dense layer formed at the surface membrane of the hyperdermal cells investing the stylet shaft posterior to the point of attachment.

The stylet of *T. dubius* is a cuticular cylinder with a central lumen. In transverse section the stylet shaft is characteristically structured. Lining the lumen is a 17 nm thick trilaminate layer. Adjacent the trilaminate layer there is a more or less well defined electron-lucent layer about 30 nm thick and then a somewhat thicker, moderately electron-dense layer which has six radial extensions dividing the thick cortex of the shaft into six sectors. This moderately electron-dense layer has a regular subunit structure which is particularly evident in tangential longitudinal sections. The sectors of the cortex between the radial extensions of this layer are composed of a homogeneous electron-lucent substance. Posteriorly, the stylet shaft expands to form the trilobed base to which the protractor muscles attach. The fine (actin) filaments of the protractor muscles terminate in hemidesmosome plaques on the surface of the stylet knobs. Anteriorly, the protractor muscles attach to the body wall just below the radial blades of the labial framework.—*Entomology Research Institute, Canada Department of Agriculture, Ottawa, Ontario, Canada.*

CARTER, W. W., H. W. REYNOLDS, AND D. R. RODNEY. *Response of debilitated grapefruit trees to manure, phosphate fertilizer and DBCP fumigation against Tylenchulus semipenetrans.*

This study was conducted to measure the effects of steer manure or phosphate fertilizer and control of *Tylenchulus semipenetrans* with 1,2-dibromo-3-chloropropane (DBCP) on recovery of severely dibilitated 42-year-old 'Marsh' grapefruit trees. Main plot treatments were DBCP at 58 and 40 kg/ha active ingredient in 1967 and 1970, respectively, and untreated controls. Subplot treatments were (i) annual application of 0.23 kg actual phosphorus per tree, (ii) annual application of steer manure at 22.4 metric tons per hectare, and (iii) no fertilizer except annual applications of 1.36 kg actual nitrogen per tree which all trees received. The average yield in kg of fruit per tree for the bloom years 1967 through 1970 were: control—70, phosphorus alone—104, manure alone—107, DBCP alone—103, DBCP plus phosphorus—133, and DBCP plus manure—162. All treated plots yielded significantly higher than untreated controls. Trees treated with phosphorus plus DBCP and manure plus DBCP yielded significantly higher than trees treated with DBCP alone. The average numbers of fruit per tree in 1969 and 1970 were: control—244, phosphorus alone—377, manure alone—345, DBCP alone—295, DBCP plus phosphorus —395, and DBCP plus manure—483. DBCP alone increased the number and percent marketable fruit (48's or larger) 18% and DBCP plus manure raised it 43% above the untreated controls. The effects of phosphorus and manure on yield were additive with DBCP.—*Cooperative investigations of U. S. Department of Agriculture, Cotton Research Center, 4207 E. Broadway, Phoenix, AR 85040 and the University of Arizona, College of Agriculture, Experiment Station, Yuma, AR 85364.*

COLLINS, R. J., AND R. RODRIGUEZ-KABANA. *Relationship of fertilizer treatments and crop sequence to populations of lesion nematodes.*

The effect of fertilizer treatments on populations of lesion nematodes in field plots was studied for two years in an established long-term fertilizer experiment with the following rotation sequence: corn, winter wheat, soybeans, fallow, cotton, and in some plots winter legume (crimson clover + vetch) as green manure. The experiment consisted of three tiers rotating every year to the three major crops: corn ('Fla-200A'), soybeans ('Bragg'), and cotton ('Auburn 56'). Each

tier, consisting of ten 30 × 5.5 m plots, received the same treatment and rotation sequence continuously for at least ten years. Fertilizer treatments varied from a complete formulation (N, P, K, lime, minor elements) to treatments deficient in one or more components. Soil and root populations of a lesion nematode species (closely resembling *Pratylenchus scribneri* Steiner) were higher in soybean and cotton plots receiving no fertilization and no winter legume than in plots receiving all major elements, lime and a winter legume; the reverse was true in corn plots. The number of lesion nematodes in soil and root samples from soybean plots receiving no N was higher than in plots with N supplied via a winter legume; this difference was not pronounced with corn or cotton. Soybeans and corn in plots receiving N through the activity of a winter legume supported significantly lower numbers of lesion nematodes than when inorganic N was used; this difference was not apparent with cotton. Yields of all crops were depressed and higher numbers of lesion nematodes developed in the P-deficient plots than in those receiving complete fertilization. In P-deficient plots, the number of lesion nematodes were in the order: corn > soybeans > cotton. Omission of K fertilization depressed yields of all crops but the numbers of lesion nematodes present were generally equivalent to those found in plots with complete fertilization. Unlimed plots had lower *p*H values (5.4) than limed plots(6.2–6.5) and generally showed greatest seasonal fluctuations in numbers of lesion nematodes. Highest populations of lesion nematodes were found in unlimed plots planted to cotton and soybean. In corn plots, nematode numbers were comparable to those of limed plots receiving complete fertilization. Well fertilized plots receiving P in the form of triple superphosphate had lower numbers of lesion nematodes and slightly higher yields than those which received superphosphate. This difference was more pronounced with corn than with cotton or soybeans. Addition of minor elements to limed plots receiving all three major elements increased yields of all crops but did not significantly affect lesion nematode populations in soybeans or cotton; with corn, plots not supplemented with minor elements had higher populations.—*Supported by Project Ala. 300 (S-19). Department of Botany and Microbiology, Auburn University, Auburn, AL 36830.*

DICKSON, D. W., AND G. C. SMART, JR. *Control of Meloidogyne arenaria and Pratylenchus penetrans on peanuts with foliar applications of a systemic nematicide.*

Two experiments were conducted on peanuts to compare foliar applications of DuPont's experimental nematicide 1410 907g/3.8 liter solution of active emulsifiable concentrate (2 EC) (S-methyl 1-(dimethylcarbamoyl)-N-{(methylcarbamoyl)oxy}thioformimidate) with soil applications of the granular formulation of 1410 and other granular materials applied either in a 30-cm band, or broadcast, and incorporated into the top 10 cm of soil with a rotary hoe. DBCP (1,2-dibromo-3-chloropropane) at 9.33 liter/ha row treatment was used as a standard, and untreated plots served as controls. The investigation was conducted in two separate fields, one heavily infested with *Pratylenchus penetrans* and the other with *Meloidogyne arenaria.* DuPont 1410 10% active granular formulation (10 G) was applied before or at planting at the rate of 4.4 and 8.8 kg/ha active. The EC at the rate of 10 kg/ha broadcast active was sprayed on the foliage in 2,473 liters of water/ha when the plants were blooming and again 4 weeks after blooming. Each treatment was replicated four times in one test and five in the other. The tests were

randomized complete block designs and each treatment consisted of two rows 9.1 m long and 76.2 cm apart. Peanut pods and roots of four plants were rated according to a galling index and five plants by a lesion index. The percentage of sound, mature kernels was determined from a 200-g sample from each treatment in accordance with Federal-State Inspection Service methods.

Peanut yields were highest and pod damage least in plots sprayed with 1410 EC than from any other treatment. In the lesion nematode test, yields from 1410 EC plots were significantly higher than from 1410 10G plots or from plots treated with six other materials, but were not significantly higher than from DBCP plots or plots treated with four other materials. In the root-knot nematode test, due to variation within treatments, no significant yield differences were detected between treatments, although plots treated with 1410 EC yielded an average of 3882 kg/ha compared with 2989 kg/ha in the control.—*Department of Entomology and Nematology, University of Florida, Gainesville, FL 32601.*

DUNN, R. A. *Comparison of two centrifugal flotation techniques with Seinhorst elutriation for efficiency of nematode extraction from soil.*

In one centrifugal flotation method (direct centrifugation), 50 cc of soil was placed directly into a 250-ml centrifuge bottle, shaken vigorously with 200 ml of water, and centrifuged sufficiently to sediment all soil particles larger than 0.5 μm (about 4 min at 1280 *g*). The supernatant was discarded and the pellet resuspended in 200 ml of 1.1 M sucrose solution (500 g sucrose in 1000 ml of tap water). After sedimentation of the soil by centrifugation 4 min at 1280 *g*, nematodes were recovered from the supernatant solution by three passages through a 400-mesh sieve.

In the second method (sieving-centrifugation), a sample of 250 cc of soil was first processed by the decanting-sieving technique after which the material retained in two passages through a 325-mesh sieve was combined in a 250-ml centrifuge bottle and processed by the direct centrifugation procedure described above. In the elutriation procedure, samples of 250 ml each were processed by Seinhorst's standard elutriation procedure.

In a typical experiment, eight samples of a sandy loam soil naturally infested with a mixture of nematodes including *Pratylenchus penetrans*, *Xiphinema americanum*, and a *Criconemoides* sp. were processed by each of the three techniques. The average numbers of *P. penetrans* recovered by direct centrifugation, sieving-centrifugation, and Seinhorst elutriation, respectively, were 106, 74, and 76; of *X. americanum*, 8, 13, and 2; and of *Criconemoides* sp., 28, 22, and 1. Variation in numbers of each species recovered was greater among the samples processed by direct centrifugation than among those processed by the other procedures; this is attributed at least in part to the use of smaller samples in the former procedure. When similar comparisons of these three techniques were made in other experiments, there were some exceptions to the above pattern of results. For instance, although direct centrifugation consistently recovered more *P. penetrans* than sieving centrifugation, elutriation sometimes recovered about equal numbers or more than direct centrifugation. In one instance when a relatively high population of *X. americanum* was present, the numbers recovered by direct centrifugation, sieving-centrifugation, and elutriation, respectively, were 325, 643, and 686 per 250 cc of soil. In general, however, the direct centrifugation procedure used in these experiments consistently recovered more *Criconemoides* sp. and about

equal numbers of *P. penetrans* and *X. americanum* as the other methods with which it was compared. Furthermore, in one case in which there was a *Paratylenchus* sp. present in the soil, 4520, 4120, and 2260 per 250 cc of soil were recovered by direct centrifugation, sieving-centrifugation, and elutriation, respectively. Direct centrifugation is the least laborious and time-consuming of the three methods and is recommended in those instances where one seeks a reasonable chance of recovering many different kinds of nematodes from soil. The processing of duplicate samples to reduce the variability inherent in the procedure requires less time and labor than does the processing of single samples by either of the other two methods.—*Department of Plant Pathology, Cornell University, Ithaca, NY 14850.*

ENDO, B. Y., AND W. P. WERGIN. *Fine-structural changes in red clover (Trifolium pratense) roots during penetration by the root-knot nematode, Meloidogyne incognita.*

Intercellular migration of root-knot larvae into the primary root tissues of 'Kenland' red clover was characterized by compression and invagination of cell walls in front of and along the penetration zone of the nematode. In contrast to the undifferentiated thin-walled cells in the root tip, many cells adjacent to and immediately in front of the infective larvae had distinct cell wall thickenings with associated dictyosome activity and presence of microtubules. Electron-dense material which may have been extruded by the nematode was often found between the nematode lip region and adjacent cell walls.

