PATHOGENICITY AND HISTOPATHOLOGY OF *PRATYLENCHUS BRACHYURUS* AND *HELICOTYLENCHUS PSEUDOROBUSTUS* ON SUGARCANE.

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ABSTRACT

Onapitan, J.A. and J.O. Amosu 1982. Pathogenicity of *Pratylenchus brachyurus* and *Helicotylenchus pseudorobustus* on sugarcane. Nematropica 12: 51-60.

In greenhouse experiments, *Pratylenchus brachyurus* (Godfrey 1929) Filipjev and Stekhoven 1941 and *Helicotylenchus pseudorobustus* (Steiner 1945) Golden 1956 as individual pathogens did not suppress the top growth and root weight of sugarcane. Histological studies of the roots showed damage to cortical and stelar tissues by *P. brachyurus* and eggs were laid in the metaxylem, while activities of *H. pseudorobustus* was limited to the cortical tissue. Although, both species are parasitic to sugarcane, only *P. brachyurus* showed pathogenic potential.

Additional key words: Cellular responses, host-parasite relationships, interaction, pathological effect.

RESUMEN

Onapitan, J.A. y J.O. Amosu. 1982. Patogenicidad e histología de *Pratylenchus brachyurus* y *Helicotylenchus pseudorobustus* en caña de azúcar. Nematrópica 12: 51-60.

Pratylenchus brachyurus (Godfrey) Filipjev & Stekhoven y *Helicotylenchus pseudorobustus* (Steiner) Golden no redujeron el peso de las raíces o el crecimiento de tallos de caña de azúcar (*Saccharum officinarum* L) en pruebas individuales de patogenicidad en el invernadero. El exámen histológico de las raíces demostró que *P. brachyurus* causó daños en los tejidos corticales y estelares y que los huevos los deposita el nematodo en el metaxilema pero que las actividades de *H. pseudorobustus* estaban confinadas a los tejidos corticales. Aún cuando ambas especies son parásitas de la caña sólo *P. brachyurus* mostró potencial patogénico.

Palabras claves adicionales: relaciones entre parásito y hospedero, interacciones, nematodo lesionador, nematodos espiraliformes, reacciones celulares, efectos patológicos.

INTRODUCTION

In Nigeria, some of the plant-parasitic nematode genera that have been found associated with sugarcane include: *Meloidogyne, Pratylenchus, Heterodera, Helicotylenchus, Xiphinema* and *Hoplolaimus* (4, 17). Among these, species of *Pratylenchus* and *Helicotylenchus* were reported to be encountered most frequently in Nigeria (1) and in other countries such as Barbados (3) and Hawaii (9). Despite the frequent occurrence of both *P. brachyurus* and *H. pseudorobustus* in sugarcane plantations, information on the specific role within the rhizosphere of sugarcane is still insufficient, especially in Nigeria. The effect of these two species on the growth and the histopathological changes each induced in sugarcane roots are reported here.

MATERIALS AND METHODS

Pathogenicity test. Stalks of the sugarcane cultivar Co 1001 were grown from cuttings treated with hot water (50C for 2 hr) to control virus and stem borer (10). Single-bud cuttings were taken from these stalks and germinated in wooden flats (46cm x 92cm x 10cm) filled with a steam sterilized potting mix of soil, sand, and weathered poultry manure (20:1:1). Two weeks after sprouting, uniform sized sugarcane seedlings were transplanted to 500ml plastic cups filled with the potting medium and allowed to grow for two weeks. Five-liter buckets were filled with potting medium and a depression was created in the center of each bucket to accomodate a 9 cm diameter (500ml) plastic cup. Each bucket was then inoculated with the appropriate nematode inoculum level. Transplants of uniform size were selected and transferred to the 5-liter buckets with as little disturbance as possible to the root system and surrounding soil.

Cultures of *H. pseudorobustus* were maintained on sugarcane using handpicked individual specimens. *Pratylenchus brachyurus* was supplied by Dr. M.O. Olowe of the National Cereals Research Institute, Ibadan, and maintained on sugarcane. Nematode inoculum was extracted and inoculated as previously described (16). Two-week old sugarcane transplants were inoculated with 1,000, 5,000, 10,000 and 20,000 of each of the two nematode species. Controls were inoculated with only the nematode supernatant (water) liquid. The treatments and controls were replicated four times. Ambient temperature of the greenhouse ranged from 25C to 30C, and average soil temperatures at 3.00 p.m. and 6.00 a.m. were 32C and 24C respectively. A recommended fertilizer mixture of nitrogen, phosphorus, and potassium (75-45-100 kg, respectively) per hectare was used as single application one week after inoculation (14). Starting one week after sprouting and throughout the experiment, the sugarcane was sprayed every two weeks with 8.5 ppm ai of Dimethoate formulated as Rogor 40 against invasion by mites and stem borer. The experiment was terminated 109 days after inoculation.

At the conclusion of the experiment the plant tops were cut off at the soil surface. Then each bucket was inverted and tapped gently so that the entire root system and all the soil was removed intact. This was gently lowered into a 10-liter bucket containing 5-liters of water. The roots were carefully freed from soil, washed and blotted dry. The final nematode populations were estimated by processing 1 liter of total soil-water suspension by a modification of (5). Two-gram samples of chopped root fragments were macerated in a blender and *P. brachyurus* extracted by a modified Baermann technique. Fresh and dry weights of roots and shoots were recorded for all treatments. Plant parts were oven-dried at 70C to constant weight.

Histopathological studies. Two week old uniform sized sugarcane transplants raised from a single bud set of cultivar Co 1001 were surface sterilized with 1% sodium hypochlorite (chlorox) for 2 min and transplanted in 500 ml-plastic cups filled with 60 g of steam sterilized river sand. Adults and larvae of *P. brachyurus* and *H. pseudorobustus* were separately surface sterilized with 100 ppm phenyl mercuric acetate (commercial fungicide phix, ai 22% phenyl mercuric acetate) for 15 min and rinsed three times in sterile distilled water before being inoculated in the sand surrounding the sugarcane roots (19). Five thousand adults and larvae of each species were drawn from a suspension of the surface sterilized populations into two separate sets, each with five cups. The five cups that served as controls received only supernatant water from the nematode sterilization. The seedlings were arranged in a Gallemkamp Compenstat IH-282 cooled incubator at 30C day and 25C night temperature with a 12-hr photo period. Five ml of nutrient solution [6 ml KNO_3 IM; 4 ml $Ca(NO_3)_2 4H_2O$ IM; 2 ml NH_4PO_4 IM, 1 ml $MgSO_4.7H_2O$ IM; 1 ml mixed micronutrients B, Mn, Zn, Cu, Mo and Fe per liter of final solution, (J.A. Adepetu, Univ. of Ife, Personal Communication)] was added to each cup every day and the plants were watered every other day with 10 ml of sterile distilled water.

At 1, 2, 8, 16 and 32 days after inoculation samples of sugarcane seedlings including the controls were removed and processed. Roots were washed free of soil and selected portions were either stained or fixed. Roots were stained with 0.1% cotton blue in lactophenol, and cleared lactophenol or Canada balsam (8). Roots were fixed in FAA, dehydrated in butyl alcohol, and embedded in molten wax (11). Longitudinal and tranverse sections of 20 mμ thickness were prepared, stained in safranin and fast green and examined microscopically.

EXPERIMENTAL RESULTS

Pathogenicity trial. Slight decreases in wet and dry weights of top growth were recorded as the initial nematode population of *P. brachyurus* increased from 1,000 to 20,000. These reductions in wet and dry weights were not significant at the 5% level (Table 1). Wet and oven dried root weights varied slightly between treatments but differences were not significant at the 5% level (Table 1). However, at the 1,000 population level of *P. brachyurus*, root weight (wet and dry) showed an increase over the other treatments (Table 1).

TABLE 1: Effect of different inoculum levels of *P. brachyurus* on sugarcane growth and nematode development after 109 days (mean of 4 replicates).

Inoculum Level	Top Growth (g) Fresh	Top Growth (g) Dry	Root Growth (g) Fresh	Root Growth (g) Dry	Final Nematode Population: Mean Population	Final Nematode Population: Multiplication Factor
0	231.25	39.37	25.12	5.31	0	0.0
1,000	210.87	37.25	32.62	6.12	35,860[a]	35.8
5,000	203.25	35.25	29.87	4.00	86,600[b]	17.3
10,000	199.37	37.12	29.37	4.12	109,410[c]	10.9
20,000	198.75	35.62	31.50	4.25	116,330[c]	5.8
SE of mean	10.14	2.95	2.79	0.71	4,010	

Means with the same letter do not differ significantly at P = 0.05 (Duncans multiple range test.)