Cells that appeared compressed by the invading larvae showed an increase in cytoplasmic density and enlargement of nucleoli. Numbers of free cytoplasmic ribosomes within these affected cells appeared higher than in adjacent cells. The altered shape and organelle complement of clover cells affected by the nematode may be related to the metabolic changes suggested in a recent histochemical study of root-knot infections of soybeans. —*Plant Science Research Division, Agricultural Research Service, U.S. Department of Agriculture, Beltsville, MD 20705.*

FASSULIOTIS, G. *Tolerance of Hoplolaimus columbus to high osmotic pressures, desiccation, and high soil temperatures.*

Hoplolaimus columbus from Swansea, South Carolina, were exposed to varying molar concentrations of potassium iodide, sodium chloride, sodium sulfate and urea for 16 hr and then transferred to distilled water. The nematodes were observed over a three day period. The nematodes ceased movement in the following concentrations of electrolytes: potassium iodide (0.2M), sodium chloride (0.4M), and sodium sulfate (0.6M). In the non-electrolyte, urea, movement stopped only in the 1M concentration. After three days in water, 10% recovered from the potassium iodide, 30% from the sodium chloride, 55% from the sodium sulfate and 90% from the urea treatments. From soil stored dry (0.2–0.3% soil moisture) in open quart containers in the laboratory for over one year and washed, using Cobb's sifting and gravity method, *H. columbus* became active in water within 24 hours. Exposure of 50 gram samples of this dry soil to 50, 60, 70, and 80 C for one hour yielded 80, 82, 65, and 0% motile nematodes, respectively, after one week in water. Both preadults and adults survived the dry soil and at the temperatures where motile nematodes were obtained. No other nematode species known to be present in the soil survived any of the treatments.—

U.S. Vegetable Breeding Laboratory, P.O. Box 3348, U.S. Department of Agriculture, Agricultural Research Service, Plant Science Research Division, Charleston, SC 29407.

GORDON, R., AND WEBSTER, J. M. *Nutritional requirements of the parasitic nematode Mermis nigrescens for protein synthesis.*

Adult desert locusts were experimentally infected *per os* with 50 *Mermis nigrescens* ova. Larval nematodes were removed 14, 17, 21 and 24 days after infection for *in vitro* radiotracer studies. At each of these times, nematodes were incubated axenically in a chemically-defined nutrient medium (*p*H 6.35) and the rates of incorporation of exogenous ^{14}C-leucine into nematode proteins compared. Such incubations were conducted at 30 C over both a 1 hr and a 24 hr period and the *in vitro* rates of protein synthesis were related to the *in vivo* growth pattern of the nematode. Proteins were synthesized at a non-linear rate by each of the larval stages. The 17-day-old larvae incorporated ^{14}C-leucine into proteins more rapidly than the 14-, 21- or 24-day-old larvae during the initial 12 hr incubation, and approximately twice as many ^{14}C-leucine moles into proteins as did the three other larval stages in a 24 hr incubation period. The 14-, 21- and 24-day-old larvae all synthesized proteins at approximately the same rates. This *in vitro* synthesis of proteins corresponded to the *in vivo* growth pattern of the nematode, because its total dry weight and protein content increased most rapidly between days 17 and 21 of infection. However, the total dry weight of the nematode increased more rapidly than the corresponding protein level during the second week of infection, indicating that the nematode synthesized relatively large amounts of non-protein reserves during that period. Such reserves were catabolized by the nematode during the latter 3–7 days of infection, when protein synthesis continued at a slower rate. Thus, the dietary requirements of the nematode may vary both quantitatively and qualitatively during its development. Nematodes at a specific stage of development (17 days) were incubated for 1 hr at 30 C in insect saline (*p*H 6.35) and the incorporation into nematode proteins of various ^{14}C and ^{3}H-labelled exogenous precursors compared. The larvae incorporated six ^{14}C-amino acids into proteins to varying degrees. On a molar basis, leucine was incorporated most effectively by the nematode, and glutamic acid was least readily incorporated into nematode proteins. The nematode, however, was unable to catabolize and incorporate more complex molecules, such as the dipeptide ^{3}H-histidyl-^{3}H-leucine or a preparation of ^{3}H-hemolymph proteins, into its own proteins. By contrast, ^{14}C-glucose was metabolized and incorporated into proteins more readily than either glycine or glutamic acid, indicating that the larval *M. nigrescens* may require a dietary supply of carbohydrates, in addition to amino acids, for protein synthesis.—*Supported by National Research Council of Canada Grant #A4679.* *Pestology Centre, Department of Biological Sciences, Simon Fraser University, Burnaby, Vancouver, British Columbia, Canada.*

HAMADA, G. H., AND H. HIRUMI. *The intestinal epithelium of Rhigonema infecta with special reference to unusual intranuclear inclusions.*

The intestine of *Rhigonema infecta* is a slightly flattened tube consisting of a single layer of cells, approximately 40 μm thick. The cells are bounded on the basal border by a basal lamina, and apically by a brush

border consisting of elongate filiform microvilli with an enteric coat. A terminal web is present adjacent to the brush border. Filaments from the terminal web project into the microvilli. Hollow microgranules, 24 nm in diameter, and larger dense granules 175 by 350 nm, are present in the terminal web. The apical cell junction is a *zonula adherens*, 2 μm long, with an 8 nm intercellular space between the adjacent plasma membranes. The basal cell junction consists of interdigitating plasma membranes. The cytoplasm contains numerous elongate mitochondria, generally concentrated in the apical half of the cell. Profiles of rough endoplasmic reticulum and dictyosomes are scattered throughout the cytoplasm, often in intimate association with one another. Microtubules are observed following aldehyde fixation. Large, circular, membrane-bound, electron-dense granules are present in the cytoplasm. Large, membrane-bound vesicles, containing a variety of circular lamellar structures, granular and membranous material, are also present. The nucleus is located near the basal plasma membrane. It contains nucleoli and has a porous nuclear envelope. Unusual elongate particles are observed in some of the nuclei. These undulating, tubular particles, 23 nm in diameter, up to 440 nm in length, may be either tightly or loosely packed within the nucleus and contain a less dense core surrounded by a dense border. They do not resemble any previously described intranuclear inclusions of animal cells. However, the morphology of these particles closely resembles the nucleocapsids of the Paramyxoviruses. Additional investigations will be conducted to determine the nature of these unusual particles.—*Supported in part by U.S. Public Health Service Research Grant No. AI-07687. Queensborough Community College, City University of New York, Bayside, NY 11364; and Boyce Thompson Institute for Plant Research, Yonkers, NY 10701.*

HANSEN, E., E. A. YARWOOD, AND E. J. BUECHER. *Temperature effects on sex differentiation in Aphelenchus avenae.*

In axenic cultures incubated at 23–27 C *A. avenae* hatched as second stage larvae and matured as parthenogenetic females. At 29–32 C males appeared and were sometimes more numerous than females. To test the effect of temperature on sex differentiation, single eggs were isolated in hanging drop micro-cultures and incubated for different periods at alternating temperatures of 23 C and 30 C. Hatching and development were observed daily. Eggs deposited at 30 C or their hatched larvae moved to 23 C before late second stage became females. After the late second stage the sex was irreversible and all maturing larvae became males whether left at 30 C or moved to lower temperatures. Conversely, eggs deposited at 23 C and moved to 30 C before the unhatched larvae began the first molt became males. After the first molt, maleness could not be induced by moving the eggs to 30 C. All adults from these eggs were parthenogenetic females even though developed at 30 C.—*Supported by U.S.P.H.S. Grant A1-07359 N.I.A.I.D. Clinical Pharmacology Research Institute, 2030 Haste Street, Berkeley, CA 94704.*

HARRISON, M. B. *Evidence of the mode of action of a systemic nematicide.*

The effect of DuPont's 1410 nematicide (S-methyl 1-(dimethylcarbamoyl)-N-[(Methylcarbamoyl) oxy] thioformimidate) upon *Heterodera rostochiensis* on *Solanum*

tuberosum was tested by foliar applications formulated 14.4 mg/L. A protective layer of vermiculite prevented contact of the spray with the soil surface. To be effective against *H. rostochiensis*, 1410 must be applied soon after potato plant emergence. Root growth, as determined by fresh root weight, was not affected by foliar-applied 1410. Potato root diffusate from treated plants stimulated less hatching from cysts, as did direct addition of 1410 to diffusate. Hatched second-stage larvae added to soil were able to develop in roots of treated plants. These results suggest that one effect of 1410 upon *H. rostochiensis* is due to interference with larval hatch.—*Nematode Research Laboratory, Cornell University, Farmingdale, NY 11735.*

HARRISON, R. E., AND J. L. MURAD. *Population dynamics of nematodes inhabiting soil in controlled-burn and unburned pine forest in central Louisiana.*

The purpose of this study was to determine whether annual surface burning affects nematode populations.

Nematode populations in two adjacent ¼-acres in Roberts' Plots in a Louisiana loblolly pine forest near Urania, Louisiana were sampled monthly for twelve consecutive months. One of the plots had been burned annually since 1915 and the other, a control plot, had remained unburned since 1912.

The burned and unburned areas were subdivided into three subplots each. Soil core samples were obtained with a 100 cm × 2 cm LaMotte auger. Cores were withdrawn at depths of 10 cm (Layer I), 20 cm (Layer II) and 30 cm (Layer III). Samples collected within each subplot were grouped by layers and 250 g subsamples were taken from each of the three homogenized layers. Each 250-g sample was processed by Seinhorst's combined elutriation and sieving method. Nematodes recovered by this procedure were counted, fixed and individuals mounted for identification.

Several soil parameters were measured at each sampling: temperature, *p*H, and soil moisture, rainfall and atomspheric temperature were noted. Organic matter, bulk density, particle density and total porosity were measured once at the end of the sampling period in 1971. Statistical treatment of these data will be reported later upon completion of the second phase of this study. Nematode numbers were highest in both the burned and unburned plots in September. Counts were minimum in January for the unburned plot and in July for the burned plot. In both plots most of the nematodes were found in the upper 10-cm of soil. Layers II and III had lower nematode numbers but did not differ appreciably from one another in total counts. Layer I was consistently highest in total numbers and generic representation.