TABLE 2: Effect of different inoculum levels of *H. pseudorobustus* on sugarcane growth and nematode development after 109 days (mean of 4 replicates).

Inoculum Level	Top Growth (g) Fresh	Top Growth (g) Dry	Root Growth (g) Fresh	Root Growth (g) Dry	Final Nematode Population: Mean Population	Final Nematode Population: Multiplication Factor
0	235.30	43.62	26.62	5.00	0,000	0.00
1,000	233.00	43.75	30.52	4.43	24,760[a]	24.76
5,000	211.37	40.00	26.62	4.62	39,640[b]	7.92
10,000	215.37	42.87	31.50	4.87	58,190[c]	5.82
20,000	216.25	43.00	27.75	4.50	88,250[d]	4.41
SE of mean	9.77	1.18	2.66	0.45	4,340	

Means with the same letter do not differ significantly at P = 0.05 (Duncan multiple range test).

Roots inoculated with *P. brachyurus* were generally darker brown in color than the uninoculated control. The dark brown color difference resulted from the dead cells in areas attacked by nematodes. The mean final populations of *P. brachyurus* increased with increases in the initial inoculum, and differences were significant at the 1% level. Population increases in treatments with 10,000 and 20,000 nematode inoculum levels were not significantly different

(Table 1). The population multiplication factor was 35.8 at 1,000 initial nematode inoculum level and 5.8 when the inoculum level was 20,000.

Slight decreases in top growth and root weights were recorded as the initial levels of *H. pseudorobustus* increased from 1,000 to 20,000. These reductions were not significant at the 5% level (Table 2). Mean final populations of *H. pseudorobustus* increased with the increases in initial inoculum level (Table 2). The increases were significant at the 5% level. At 1,000 inoculum level the multiplication rate was 34.7 and 4.41 at the 20,000 inoculum level.

Histopathological Studies. Pratylenchus brachyurus was found in both the secondary and tertiary roots of sugarcane 2 days after inoculation. No trace of discoloration was observed, but cell destruction due to nematode movement was noticeable in the cortex (Fig. 1). At the 8th day and 16th day *P. brachyurus* was found around the vascular bundle. On the 16th day *P. brachyurus* was observed partly buried in the cortex and partly in xylem vessels of the vascular bundle (Fig. 2). Stelar tissue distortion and eggs in the metaxylem were observed at the end of the 32nd day in the roots (Fig. 3). *Helicotylenchus pseudorobustus* was not observed on or within the roots until the 8th day where it was observed completely buried in the cortex (Fig. 4). Thirty-two days after inoculation, *H. pseudorobustus* were not observed beyond the cortex of the roots.