The ratio of total larvae to total adults was relatively constant from month to month and was approximately 4:1. Nematode genera recovered from both the burned and unburned plots were: *Acrobeles, Acrobeloides, Alaimus, Aphelenchus, Axonchium, Cephalobus, Criconemoides, Cryptonchus, Diplogaster, Ditylenchus, Dorylaimellus, Dorylaimus, Eucephalobus, Hemicycliophora, Mononchus, Mylonchulus, Pelodera, Plectus, Pungentus, Rhabditis, Tripyla, Tylenchus,* and *Xiphinema.* Genera recovered from the burned plot only were: *Discolaimus, Helicotylenchus,* and *Miconchus. Longidorus* was found only in the unburned area.—*Supported by McIntire-Stennis Cooperative Forestry Research Project MRP-22.* *Departments of Forestry and Zoology, Louisiana Tech University, Ruston, LA 71270.*

HIRSCHMANN, H., AND A. C. TRIANTAPHYLLOU. *Development and sex differentiation of Meloidodera floridensis.*

Development of *Meloidodera floridensis* was investigated by incubating larvae in chlorine-free tap water and by inoculating pine seedlings. Very few or no male nematodes developed in pine roots, whereas second-stage larvae kept in water molted and most developed into adult males. After 3 months, 56% of the larvae had developed to adult males, 27% had undergone at least the second molt but died before becoming adults, and 13% were still in the second larval stage. It appears that larvae of the polyploid, parthenogenetic *M. floridensis*, develop and differentiate as males under conditions of starvation, but grow, develop and differentiate as females when they infect a suitable host plant. Cholesterol added to the water increased the percent of larvae developing to adult males, but not consistently in the various tests. Similar but less striking effects were detected when testosterone propionate or beta-estradiol were added to the water. Development of males in water included three superimposed molts and no metamorphosis. The cuticles of third-stage male larvae were well developed with transverse markings, whereas those of fourth-stage larvae were very thin. The stylet became progressively smaller, and the cephalic framework fainter with each successive molt. The subventral esophageal glands lost their identity with the onset of molting. All these structures, however, were again well developed in adult males. Third- and fourth-stage larvae had broadly rounded tails with small papilloid phasmids. The reproductive system gradually increased in size from the four-celled primordium in second-stage larvae to the single male gonad, which in the adult consisted of a short testis and an extended *vas deferens* filled with spermatozoa. Formation of the spicule primordia began during the second molt. Development of females in pine roots included three molts and a substantial increase in body width following feeding. Cuticle and hypodermis increased in thickness. The stylet of third-stage larvae was smaller, but that of fourth-stage larvae was larger than that of infective larvae. The esophageal metacorpus and dorsal gland increased in size progressively, but the subventral glands atrophied. The tail was rounded and inconspicuous in fourth-stage larvae. The reproductive system in third-stage larvae extended anteriorly and posteriorly to form the elongated and twisted gonads of the didelphic female. Specialized ventral chord nuclei, instrumental in vagina formation, were present in third-stage larvae. In growth chamber tests at 28/24 C day/night temperatures, third-stage female larvae developed in 18 days, fourth-stage in 25, and adult females in 45 days.—*Department of Plant Pathology and Department of Genetics, respectively, N. C. State University, Raleigh, NC 27607.*

HOPPER, B. E. *The taxonomic status of Pratylenchus neglectus (Rensch).*

In 1960, Loof designated a neotype for the taxon *Pratylenchus neglectus* (Rensch, 1924) Chitwood and Oteifa, 1952 from among five isolates of *Pratylenchus* populations extracted from the root systems of type host plants collected at the type locality in Germany. Subsequent analysis of the results from propagation trials suggested the conspecificity of *P. minyus* Sher and Allen, 1953 with *P. neglectus*. Consequently, the two species were synonymized. Seinhorst (1968) rejected this synonymy and relegated *P. neglectus* to *species inquirendae* on the grounds that more than one species was dis-

covered in the type localities and that diagnostic features in Rensch's original figures were inconclusive. Corbett (1969) questioned Seinhorst's proposals and felt that the synonymy "required clarification, as Loof's neotype of *P. neglectus* went unchallenged for eight years." While the synonymy of the two species may require clarification, the recognition and acceptability of Loof's neotype is the more important issue to be considered. As Loof's procedures were in compliance with the qualifications specified under Article 75, Section C, of the International Code of Zoological Nomenclature, the neotype must be recognized and accepted scientifically and *P. neglectus* regarded as a valid species.—*Nematology Section, Entomology Research Institute, Canada Department of Agriculture, Ottawa, Ontario, Canada.*

R. S. HUSSEY. *Serological relationships in root-knot (Meloidogyne* spp.) *nematodes.*

The purpose of this study was to investigate serological differentiation of *Meloidogyne* species. Adult females (ca. 7,000/cc) of two populations each of *M. incognita* (190-Taiwan, 189-Peru) and *M. arenaria* (54-Virginia, 17-Greece) were suspended (1:1½ v/v) in cold buffer at *p*H 7.4, freshly prepared from 0.05 M potassium phosphate, 0.85% NaCl and 0.02 M $MgCl_2$. Nematodes were homogenized with the aid of powdered glass in a Ten-Broeck power-driven tissue homogenizer. The homogenate was centrifuged 30 min at 20,000 *g* at 4 C, and the supernatant fluid served as the source of antigens. Antigen preparations were emulsified (1:1 v/v) with Freund's incomplete adjuvant, and 1 ml was injected into the foot pad of each hind foot of a rabbit to induce antibody formation. Rabbits were injected at 3–4 week intervals and bled 8–12 days after the third and subsequent injections. Eight to 10 immunoprecipitates occurred in a double immunodiffusion system. Precipitin bands characteristic for *M. incognita* were observed. Most of the precipitin bands, based on band position and coalescence, however, were common for both *Meloidogyne* species. Antisera specific for *M. incognita* was prepared by cross-absorbing antisera to *M. incognita* with antigens from *M. arenaria.* These results suggest that serological techniques can be successfully applied to taxonomic studies and be useful for identification of root-knot nematodes.—*Plant Pathology Department, N. C. State University, Raleigh, NC 27606.*

JOHNSON, A. W., AND G. W. BURTON. *Effects of two nematicides on a mixed population of five species of plant-parasitic nematodes and on yield of selected millet and sorghum-sudangrass hybrids.*

A 15% granular formulation of ethyl 4-(methylthio)-m-tolyl isopropylphosphoramidate (Bay 68138) and a 10% granular formulation of 2-methyl-2-(methylthio) propionaldehyde *O*-(methylcarbamoyl) oxime (UC 21149) were surface applied to the soil (11.4 kg active/ha) and immediately disk-incorporated on May 13, 1969. These materials were field-plot tested for control of a mixed population of nematodes and effect on yield of pearl millet varieties, 'Gahi 1', 'Tift 23 A × 186', 'Millex 22', 'Tift 23 A × 1268', 'Tiflate', 'Pennington's Haygrazer', and the sorghum-sudangrass hybrids, 'Funk's sorghum × sudangrass hybrid 78' and 'Haskell Harris 1746E'.

The plant-parasitic nematode population of test plots initially averaged 8 (5–45) *Criconemoides* sp. (ring), 38 (5–115) *Pratylenchus* sp. (lesion), 7 (1–40) *Trichodorus* sp. (stubby-root), 16 (5–120) *Belonolaimus*

sp. (sting), and 12 (5–190) *Xiphinema* sp. (dagger) nematodes.

Experimental design was a split-plot with nematicide treatment as whole plots (4.6 × 15.8 m) and host species and varieties the subplots with five replications. Treated and control plots were arranged in five randomized blocks. Single-row plots (on 1.8 m centers) of eight sorghum-sudangrass or millet hybrids were randomized within whole plots and seeded (approximately 45 seed/30.8 cm in drill) May 21.

Plant-parasitic nematodes were separated from 150-cc soil samples (composite of 10 cores, 2.5 × 20 cm, from each plot) by centrifugal-flotation on June 23, August 7, and September 25.

The nematode count data indicated that by June 23, nematicide treatments had significantly reduced the number of ring, lesion, stubby-root and sting nematodes in the soil. This reduction in relation to the control increased by August 7. By September 25, however, plots treated with UC 21149 contained significantly more ring nematodes than the control plots, suggesting that its nematicidal properties had disappeared and the ring nematode, possibly freed from competitors by UC 21149, was able to increase rapidly in number and actually exceed the control. By September 23, there were no longer differences in numbers of stubby-root nematodes as a result of soil treatment. Control of sting nematode by both nematicide treatments continued to be effective through September 25.

Dry matter forage yields were determined on July 30 and September 16. Total yield data revealed that UC 21149 and Bay 68138 were equally effective in increasing average yields of the eight entries by 35%. When all soil treatments were averaged 'Tift 23 A × 186' and 'Gahi 1' were the top-yielding varieties. When nematicides were applied, these top millets yielded only 12% more than Haskell Harris' '1746E' sorghum-sudangrass hybrid and where nematicides were not applied these millets yielded 41% more than the sorghum-sudangrass hybrid.

Nematode counts suggested that 'Tiflate' pearl millet was a poorer host for ring, lesion, stubby-root, and perhaps sting nematodes than the other varieties. Nematode data also indicate that lesion and sting nematodes were primarily responsible for reduction in yield of these sorghum-sudangrass hybrids and millet varieties.—*Plant Science Research Division, Agricultural Research Service, U.S. Department of Agriculture, University of Georgia, Coastal Plain Experiment Station, Tifton, GA 31794.*

JOHNSON, S. R., AND J. M. FERRIS. *Nematode community structure of selected deciduous woodlots.*

Eighteen Indiana deciduous hardwood stands of varying composition, soils, physiography, and past management practices were sampled for nematodes in April, July, and October of two successive years. Woodlots with similar nematode species were found by means of a resemblance equation to be similar in dominant tree species and soil type. Sites that had undergone major disturbances were the most dissimilar. A community ordination utilizing density and frequency for each species yielded results similar to those based on the resemblance equation but provided more information on species relationships and ecological distance between sites. Separate ordinations of the orders Tylenchida and Dorylaimida gave results similar to the ordination of all species combined. The dorylaimid ordination showed the greatest degree of dissimi-

larity among sites of the ordinations calculated. This indicated that dorylaimids were more sensitive to habitat changes and that changes in their communities were more indicative of disturbance. An ordination based on biomass of each species showed a remarkable degree of similarity to the dorylaimid ordination, indicating the influence of this order when size is a consideration.—*Supported by National Science Foundation Grant GZ-416. Department of Entomology, Purdue University, Lafayette, IN 47907.*

McKENRY, M. V., AND I. J. THOMASON. *Soil porosity, moisture, and temperature effects on persistence and degradation of Telone nematicides.*

By direct sampling and gas chromatography the diffusion patterns and persistence of the components of Telone® (Dow Chemical Co., Midland, Michigan) were monitored in several soils for periods ranging 3 to 12 weeks. The degradation of both *cis*- and *trans*-1,3-dichloropropene (1,3-D) was also estimated. Soil porosity and moisture level had the greatest influence on diffusion and persistence. In relatively dry soils the greatest loss of the chemical was to the atmosphere. The degradation of 1,3-D is a gradual process occurring most rapidly at higher temperatures (25 C). Degradation is only one of several factors affecting persistence and its importance varies with soil conditions. In moist, warm (25 C) soils the 1,2-dichloropropane (1,2-D) component persists for longer periods than *cis*- or *trans*-1,3-D. In contrast, degradation of 1,3-D in moist, cold soils (5 C) is slowed and thus it persists for as long as 1,2-D. Fumigation of extremely wet soil results in essentially no diffusion and a persistence of the chemical in a restricted volume of soil for many weeks. In an extremely dry soil (0.4% moisture by weight in a sandy loam), Telone is sorbed to the soil particles and no diffusion occurs. However, this degree of dryness would rarely occur in field soils except on a sun-dried soil surface.—*Supported by NIH Grant ES 00084. Department of Nematology, University of California, Riverside, CA 92502.*