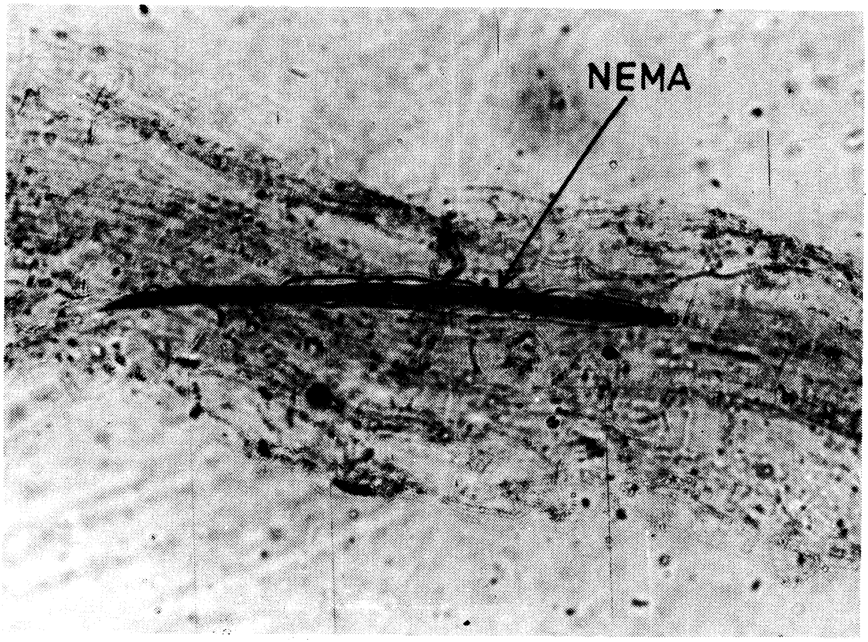
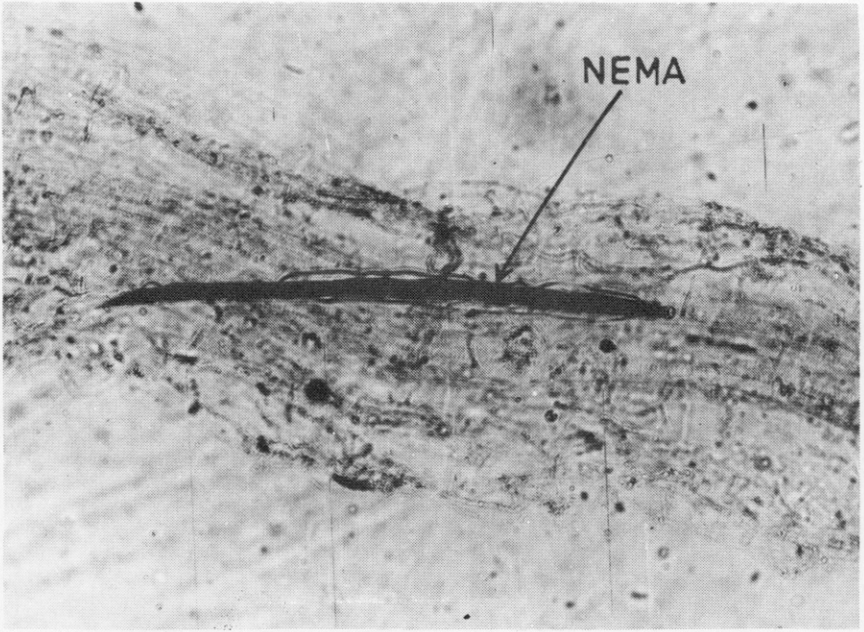


Fig. 1: Damage to the cortex of sugarcane root by *P. brachyurus* two days after inoculation.



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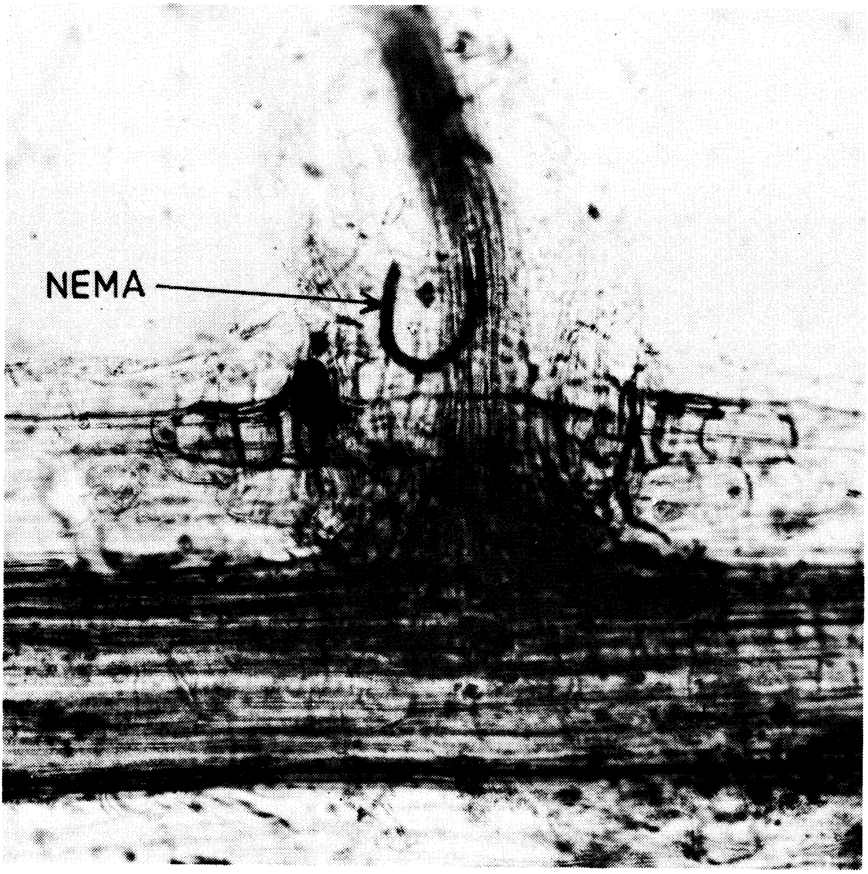

Fig. 2: *Pratylenchus brachyurus* partly buried in the cortex and partly in the vascular bundle of sugarcane root 16 days after inoculation.