MAI, W. F., AND G. S. ABAWI. *Effectiveness of the double-plant and nematode-transfer techniques for testing transmission of tobacco rattle virus by Trichodorus christiei at 20, 25, and 30 C.*

In transmission tests with a California strain of tobacco rattle virus (TRV) by a New York isolate of *Trichodorus chistiei* Allen, *Nicotiana glutinosa* L. was found more suitable than tomato (*Lycopersicon esculentum* Mill. 'Bonny Best'), pepper (*Capsicum frutescens* L. 'California Wonder') or tobacco (*N. tabacum* L. 'Turkish' and 'Samsun NN') and was used exclusively for virus source assay. TRV was recovered by mechanical inoculation from 37 of 37 newly formed leaves of source plants but from only 13 of 37 distal 1-cm segments of roots of the same plants. *N. glutinosa* and tomato were used as bait plants. Three-to-four-week-old seedlings of *N. glutinosa* were transplanted into 6.25-cm pots filled with a mixture of sterilized sand and sandy loam soil (3:1). Two weeks later they were mechanically inoculated with TRV and after an additional week about 500 *T. christiei* were added. After an acquisition period of 2 weeks, nematode transmission of TRV was tested either by a double-plant technique (plant cut at soil level and bait plant transplanted into pot) or a nematode-transfer technique (nematodes extracted and added to bait plants) at 20, 25, or 30 C. Two to

3 weeks after nematodes were added, bait plants were assayed for TRV by mechanical inoculation of crude root extracts on *N. glutinosa*. A high percentage of transmission of TRV (as high as 11/12 plants) was obtained by the double-plant technique. When results of four experiments conducted under 14 hr fluorescent light/day at 2000 ft-c and about 70% relative humidity were combined, highest transmission (32/70) was obtained at 25 C; 21 of 55 were infected at 20 C and 6 of 28 at 30 C. Low transmission (2/113) of TRV was obtained by a nematode-transfer technique. Also high transmission (22/53) was obtained by the double-plant method under variable greenhouse conditions. The double-plant and nematode-transfer methods also were compared with infected source plants growing in liquid culture in wide-mouth vials, each containing about 2.5-cm of water, and enough sand to cover the bottom. The acquisition and transmission periods each were 48 hr. Then seedlings were planted in 10-cm pots filled with greenhouse soil. High transmission (up to 10/16) was obtained by the double-plant technique but no transmission (none in four experiments) was obtained by the nematode-transfer technique.—*Supported by NSF Grant Number GB 6928.* *Department of Plant Pathology, Cornell University, Ithaca, NY 14850.*

MARKS, C. F., J. M. ELLIOT, AND C. M. TU. *Control of Pratylenchus penetrans on flue-cured tobacco in Ontario.*

Five nematicides were compared for control of the root-lesion nematode *Pratylenchus penetrans* on Fox loamy sand. Four replicates of each treatment were applied to .0104 ha plots in a randomized block design. Tobacco seedlings, cv. 'Hicks Broadleaf', were planted on May 31, 4 weeks after application of the fumigants and 10 days after application of the non-fumigant nematicides. Chemicals, and their rates (active ingredient) of application were: 1,3-dichloropropene, 1,2-dichloropropane and related C_3 hydrocarbons, D-D®, 224.6 l/ha broadcast, 89.9 l/ha row; methyl isothiocyanate 20% + 1,3-dichloropropene and related C_3 hydrocarbons 80%, Vorlex®, 56.2 l/ha broadcast, 22.5 l/ha row; 0,0-diethyl 0-(P-(methyl sulfinyl) phenyl) phosphorothioate, Dasanit® E.C. 6, 11.2 kg/ha broadcast; 3-dihydro-2,2-dimethyl-7-benzofuranyl N-methylcarbamate, Furadan® 75% W.P., 11.2 kg/ha broadcast in 1969 and 6.7 kg/ha broadcast in 1970; and 0-ethyl S,S-dipropyl phosphorodithioate, Mocap® 10% G., 11.2 kg/ha broadcast. D-D and Vorlex were injected 15–18 cm deep with an additional 18-cm soil ridge to seal in the chemical in the row applications. All other chemicals were applied to the soil surface and incorporated to a depth of 18–20 cm with a rotovator. Nematode population densities in the soil were assayed by periodic sampling throughout the growing season. Nematode numbers in the roots were determined at harvest.

Mocap was the most effective nematicide in terms of nematode control and crop response. When initial nematode population densities were relatively high the D-D, Vorlex and Mocap-treated plots produced higher yields ($P = 0.05$) than the controls. At relatively low initial population densities there were no significant yield differences. This suggests that the use of nematicides at low populations of *P. penetrans* is not warranted under Ontario conditions. Crop yield was not consistently correlated with either the numbers of nematodes in the soil or in the roots at harvest. Control of *P. penetrans* during the first few weeks after transplanting is most important. Once the plant is well established, it can tolerate relatively large num-

bers of these nematodes.—*Research Station, Canada Department of Agriculture, Vineland Station, Delhi, and London, respectively, Ontario, Canada*

MILLER, L. I. *Physiologic variation within the Virginia-2 population of Heterodera glycines.*

Physiologic variation of single cyst cultures of *Heterodera glycines* from seven selected areas within a field of the Va.2 population was compared to a composite culture (CVa.2 = Race 2, designated by the Terminology Committee at the 1969 Soybean Cyst Nematode Workshop) started from cysts from the entire field. Comparisons were made of the ability of the cultures to develop egg-bearing females in interaction with the following varieties or plant introductions of *Glycine max*: 'Lee', 'Peking', P. I. 90763, and P. I. 79693. Seventy cysts each containing 185–250 eggs/cyst, from 8-week-old cultures of single-cyst isolates (C1, C2, C3, C4, C5, C6, C7) and CVa.2 propagated on 'Lee', were introduced into methyl bromide-fumigated Ruston loamy fine sand soil in 10-cm pots. A single seed of each soybean variety or plant introduction was planted in each pot and greenhouse-grown at air temperatures 24–29 C. Each treatment was replicated four times. After 5 weeks the soil in each pot was screened, and the numbers of mature females were counted. Based on the criteria established by the 1969 Terminology Committee [positive rating (+) more than 10%, negative rating (–) less than 10% of the reproduction on 'Lee'], five races could be distinguished among the seven single-cyst cultures and the CVa.2 culture. The average reproductive rating of the cultures on 'Peking', P. I. 90763, and P. I. 79693, respectively, were C1 = –, –, +; C2, C4, C7 = +, +, +; C3, C5 = –, –, –; C6 = +, –, –; CVa.2 = +, –, +. The average number of females/pot produced by the cultures on 'Lee' were C4 = 34; CVa.2 = 63; C2 = 81; C7 = 107; C6 = 196; C3 = 247; C1 = 605; C5 = 750. Four different ($P < 1\%$) groups could be distinguished with respect to the number of females formed on 'Lee' from these data: (i)C4, CVa.2, C2, C7; (ii)C6, C3; (iii)C1; (iv)C5. Since levels of reproduction of different isolates of *H. glycines* may vary greatly on 'Lee', it is questionable whether this variety should be used as a standard to compute the reproductive rating of other varieties and lines of *G. max*.—*Department of Plant Pathology and Physiology, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061.*

MINTON, N. A., AND E. D. DONNELLY. *Field reaction of sericea lespedeza to root-knot nematodes.*

Several sericea lespedeza (*Lespedeza cuneata*) breeding lines and varieties were evaluated for two years in both root-knot (*Meloidogyne* spp.)-infested and non-infested replicated field plots at five widely separated locations in Alabama and Georgia. Soils ranged from a loamy sand to a heavy clay. The level of root-knot nematode larvae in the soil at all locations was determined periodically. Root-knot nematode species determinations were made. Roots from the southern Georgia test were rated periodically for galling, and the relative numbers of the different root-knot species present in the roots were determined in 1970. Forage yields were obtained by haying two or three times each year. Several sericea breeding lines were classed as resistant to *M. incognita*, *M. incognita acrita*, and *M. hapla* in greenhouse tests and two varieties were found resistant to *M. incognita acrita*. Root-knot galling and larvae numbers were less and for-

age yields were as much as 57 times greater for resistant entries than for the susceptible check grown in root-knot infested field soil for three growing seasons. Lines resistant to *M. incognita acrita* generally had higher tolerance to *M. javanica* than the susceptible checks. A predominantly *M. incognita acrita* population following cotton (*Gossypium hirsutum*) and hairy vetch (*Vicia villosa*) on sandy soil in southern Georgia shifted to *M. javanica* under sericea.—*Plant Science Research Division, Agricultural Research Service, U.S. Department of Agriculture, University of Georgia, College of Agriculture Experiment Stations, Coastal Plain Station, Tifton, GA 31794, and Auburn University Agricultural Experiment Station, Auburn, AL 36830.*

MONGKOLKITI, S., AND R. M. HOSFORD, JR. *Biological control of the grasshopper Hesperotettix viridis pratensis by the nematode Mermis nigrescens.*

An epizootic occurrence of *Mermis nigrescens* was related to decline and disappearance of the grasshopper *Hesperotettix viridis pratensis*. The high infection rate was related to the low, wet habitat where the grasshopper fed primarily on *Solidago missouriensis*. *Melanoplus bivittatus*, feeding in alfalfa fields on drier ground, was only slightly infected. *M. sanquinipes*, *M. differentialis* and *M. femur-rubrum*, feeding on mixed plants on drier ground, were not infected. Infected females of *H. viridis pratensis* failed to develop ovaries and males remained sexually immature. This was the first known occurrence of a nematode infecting *H. viridis pratensis*. The juvenile nematodes left the dead grasshoppers through holes in the sides of the thorax and entered the soil within 30 to 60 minutes. Photographs were made of the previously undescribed juvenile stoma, the stylet, cephalic sensory organs and paired oval structures in the cerebral region. The results indicated that under certain localized environmental conditions *Mermis nigrescens* can control a grasshopper population.—*Departments of Entomology and Plant Pathology, North Dakota State University, Fargo, ND 58102.*

MYERS, R. F. *Effects of sub-lethal concentrations of cholinesterase-inhibiting nematicides in the diet of Aphelenchoides rutgersi.*