DISCUSSION

Reduction in the top growth and root weights of sugarcane inoculated with *P. brachyurus* when compared with the uninoculated control was not significant. *Pratylenchus brachyurus* did not suppress the top growth and root development of the sugarcane cultivar used, which is similar to the results reported by (10, 16). The significant increase in populations of *P. brachyurus* with increases in inoculum levels illustrates that sugarcane supports the reproduction of *P. brachyurus,* and adults were found in both the secondary and tertiary roots. Although, no discoloration was observed in the histopathological studies, *P. brachyurus* did invade and damage cells in the cortex and moved through the endodermis into the stele causing extensive destruc-

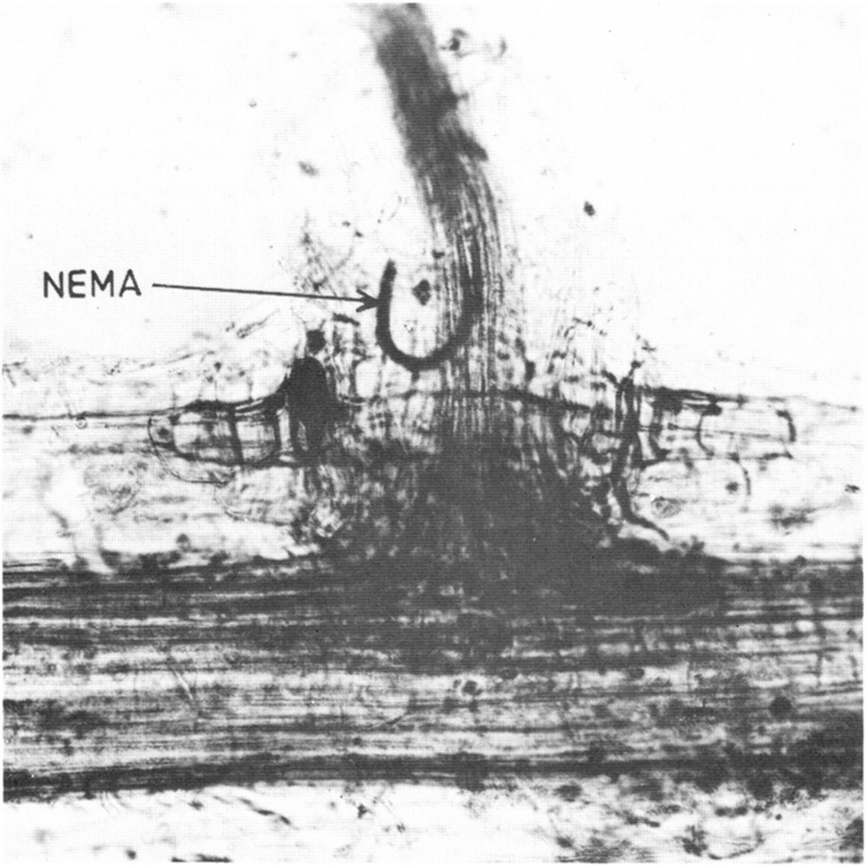

Fig. 2: *Pratylenchus brachyurus* partly buried in the cortex and partly in the vascular bundle of sugarcane root 16 days after inoculation.