Aphelenchoides rutgersi was axenically cultured in an aqueous medium containing 2% yeast extract (NBC), 3% Bacto Soytone (Difco Lab.), and 10% chick embryo extract (GIBCO, Cat. #511). Various concentrations of cholinesterase-inhibiting nematicides were incorporated into this diet and total numbers, number of eggs, percent females, and percent mortality were determined at several intervals up to 21 days. In all cases reproduction was significantly retarded or totally stopped at 10 ppm (w/v) and only Nemacur (Ethyl 4-(methylthio-*m*-tolyl isopropylphosphoramidate) increased mortality at this concentration. Reproduction was normal when one ppm Nemaphos (*O, O*-diethyl *O*-2-pyrazinyl phosphorothioate), Temik (2-methyl-2-(methylthio)propionaldehyde *O*-(methylcarbamoyl)oxime), or Du Pont 1410 (s-methyl 1-(dimethylcarbamoyl)-N-[(methylcarbamoyl)oxy]thioformimidate) were added to the substrate, slightly retarded by one ppm Furadan (2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate) was added, but severely retarded when the same amount of Dasanit *O,O*-diethyl *O*-[*p*-(methylsulfinyl)phenyl] phosphorothioate) or Nemacur were included. Temik, Nemacur, and Du Pont 1410 at concentrations of 0.1 and 0.01 ppm in the

nematode's diet, significantly increased production of eggs compared with controls. Numbers of nematodes also significantly increased. If the rough approximation of 1 ppm = 2 lbs/acre is accepted, and the recommended lbs/acre active ingredient of these nematicides are applied, then it would appear that control with this group of cholinesterase inhibiting nematicides is mainly effected by retarding or eliminating reproduction instead of killing nematodes.—*Supported by NSF Grant GB-18012.* *Department of Entomology and Economic Zoology, Rutgers University, New Brunswick, NJ 08903.*

NICKLE, W. R. *A contribution to our knowledge of the Mermithidae.*

The family Mermithidae represents a large group of useful nematode parasites of insects. There are about 45 genera in this family, approximately 30 of which are poorly described. Some of these descriptions were based only on larval stages, occasionally only one sex was known, and often no host information is given. This situation has led to a great deal of guesswork which has complicated the systematics of this group. Entomologists, parasitologists and phytonematologists have published articles over the last 225 years in a dozen different languages on mermithid taxonomy. Accessibility of described specimens for comparison has been very limited. I have been fortunate enough to study adult material from the Steiner Mermithid Collection, the Petersen Louisiana material, and other collections, and have described and illustrated about 15 of the more important genera. These up-to-date descriptions, usually of the type species material, show the morphology of the males, females, and eggs, and also host information and other biological information necessary for identification at least to genus. Certain mermithids, *Hexamermis albicans*, *Mermis nigrescens*, *Reesimermis nielseni*, and *Agamermis decaudata*, are worldwide in distribution and are not host-specific. *A. decaudata* vary in length from 3 to 45 cm with corresponding variability in other measurements. Other species vary equally as much. Two new genera are proposed. One is based on the adult stages of *Agamomermis culicis*, which kills a large percentage of the salt marsh mosquito, *Aedes sollicitans* on the East and Gulf coasts of the USA. The other new genus is parasitic in *Anopheles* mosquitoes.—*Plant Science Research Division, Agricultural Research Service, U.S. Department of Agriculture, Beltsville, MD 20705.*

O'BANNON, J. H., AND A. T. TOMERLIN. *Susceptibility of Radopholus similis-resistant citrus species to Pratylenchus coffeae and Tylenchulus semipenetrans.*

Ten citrus rootstocks with differing resistance, tolerance, or susceptibility to *Radopholus similis* were separately infected with *R. similis*, *Pratylenchus coffeae*, or *Tylenchulus semipenetrans* and grown for study of individual nematode response and behavior under comparable conditions. Six-month-old seedlings were uniformly infected by transplanting them into separate soil bins heavily infested with each nematode. After 6 weeks, replicated infected and noninfected seedlings were individually transplanted into 20-cm pots. Plants were grown in the greenhouse at ambient temperatures for 1 year. Growth measurements and nematode counts were made at intervals and at harvest. Numbers of nematodes/g of root (in parentheses) and percent growth reduction of *R. similis*, *P. coffeae* and *T. semipenetrans*-infected seedlings were, respectively: 'Argentine TO' (*P.*

trifoliata, *R. similis*-susceptible) (50) 0, (3000) 49, (8) 25; 'Carrizo' citrange (*P. trifoliata* × *C. sinensis*, *R. similis*-resistant) (100) 33, (1500) 70, (300) 14; 'Troyer' citrange (*P. trifoliata* × *C. sinensis*, *R. similis*-susceptible) (40) 42, (2600) 68, (250) 37; 'Yuma' citrange (*P. trifoliata* × *C. sinensis*, *R. similis*-susceptible) (300) 45, (2100) 68, (100) 12; 'Algerian' navel (*C. sinensis*, *R. similis*-resistant) (40) 0, (1000) 60, (200) 36; 'Ridge' pineapple (*C. sinensis*, *R. similis*-resistant) (20) 0, (1800) 53, (50) 47; 'Smooth flat Seville' sour orange (*C. aurantium*, *R. similis*-susceptible) (90) 45, (2500) 63, (600) 35; 'Florida' rough lemon (*C. jambhiri*, *R. similis*-susceptible) (450) 80, (1100) 60, (30) 57; 'Estes' rough lemon (*C. jambhiri*, *R. similis*-tolerant) (500) 84, (2900) 62, (40) 59; and 'Milam' lemon (*Citrus* sp., *R. similis*-resistant) (0.1) 20, (2000) 73, (400) 1.

All *R. similis*-resistant varieties were attacked by *P. coffeae* and *T. semipenetrans*. Most severe infections and greatest growth reductions were caused by *P. coffeae*. 'Milam' lemon showed the highest degree of resistance to *R. similis*. 'Estes' rough lemon, an *R. similis*-tolerant variety, was severely damaged by all nematodes in this study. 'Argentine TO' showed the highest degree of resistance to *T. semipenetrans*. No variety showed any resistance to *P. coffeae*. There was a marked variation in the degree of susceptibility between each rootstock with each nematode. In this experiment, none of the *R. similis*-resistant or tolerant rootstocks could be classified resistant to either *P. coffeae* or *T. semipenetrans*. 'Milam' lemon, a vigorous growing rootstock, exhibited tolerance to *T. semipenetrans*.—*Plant Science Research Division, Agricultural Research Service, U.S. Department of Agriculture, Orlando, FL 32803.*

ODIHIRIN, R. A. *Effects of root-knot and lesion nematodes on transpiration and water utilization by tobacco plants.*

Transpiration rates of nematode-infected and healthy tobacco (*Nicotiana tabacum* L.) plants were investigated. Measurements, based on total loss of water per unit area of leaf per hr, were made every 2 weeks for 12 weeks. Two inoculum densities of the root-knot nematodes, *Meloidogyne incognita* and *M. hapla*, and one density of the lesion nematode, *Pratylenchus brachyurus*, were used. For *M. incognita* transpiration, rates were compared between infected and noninfected plants of susceptible and a resistant cultivars. A nematode-infected plant may transpire twice as much water as a healthy plant, depending on the stage of infection. During the first 8 weeks, infected plants used more water per day than healthy plants. After 12 weeks, the situation was reversed. The observed effects are influenced by temperature and also vary significantly with nematode species, inoculum density, and tobacco cultivar. Plant changes causing increased transpiration seemed to be more persistent when induced by *M. hapla* than by *M. incognita* and less persistent when induced by *P. brachyurus*. During the first 8 weeks, wilting was due to shortage of available water resulting from excessive transpiration. After 12 weeks, wilting occurred in most of the infected plants, even when moisture was at field capacity, apparently due to root damage. Leaf measurements, as well as dry weights and stem diameter of infected and noninfected plants, revealed that nematodes may stimulate growth in tobacco so long as soil water is not in short supply. The reaction of the resistant cultivar 'NC 95' was similar to the susceptible 'Coker 319' relative to excessive transpiration. Water use efficiency is less in nematode-infected

plants than in healthy plants; more water was required per unit increase of dry weight. The stomata seem to open faster during the early stages of infection. Another possible mechanism by which nematodes may damage plants is dehydration of the tissues, especially the leaves, resulting from rapid dehydration of the soil immediately adjacent to the roots.—*Supported by Rockefeller Fellowship. Department of Plant Pathology, North Carolina State University, Raleigh, NC 27607.*

OLTHOF, TH. H. A., AND J. W. POTTER. *The relation between preplant populations of Meloidogyne hapla and crop losses in field-grown vegetables in Ontario.*

From May until August, 1970, five kinds of vegetables were grown in clay drainage tiles of 20-cm diameter and 30-cm length, buried vertically on 1.2 m centres in the field, filled with 9 kg of Vineland loam infested with either 0, 666, 2000, 6000 or 18000 *Meloidogyne hapla* juveniles/kg of soil. Different infestation levels were established by diluting nematode-infested soil from a greenhouse groundbed with steam-sterilized soil. Each nematode population density was replicated 20 times for each crop. Planting, fertilization, insect control and grading followed commercial recommendations and standards. Counts of nematodes in the soil were made at planting time, at mid-season, and at harvest. The weight of marketable 'Pennlake' lettuce heads decreased progressively with increasing nematode population up to a 46% decrease at the 18000 density. The highest density reduced yields of 'Market Prize' cabbage and 'Igloo' cauliflower by 9 and 24%, respectively; cauliflower curd maturity was delayed 3 days. With 'Sebago' potatoes and 'Copper Gem' onions, the number and weight of marketable produce decreased with increasing nematode population densities, while number and weight of culls increased. Commercial losses with these crops amounted to 46% and 64%, respectively, at the highest nematode density. Extrapolated plots of the relationship between initial nematode density *vs* % marketable yield shows that an economic loss (5% or more of marketable yield) of cabbage would occur at a preplant density of 15000 *M. hapla*/kg of soil, from 5000–5500/kg for cauliflowers, 1000/kg for onions, and from 100–200/kg for lettuce and potatoes. Midseason soil populations determined 44 days after transplanting showed an average for all vegetables of 18, 79, 66 and 46%, respectively, of the initial four densities. With potatoes, final population densities exceeded initial densities at all four levels of preplant infestation. This was also the case for onions, except at the highest density. In contrast, preplant nematode densities were not maintained under lettuce and, especially, cabbage and cauliflower. With the latter two crops, only about 25% of the initial densities were recovered at harvest time.—*Research Station, Canada Department of Agriculture, Vineland Station, Ontario, Canada.*

ORBIN, D. P., AND E. J. CAIRNS. *Histopathology of soybean roots infected with Helicotylenchus dihystera (Cobb) Sher.*

Soybean roots artificially infected with *H. dihystera* in the greenhouse were stained in acid fuchsin-lactophenol or sectioned and stained with safranin and fast green. *H. dihystera* was found in all roots examined. Adults and larvae were observed both in semiendoparasitic feeding positions and completely within the roots. Adults, larvae, and eggs were observed within the root cortex posterior to the region of maturation. Small brown lesions were observed around the

nematodes but were limited to those six to ten cells in the immediate vicinity of the nematode. Endoparasitic nematodes were usually coiled within the walls of one or two cells. Cytoplasm of the infected cells appeared to be normal, and there was no indication of nuclear proliferation. The walls of the infected cells and those immediately adjacent were thickened and lignified. Nematodes were usually aligned parallel to the vascular tissue, but were not consistently oriented with respect to the root apex. Nematodes moved through cell walls rather than between them. However, no persistent burrows were observed in the path of the nematode, and there was no indication of swelling or giant cell formation.—*Supported by Project Ala 137 (S-19). Department of Botany and Microbiology, Auburn University, Auburn, AL 36830.*

PAULSON, R. E., AND J. M. WEBSTER. *Ultrastructure of the hypersensitive reaction of tomato root cells to Meloidogyne incognita.*

Hypersensitivity may be a mechanism for resistance of tomato (*Lycopersicon esculentum*) 'Nematex' to infection by *Meloidogyne incognita.* The purpose of this study was to define the changes in cell structure which occur during development of hypersensitivity.

Axenic seedlings, with radicles about 1 cm long, were infected with freshly hatched, sterile *M. incognita* larvae. Samples for microscopy were taken every 12 hr after infection.

Migration of larvae in root tissues resulted in some breakage of cell walls and displacement of cytoplasm. The hypersensitive reaction became most extensive in cells adjacent to the nematode after it had migrated to a more central position within the root tip. The earliest indication of hypersensitivity was increased affinity for stains used in light microscopy and increased electron density of the cytoplasm. The increased affinity of cells for stains was not due to increased numbers of cytoplasmic organelles.

Most cells in uninfected roots and those adjacent to hypersensitive cells within infected roots contain within their vacuoles an electron-dense deposit. This material, possibly some phenolic compound, disappears from the vacuoles of hypersensitive cells at about the same time cytoplasmic density changes were observed. In contrast, this material remains highly condensed within the vacuoles of adjacent cells. Increased cytoplasmic density and disappearance of vacuolar inclusions is accompanied by a loss of membrane distinctness throughout the cell; thus, membrane-bounded organelles such as Golgi bodies and mitochondria tend to disappear at an earlier stage. In contrast, plastids and nuclei with a more dense inner structure remain visible for some time after loss of all internal or limiting membranes.

No significant increase in abundance or size of organelles was observed with the exception of the endoplasmic reticulum which increased from short, isolated segments to long, branched channels throughout the cytoplasm as the hypersensitive reaction progressed.

Eventually the cytoplasm of hypersensitive cells became so electron-dense that resolution of fine structure was prevented. The disorganized parts of nuclei and plastids and conspicuously electron-permeable endoplasmic reticulum channels were the only recognizable cellular components.

Cells adjacent to hypersensitive cells showed no changes in ultrastructure. The hypersensitizing stimulus did not appear to induce a graded cell response away from the hypersensitive site.

In 'Nematex' seedlings infected by *M. incognita* at higher temperatures (33 C) the hypersensitive reaction was almost completely inhibited. Cells adjacent to the nematode showed no evidence of membrane breakdown or loss of the electron-dense material from the vacuoles, and giant cell formation resembled that observed in a susceptible interaction.

When *M. hapla*, to which 'Nematex' tomato is susceptible, infected the seedlings none of the characteristic hypersensitivity changes were observed.—*Department of Biological Sciences, Pestology Centre, Simon Fraser University, Burnaby, 2, B. C., Canada.*

PERRY, V. G., G. C. SMART, JR., AND G. C. HORN. *Nematode control on golf courses by injected DBCP.*

Surface applications of the nematicides Dasanit (O,O-diethyl O-[p-(methylsulfinyl) phenyl] phosphorothioate), Sarolex (O,O-diethyl O-(2-isopropyl-4-methyl-6-pyrimidinyl) phosphorothioate), and DBCP (1, 2-dibromo-3-chloropropane) are currently used for nematode control on golf courses in Florida. However, only *Belonolaimus longicaudatus* is effectively controlled by Dasanit and Sarolex, and phytotoxicity sometimes occurs with drench treatments of DBCP which must be rather high (45–95 kg/ha = 40–85 lb/acre) since significant amounts of surface-applied DBCP are lost. In exploratory tests, DBCP at 20–96 kg/ha (18–86 lb/acre) injected 5–13 cm (2–5 inches) deep on 30 cm (12-inch) centers was not phytotoxic and turf response was excellent at all rates. Thus, increased efforts have been made to develop satisfactory application equipment and to determine practical rates of DBCP. For treatments of fairways, tractor-mounted pressure or gravity flow injectors have proved satisfactory. In order to minimize damage to the turf, large coulters (61 cm = 24-inch diameter or greater) were used ahead of thin-bladed delivery shanks, and flat press wheels behind the shanks closed the slits. For application to golf greens and tees, a Jacobson "Sod Master Sub-Air" aerator was fitted with a pressure fumigant injector. The slow forward speed of this machine requires water dilution of the DBCP and capillary tube flow control. Excellent turf response was obtained by injecting 22–34 kg/ha (20–30 lb/acre) of DBCP with either machine. The lower rate was as effective as the higher rate. In our oldest (September 1969) fairway test turf response was maximum a year later and was still obvious after almost two years.—*Departments of Entomology and Nematology, and Ornamental Horticulture, University of Florida, Gainesville, FL 32601.*

PITCHER, R. S., AND D. G. MCNAMARA. *In vivo subterranean time-lapse photographic studies on the feeding of Trichodorus viruliferus on apple roots.*

The behavior of colonies of *T. viruliferus*, feeding under near-natural conditions, was photographed with pre-war Ciné Kodak Model-B cameras, adapted by Mr. E. Yoxall Jones to expose single frames, illuminated by electronic flash at chosen intervals. The camera was focused through the glass observation panels of an underground laboratory which was primarily designed to study the root growth of fruit trees planted adjacent to the plate glass windows. In making this laboratory, the soil profile was disturbed as little as possible. As a result, the nematodes shown were part of the natural population of the orchard in which the laboratory was built.

Two techniques were employed. The first, designed principally to show nematode

behavior, was taken at time-lapse intervals of 10–20 seconds between successive frames, accelerating the action about 200 times when projected at a speed of 16 frames per second. The second type was designed to illustrate root reaction to nematode attack and was usually photographed at a lower magnification with a lapse time of 7½ or 15 minutes, accelerating the action 7,000 to 15,000 times. Vividly illustrated root tip damage caused by the mass feeding of colonies of nematodes numbering up to 300 or more, clustered 1–2 mm behind the growing point. Stubby root symptoms (*sensu stricto*, as caused by *T. christiei*) did not develop on the 1–2 mm thick apple roots, but when moderate numbers (25–75) of *T. viruliferus* are present the epidermis is browned and the cortex split, allowing sub-epidermal feeding. Colonies numbering 100–300 usually kill the growing point, after which the nematodes soon leave the root and disperse into the soil.—*East Malling Research Station, Maidstone, Kent, England.*

POTTER, J. W., AND C. F. MARKS. *Effect of DuPont® 1410-X on rate of development of Heterodera schachtii on cabbage.*

The relative effectiveness of drench and foliar applications, and the relationship between method of application and type of nematicidal action, of the systemic nematicide DuPont® 1410-X 90% WP (S-methyl-1-(dimethylcarbamoyl)-N-[(methylcarbamoyl)oxy]thioformimidate) were studied with *Heterodera schachtii* on cabbage. The chemical was applied at 6 kg (actual)/ha as a drench or at 0.04 kg (actual)/100 litres as a foliar spray. Drench, pre-transplant spray and post-transplant spray treatments, alone and in various combinations, were applied in each of three successive experiments. Fresh root weights and numbers of cysts per root system were determined at the end of each experiment. Numbers of larvae in the soil were also determined at the termination of the second and third experiments. During the latter experiment, numbers of larvae in soil and roots, and cysts per root system were determined by three destructive samplings.

Drench treatments at transplanting reduced numbers of cysts per root by 50–90%. Drenches combined with post-transplant foliar sprays, at 2 weeks, reduced cysts per root by 95–98%. As a drench, the chemical did not kill nematodes in the soil but reduced the number of larvae penetrating roots and retarded the rate of development of those larvae which did establish feeding relationships. As a pre-transplant foliar spray, the chemical seemed to prevent larval penetration of roots or killed larvae during penetration. After 9 days exposure to roots of cabbage that had received a pre-transplant foliar spray, no larvae had entered although active larvae were numerous in the soil. Although post-transplant foliar application of 1410-X reduced the number of developing larvae, this would not be a satisfactory chemotherapeutic method against *H. schachtii*.—*Canada Department of Agriculture, Research Branch, Research Station, Box 185, Vineland Station, Ontario, Canada.*

PRIEST, M. F., AND C. J. SOUTHARDS. *Comparative morphology of sixteen isolates of Meloidogyne incognita.*

Males and day-old larvae of 16 isolates of *M. incognita* were heat relaxed, transferred to 2.5% formalin for 10 min, and mounted in type-B polyvinyl alcohol. Morphological measurements of 25 larvae and 10 males of each isolate were made with a phase contrast microscope. Highly significant differences ($P = 0.01$) were found among some isolates

for certain taxonomic characters, but not for all characters measured. In general, physiologic races were not distinct morphologically. Larvae of Isolate-10 were significantly different from all other isolates in total length (L), L/tail length (c), and, with one exception, in stylet length. Three other isolates of the same physiologic race as Isolate-10 were significantly different from Isolate-10 and from each other. The greatest variation of larvae of all isolates occurred in measurements of L, tail length (t), t/anal width (c′), and in the distance from the stylet base to the dorsal esophageal gland orifice (D.G.O.), in that order. The least variable larval characters were anal width, ratio c, and the ratio of esophagus length to the center of the median bulb/total esophageal length. Male measurements were less variable than larval measurements. The spicule lengths and total lengths of males were most variable. In a comparison of measurements of the 16 isolates of *M. incognita* with *M. javanica* and *M. hapla*, there was considerable overlap of several characters; however, the three species could usually be distinguished by one or more of the following measurements: D.G.O., c′, stylet length, or width of stylet knobs. Perineal patterns of all isolates were also quite variable, but, in general, were within the range reported for the species.—*Department of Agricultural Biology, University of Tennessee Institute of Agriculture, Knoxville, TN 37901.*

REBOIS, R. V. *The effect of Rotylenchulus reniformis inoculum levels on yield, nitrogen, potassium, phosphorus and amino acids of seed of resistant and susceptible soybean (Glycine max).*

Resistant and susceptible soybean cultivars, inoculated with *Rotylenchulus reniformis*, were grown to maturity in a greenhouse. Harvested seed were compared with those from non-infected controls for yield, nitrogen, potassium, phosphorus, and acid-hydrolyzable amino acids. The inoculum levels (IL) per 3.8-liter pot were as follows: 0, 5,000, and 25,000 nematodes for 'Bragg', 'Hampton 266', and 'Jackson'; 0, 1,000, 5,000, 10,000, and 25,000 nematodes for 'Hood', 'Lee', 'Dyer' and 'Pickett'. Reniform nematode-resistant cultivars, 'Dyer' and 'Pickett', did not receive the 1,000 IL. Inoculum levels of 1,000 to 10,000 were considered low and the 25,000 IL as high. Treatments were replicated and applied each year for three years. The highest IL was applied only in the last year.