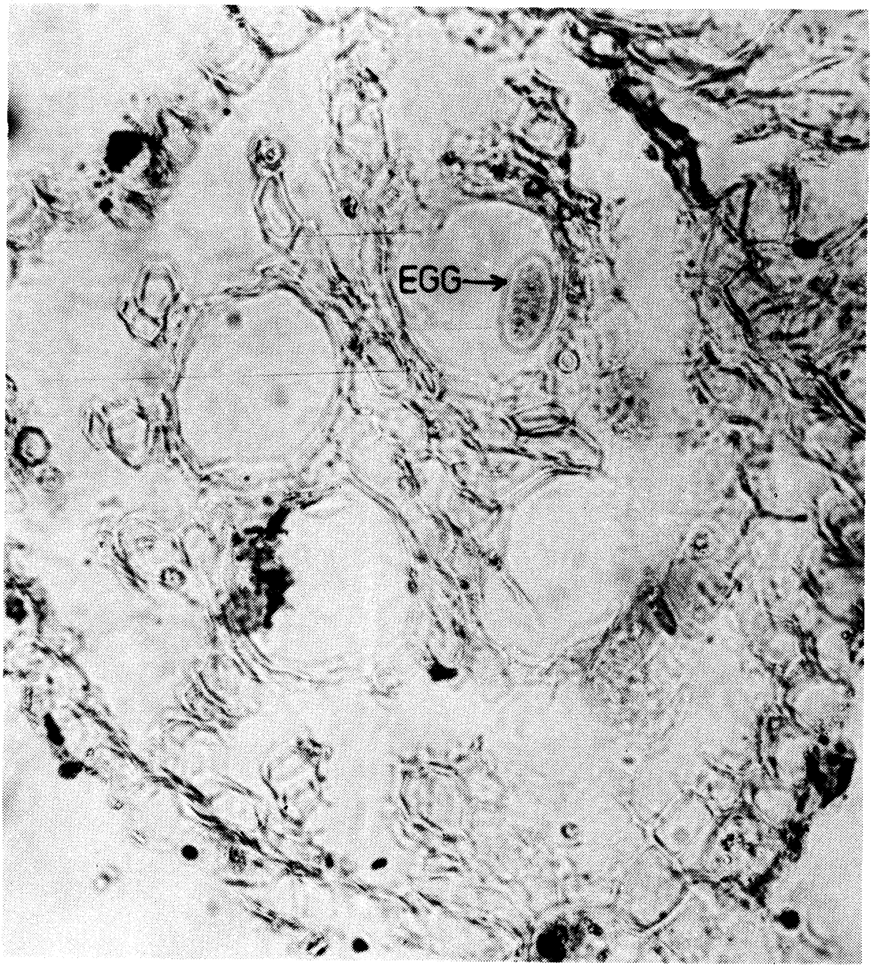

Fig. 3: Egg of *P. brachyurus* in metaxylem 32 days after inoculation.

tion. The presence of *P. brachyurus* in the vascular bundle coupled with the stelar tissue destruction indicates its pathogenic potential. The stelar tissue destruction observed here is contrary to the observations that *Pratylenchus* seemed to invade only the cortical tissue and not the stele of sugarcane (9, 20). However, stelar tissue destruction by *P. brachyurus* in maize has been reported (15). It has been found that *P. brachyurus* can invade the vascular tissues of soybean roots without causing necrosis (12).

Because the root system of sugarcane is fibrous and extensive, the rate of compensation for damaged root is expected to be high. At a 1,000 inoculum level of *P. brachyurus*, more food is available, thus accounting for the higher