Cultivars varied in their growth response and tolerance to ILs of 10,000 or less. At the 10,000 IL, 'Hood' dry-seed yields decreased 19.4% while 'Pickett' yields increased 18.1% over their respective controls. No significant changes were observed in the dry-seed yields of other cultivars at low ILs. Increasing the IL to 25,000 reduced the average dry-seed yields for resistant and susceptible cultivars by 33.1%.

In one test, only seed from infected 'Hood' and 'Jackson' plants showed a decreased nitrogen content. In this same test, seed nitrogen for all cultivars at the 5,000 IL was significantly lower than the controls by an average of 2.3%. However, when three years test data were averaged the seed nitrogen from all infected plants was not significantly lower than in the controls.

One year the total seed potassium and phosphorus was determined for all cultivars at inoculum levels of 0 to 10,000. The total potassium increased over the controls in 'Lee' and 'Hood' and was highest at 5,000 IL. When all cultivars were grouped by nematode treatments, the average increase in potassium was 5.5 and 4.6% at the 5,000

and 10,000 ILs, respectively. Phosphorus content of seeds varied significantly only in infected 'Lee', 'Dyer', and 'Pickett'. Compared with uninfected controls, 'Lee' seed contained more phosphorus at the 1,000 IL and less at the 10,000 IL. Phosphorus content of 'Pickett' and 'Dyer' seeds was lower than the controls at the 5,000 and 1,000 ILs. When all cultivars were grouped by nematode treatments, the phosphorus content was 11.1 and 11.6% lower in the 5,000 and 10,000 ILs, respectively, than in the controls.

Of the 17 amino acids analyzed, nematode infection consistently lowered the average leucine content of seeds in all cultivars receiving the 25,000 IL treatment. Amino acid analyses were not sufficiently replicated to detect significant changes. The average percent leucine in seeds from resistant plants was slightly less than the average decrease of 0.71% for all cultivars.

The greatest soybean losses from *Rotylenchulus reniformis* infections were due to reduced dry-seed yield and phosphorus content of seeds.—*Partially supported by U.S. Department of Agriculture Cooperative Research Agreement 12–14–100–5600 (34) with Auburn University, Auburn, AL 36830.* *Plant Science Research Division, Agricultural Research Service, U.S. Department of Agriculture, Beltsville, MD 20705.*

RIEDEL, R. M. *Cellulases in aqueous extracts of Ditylenchus dipsaci.*

Cellulase activity was studied in extracts of population of *Ditylenchus dipsaci* isolated from infected onion. Nematodes were cultured monoxenically on onion callus (*Allium cepa* L.) and collected aseptically in modified Baermann funnels containing Merthiolate Solution N.F.® Carboxymethylcellulase (C_x) activity was detected at *p*H 4.0 through 9.0 in viscometric tests at 30 C. C_x activity was optimum at *p*H 5.0 in 0.2 M citrate-phosphate buffer containing 1.0% sodium carboxymethylcellulose (CMC). Under these conditions detectable quantities of reducing groups were not released by C_x activity. Neither glucose nor cellobiose were detected by paper chromatography of buffered 1.0% CMC incubated with nematode extracts 24, 48, or 72 hr at 30 C under toluene. In unbuffered extracts heated at *p*H 7.0, C_x activity in viscometric tests was stable to 70 C for 10 min. Enzyme activity was decreased above this temperature, although some remained after heating at 100 C for 30 min.

Active nematode extracts released reducing groups from and caused reductions in tensile strength of insoluble, regenerated cellulose substrates at *p*H 4.0 through 7.0. At 30 C release of reducing groups by enzyme activity (C_1) was optimum at *p*H 5.0 in 0.2 M citrate-phosphate buffer. C_1 activity was destroyed in unbuffered extracts heated at 70 C for 10 min. C_1 activity on dewaxed cotton fibers was not detected. Aqueous extracts of *D. dipsaci* lacked detectable cellobiase activity.

Pathogen cellulases could aid penetration of onion tissues by *D. dipsaci*. Unbuffered nematode extracts heated at 70 C for 10 min lose their ability to macerate disks of potato (*Solanum tuberosum* L.) tuber tissue.

Data in this study suggest, therefore, that C_x cellulase activity alone probably does not cause maceration of host tissues. The possibility of maceration by interactions between C_x activity and other cell wall degrading enzymes as well as that of maceration caused by C_1 cellulase activity should be explored.—*Supported by Hatch Project 386.* *Plant Pathology Department, Ohio State University, Columbus, OH 43210.*

ROBBINS, R. T., AND K. R. BARKER. *Reproductive responses of Belonolaimus longicaudatus to soil type and temperature.*

Effects of soil type and soil temperature on reproduction of *Belonolaimus longicaudatus* were investigated under greenhouse conditions. 'Lee' soybean and 'Earlibelle' strawberry were used as host plants in separate experiments designed to determine the influence of soil type on the reproduction of *B. longicaudatus*. Treatments were replicated five times and included noninoculated controls and 1000 nematodes per 20-cm pot for each of the following "soils": (i) clay soil (42% sand, 24% silt, 34% clay); (ii) fine sandy loam (88, 4, 8); (iii) coarse sandy loam (80, 10, 10); (iv) a 1:1 mixture of fine sandy loam and 65-mesh screened silica sand (94, 2, 4); (v) 65-mesh screened silica sand (100, 0, 0); (vi) beach sand (100, 0, 0); and (vii) muck. Ninety days after inoculation, nematodes were extracted by the sugar-flotation-sieving method and counted. For soybean the reproduction factors ($Population_{final}/Population_{initial}$) for muck, clay, coarse sandy loam, fine sandy loam, 1:1 mixture, beach sand and 65-mesh sand were: 0.1, 0.3, 2.5, 15.9, 18.9, 18.9 and 28.9, respectively. The reproduction factors on strawberry were: 0.4, 0.4, 2.8, 13.9, 27.6, and 34.6, respectively. For the temperature study, 'Albritton' strawberry was used as the host in 20-cm pots filled with steamed Norfolk fine sandy loam. These pots were placed in 26-cm waterproof plastic pots, and the space between them was then filled with small gravel. Treatments included three replicates of 0, 100 and 1000 nematodes in water baths at 15, 20, 25 and 30 C. Sixty days after inoculation, the number of nematodes per pot was determined, using the sugar-flotation-sieving method of extraction. The reproduction factors were: .8, 1.0, 1.0, and 10.8 for the treatments with 100 nematodes at 15, 20, 25 and 30° C, respectively. Where 1000 nematodes were used, the reproduction factors were: .4, 1.1, 1.8 and 5.6 for the temperatures in the same order as above. Thus, *B. longicaudatus* requires a sand content of 80% or greater and a rather high soil temperature for high rates of reproduction.—*Department of Plant Pathology, North Carolina State University, Raleigh, NC 27606.*

RUEHLE, J. L. *Reaction of two races of sand pine (Pinus clausa) to parasitism by the lance nematode, Hoplolaimus galeatus.*

Soil and root samples from pine plantations in the sand hills areas of west Florida revealed lance nematodes (*Hoplolaimus galeatus*) parasitizing and causing damage to the roots of the Ocala race of sand pine (*Pinus clausa*), a native of eastern Florida characterized by closed cones. The Choctawhatchee race of sand pine, a native of western Florida characterized by open cones, has recently been recommended for west Florida plantations because it is apparently resistant to *Clitocybe* root rot, a highly destructive disease in the sand hills.

A study was conducted to determine the relative susceptibility of seedlings of the two races of sand pine to lance nematode. Single 4-month-old seedlings of uniform size were planted in fine sandy loam in 20-cm clay pots. Nematodes were added 10,000 or 1,000 per pot; controls received no nematodes. Treatments for each race were replicated five times.

After 4 months in the greenhouse, the heights and the foliar-stem and root weights of seedlings of both races infested with 10,000 nematodes per pot were significantly less than those of control seedlings but not significantly different from each other. Seedlings of the Ocala and Choctawhatchee races

that received the high inoculum level had less height (25 percent and 18 percent), foliar-stem weight (35 percent and 42 percent), and root weight (45 percent and 54 percent) than their respective controls. There was also no difference in numbers of nematodes recovered from the roots of seedlings of both races. These data suggest that resistance to lance nematode parasitism is lacking in both races of sand pine.—*Forestry Sciences Laboratory, Southeastern Forest Experiment Station, Forest Service, U.S. Department of Agriculture, Carlton St., Athens, GA 30601.*

SAYRE, R. M., AND W. A. HABICHT. *An amoeboid organism preying on root-knot nematodes.*

An amoeboid organism isolated from greenhouse soil was found preying on larvae of the root-knot nematode, *Meloidogyne incognita.* The amoebae had many of the characteristics of *Theratomyxa weberi,* the species Zwillenberg described preying on larvae of *Heterodera rostochiensis.* The organism was cultured on root-knot larvae. Various stages in its life cycle were recorded by cinéphotomicrography.

The creeping trophic form of the organism was granular in appearance, with no visible nucleus. It moved at a rate of about 70μ/ minute in the plastic culture dishes. Its pattern of locomotion was a departure from the usual description of amoeboid movement. There were no broad psuedopodia at the advancing margin, only numerous very fine filaments of protoplasm called reticulopodia. There were, however, long slender arms of protoplasm that trailed far behind the advancing cell. These arms quickly flowed back into the main cell when stretched to a point where contact with the bottom of the culture plate was lost.

After an amoeba made contact with a nematode, it was literally pulled off the bottom of the culture dish by the nematode's struggle to free itself. The amoeba covered the larva in about 30 min, gradually folding the larva into a tight packet that became the digestive cyst. A cyst wall was laid down by the amoeba and then digestion of the nematode occurred within the cyst. At room temperature, the entire cycle from creeping form to creeping form was completed in less than 24 hr. Depending on the initial size of the digestive cyst, from four to ten amoebae emerged from the cyst, leaving behind an empty cyst wall and what appeared to be the remnant of the original cell nucleus.