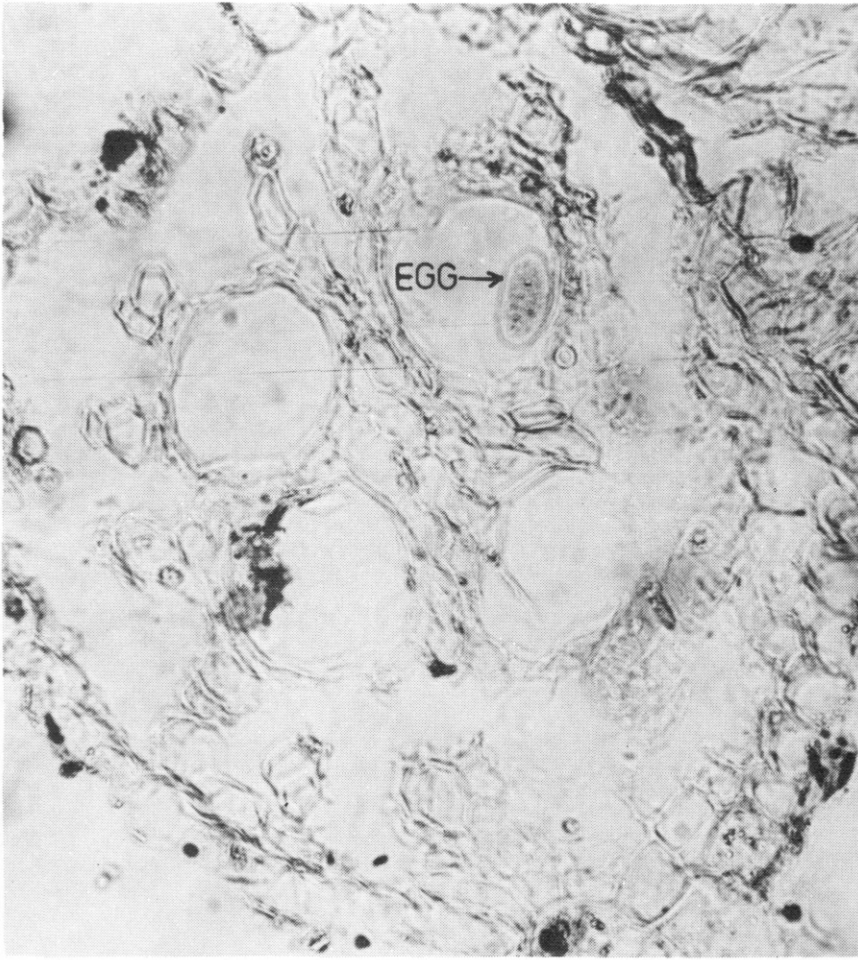

Fig. 3: Egg of *P. brachyurus* in metaxylem 32 days after inoculation.

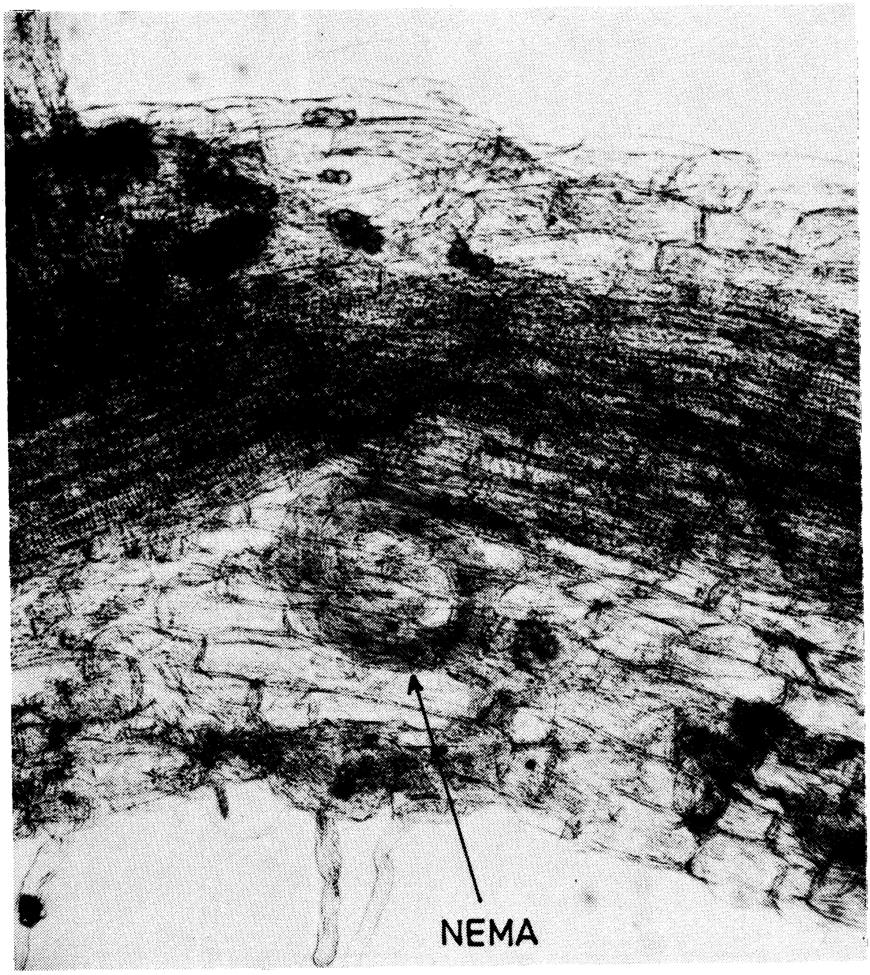

Fig. 4: *Helicotylenchus pseudorobustus* in the cortex of sugarcane root 8 days after inoculation.

and more rapid growth rate. This suggests that at a low population level, there is less competition and population and growth rate is increased. As the inoculum increased, competition for survival occurs and reduced growth rate was observed and no overall significant increase in population was recorded at high inoculum levels. As the population increased infestation on the plant becomes heavy, less food is available and the sex ratio of the nematode probably was upset, resulting in less reproductive capability. This perhaps explains the decrease in multiplication factor as inoculum increases. Similar reports on the effect of food shortage on sex ratio reversal and overall population have been reported (6, 7, 13).

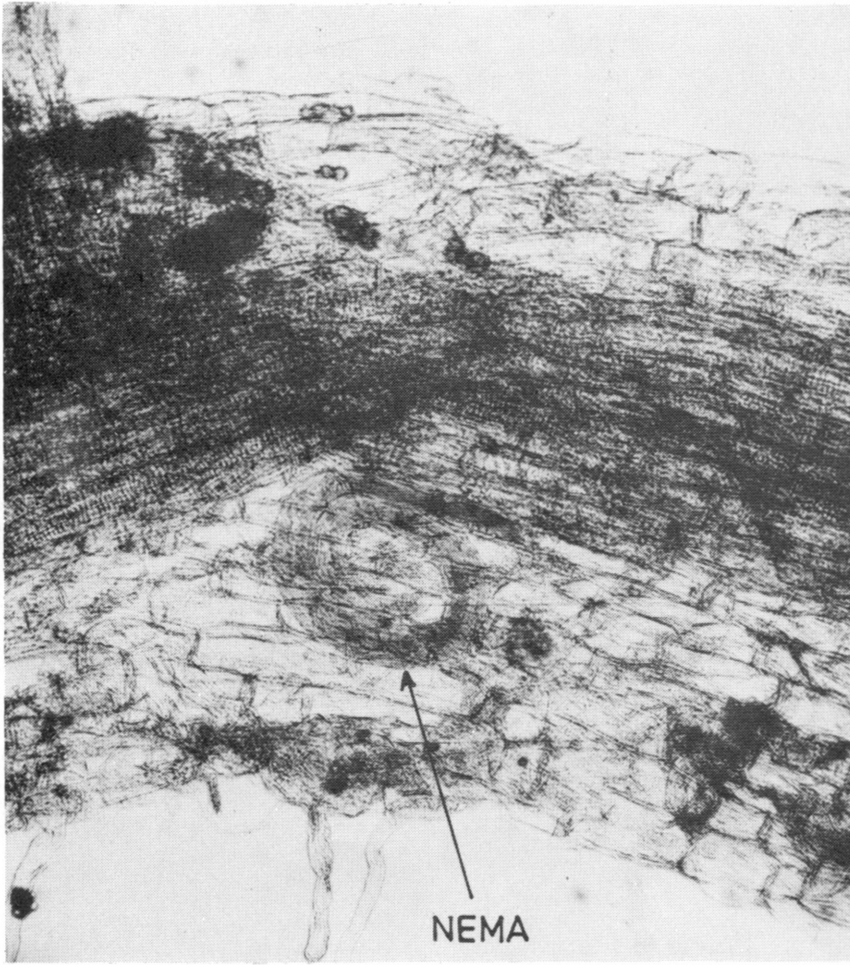

Fig. 4: *Helicotylenchus pseudorobustus* in the cortex of sugarcane root 8 days after inoculation.

Top growth weights of *H. pseudorobustus* inoculated plants were not significantly different from the control. This result is contrary to previous findings with *H. nannus* and *H. dihystera*, when significant reductions in yield were recorded (2, 18). The effect that a given population of parasitic nematode will have on sugarcane yields, decreases as the amount of clay and organic matter increases (17). This tends to suggest that the more the optimum nutrient requirement of sugarcane is met the less effect parasitic nematodes will have on sugarcane development. Based on this assumption, the optimum fertilizer supplied to the sugarcane cultivar used in this test, may have contributed to the insignificant difference in top growth weights and root development of *H. pseudorobustus* inoculated plants. *Helicotylenchus* is ectoparasitic and has been reported actually feeding on roots of sugarcane (9), and the significant increases in populations of *H. pseudorobustus* at the higher inoculum levels illustrates that sugarcane will support the reproduction of *H. pseudorobustus*. *Helicotylenchus pseudorobustus* were only found within the cortical area of the roots and the type and the extent of damage was not obvious. Although the presence in the cortex region would have resulted in the destruction of some cells, the effects were not obvious as in the case of *P. brachyurus* and therefore not conclusive.

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