Amoebae failing to ensnare a nematode within a few hr ceased to move, and within eight to twelve min collapsed inward, formed a cyst wall and entered a resting stage.—*Plant Science Research Division, Agricultural Research Service, Beltsville, MD 20705.*

SPASOFF, L., J. A. FOX, AND L. I. MILLER. *Multigenic inheritance of resistance to Osborne's cyst nematode in tobacco.*

The inheritance of resistance to Osborne's cyst nematode (OCN) was studied in a cross between 'BVA 523' (a burley tobacco breeding line in which resistance to wildfire and OCN is apparently linked) and 'NC 2326' (a flue-cured variety susceptible to wildfire and OCN). Twenty plants of each parent, 20 plants of the F_1, 60 plants of the F_2, and 20 plants each of 18 F_3 lines were inoculated in 5-cm pots each containing approximately 20,000 eggs and larvae of OCN in a 1:1 (v:v) soil-Weblite® mixture. One month after transplant into infested soil, leaves of each plant were inoculated with *Pseudomonas tabaci* and rated for wildfire resistance one week later. Mature OCN females were washed from the roots and

counted two months after transplant. The means and ranges of OCN female counts in the following plant lines were: 'BVA 523' 4.6 (0–16); 'NC 2326' 470.4 (129–754); F_1 110.0 (52–155); and F_2 163.5 (0–899). Wildfire resistance segregated 3:1 resistant: susceptible in the F_2. The F_2 exhibited a continuous range of variation in OCN females/plant but low OCN counts were correlated with wildfire resistance. The means and ranges of OCN females in selected F_3 lines were as follows: R_1 1.4 (0–4); R_2 7.2 (0–25); R_3 20.9 (7–52); R_4 29.2 (2–185); S_1 295 (52–649) and S_2 563 (264–921). The F_3 data indicated a continuous range of variation in mature OCN females per plant with at least four levels of OCN resistance in lines homozygous for resistance to wildfire R_1, R_2, R_3, and R_4. Two degrees of susceptibility of OCN were found in lines homozygous for susceptibility to wildfire (S_1 and S_2). The (i) intermediate resistance in the F_1, (ii) continuous range of resistance in the F_2, (iii) transgressive inheritance noted in the F_3 lines and (iv) occurrence of four levels of resistance to OCN in lines homozygous for wildfire resistance indicated multigenic inheritance. It was concluded that an unknown number of genes linked to wildfire resistance from 'BVA 523' and that at least one gene introduced from the susceptible parent 'NC 2326' conditioned the reaction to OCN. There was evidence of linkage group breakage in the F_3.—*Department of Plant Pathology and Physiology, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061.*

SOUTHARDS, C. J., AND M. F. PRIEST. *Physiologic variation of seventeen isolates of Meloidogyne incognita.*

The pathogenicity of 17 isolates of *M. incognita* collected in Tennessee were studied on six different hosts: *Lycopersicon esculentum* 'Rutgers', *Nicotiana tabacum* 'N.C. 95', *Vigna sinensis* line 'M57–13N', *Gossypium hirsutum* 'McNair 1032', *Citrullus vulgaris* 'Dixie Queen', and *Capsicum frutescens* 'California Wonder'. Each host, replicated four times, was inoculated with eight egg masses derived from single egg-mass colonies of each nematode isolate and grown in the greenhouse for 50 days at 26–30 C. On the basis of root-knot indices and egg mass production, six distinct physiologic races were differentiated among the 17 isolates. All isolates produced numerous galls and egg masses on tomato, but none developed on 'N.C. 95' tobacco. Isolates 8, 10, 11, and 16 reproduced well on watermelon and pepper, and produced a low-to-moderate number of galls and egg masses on cotton and cowpea. Isolate-9 formed numerous galls and egg masses on watermelon, but none on pepper or cowpea. Isolate-12 reproduced well on cotton, watermelon and pepper, but did not reproduce on cowpea. Isolate-15 reproduced on pepper and cowpea, but did not reproduce on cotton and watermelon. Isolates 4 and 14 reproduced on watermelon, pepper and cowpea, but not on cotton. Isolates 1, 2, 3, 5, 6, 7, 13 and 17 reproduced well on watermelon and pepper, but did not reproduce on cotton and cowpea.—*Department of Agricultural Biology, University of Tennessee, Institute of Agriculture, Knoxville, TN 37901.*

TARTE, R. *The relationship between preplant populations of Pratylenchus zeae and growth and yield of corn.*

Single corn plants were greenhouse-grown in 10-cm clay pots each containing 500 cc of clay loam soil inoculated with 0, 5, 10, 40, 60, 170, 270, 1900, 5350, or 10350 *Pratylenchus zeae*; the various populations

were obtained by proportionate mixing of steam-sterilized and naturally-infested soil. Within two weeks after planting, seedlings growing in the heavily infested mixtures were visibly stunted. After 6 weeks, a highly significant negative correlation between initial population of *P. zeae* and dry weight of tops was found. Top weight reduction ranged from 11.9 to 38.3%. Rates of population increase were linear up to 270 *P. zeae* per pot, decreased for the 1900 group, and the 5350 and 10350 groups did not maintain the initial inoculum levels.

In a field block initially infested with *P. zeae*, plot populations were adjusted to a range of levels by previously cropping to hosts of different suitability. The graded population levels detected by sampling ranged from 1 to 1225 nematodes per 500 cc of soil. A highly significant negative correlation between preplant numbers of *P. zeae*, and yield of corn was found at the end of the experiment. Higher yields were obtained following poor host crops such as okra, cowpea, and cotton. Lower yields were obtained after crops of corn, sorghum, and rice which are very good hosts of this nematode. Since no plot was completely free of *P. zeae*, yield reduction due to nematode damage was difficult to measure. However, the lowest yield, obtained after a crop of rice, was 20% less than the highest yield obtained after a crop of okra.—*Faculty of Agriculture, University of Panama, Panama City, Panama. Author presently at Department of Plant Pathology, Cornell University, Ithaca, NY 14850.*

THOMASON, I. J., C. E. CASTRO, N. BELSER, AND M. BELD. *Osmoregulation and the kinetics of permeation of Aphelenchus avenae by water.*

In order to sort out the sequence of events in osmoregulation, a study was made of the influence of osmotic pressure upon the rates of permeation of *Aphelenchus avenae* by water. Glucose was used to control the osmotic pressure because it was previously found to be a slow permeator ($k_{in} \cong 1.3 \times 10^{-4} min^{-1}$). Thus glucose moves into *A. avenae* about 2,000 times slower than water.

The permeation rates of water were not markedly influenced by osmotic pressure. The k_{in} for water in the presence of 0.05–0.5M glucose ranged 0.12–0.17 min^{-1} which is in good agreement with the K_{in} of 0.2 min^{-1} for water in the absence of osmotic stress.

In glucose solutions, the volume of the nematode changes but at a slower rate than water enters or exits. Thus in 1M glucose in the first 30–60 min the nematodes shrink 50 percent (avg. vol. change/min ~1.6%). At lower glucose concentrations the average volume change per min is ~0.5 percent. An equilibrium volume is reached in about 30 min. Experiments with animals pre-exposed to glucose for 18–20 hr showed the same rates of water uptake.

Thus *A. avenae* adjusts to osmotic stress mainly by altering its size. The rate of size change is, however, slower than the rates of permeation by water. These rates are not appreciably altered by osmotic pressure in the range of 0–26 atm.—*Supported by U.S. Department of Agriculture Grant No. 12-14-100-9162 (34).* *Nematology Department, University of California, Riverside, CA 92502.*

TOWNSHEND, J. L. *Effect of forage components on populations of selected plant-parasitic nematodes.*

Ten species in six plant parasitic nematode genera were found associated with forage

crops in southwestern and central Ontario. These species occurred in combinations of two or more. In 7 months of greenhouse culture, *Helicotylenchus digonicus* under 'Vernal' alfalfa increased from 353 to 663 per 25 g of soil and *Paratylenchus projectus* under 'Climax' timothy increased from 37 to 3200 per 25 g of soil and under 'Viking' trefoil from 67 to 866 per 25 g of soil. *Pratylenchus minyus* maintained itself on alfalfa, trefoil, timothy, and 'Canadian Double Cut' clover while *Meloidogyne hapla* maintained itself only on the legumes.—*Research Station, Canada Department of Agriculture, Vineland Station, Ontario, Canada.*

TURNER, D. R., AND R. A CHAPMAN. *Infection of alfalfa and red clover by concomitant populations of Meloidogyne incognita and Pratylenchus penetrans.*

Two-day-old seedlings of 'Buffalo' alfalfa and 'Kenland' red clover were exposed to combinations of second stage larvae of *Meloidogyne incognita* and a 1:1 mixture of male and female *Pratylenchus penetrans* during 24–72 hr of incubation at 24 C on 1% agar *in vitro*. Over the range of 3–200 nematodes in the inoculum, both species penetrated both hosts arithmetically when introduced singly. Introduced simultaneously, neither nematode affected the penetration of the other either in combinations of 50 of each species or 50 of one species and 200 of the other. Prior incubation of seedlings with 200 *P. penetrans*, however, significantly reduced invasion by 50 *M. incognita* but the reciprocal treatment had no affect on the invasiveness of *P. penetrans*.—*Department of Plant Pathology, University of Kentucky, Lexington, KY 40506.*

WEBBER, A. J., JR., AND J. A. FOX. *Variation in sex differentiation among single larval and single egg mass isolates of Meloidogyne graminis.*

Sex differentiation of *Meloidogyne graminis* on *Cynodon* sp. ('Tifgreen' bermudagrass) was found to differ among populations initiated with single larval (SL) and single egg mass (EM) isolates. One to four SL populations were initiated from each egg mass of 22 females. The remaining eggs of each female were used to initiate EM populations. Males among EM populations 1 month after initiation averaged 12.4% (range 0.0 to 46.9%) but the proportion averaged 26.6% (range 0.0 to 69.6%) after 4 months. SL populations 4 months after initiation averaged 27.5% (range 0.0 to 67.1%) males. Differences between sex ratios of some SL populations derived from individuals isolated from a given egg mass indicated heterogeneity. In other cases, all SL populations derived from a given egg mass, as well as the corresponding EM populations, had similar low or high percentages of males. A test of the heritability of factors influencing sex ratios was initiated. Two of 16 single larval isolates (SL-2 populations) were derived from each of six selected SL populations representing graded sex ratios and apparent heterogeneity. The graded series thus established consisted of three SL populations with low percentages of males but with progressively greater apparent heterogeneity, and three populations with high percentages of males but with progressively less apparent heterogeneity. Males among all SL-2 populations after 4 months averaged 28.9% (range 0.0 to 73.4%). Among SL-2 populations from the six graded SL populations, a progression from low percentages of males to high percentages of males occurred. The average percent males in each series was 8.9,

18.5, 25.8, 37.0, 55.9, and 53.4 respectively (ranges 0.0 to 22.4%, 0.0 to 33.3%, 14.7 to 40.9%, 20.8 to 50.0%, 35.5 to 73.6%, and 44.4 to 62.3% respectively). None of the larvae used to initiate single larval isolates differentiated as males at the end of the first generation. Proportionate sex differentiation in two SL populations characterized by low and high percentages of males was then tested by inoculating 'Tifgreen' bermudagrass with 15, 150 and 1500 larvae/rooted sprig. The differentiation of larvae as males was low and did not differ between populations with 15 larvae/sprig. Percentage of males was considerably greater with the SL population characterized by a high percentage of males but only at the higher inoculum levels. The continuous variation among populations and the influence of nematode population density suggest that sex in *M. graminis* is polygenic in inheritance but that heritability is low. Although an isolated larva differentiates as a female, differences exist in the potential of larvae to become males. The expression of potential male differentiation, as influenced by nematode population density, may involve genotypic variation in a hormonal sex-determining mechanism.—*Department of Plant Pathology and Physiology, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061.*