STUDIES ON HOST-PARASITE RELATIONSHIPS OF *MELOIDOGYNE INCOGNITA* AND *COFFEA ARABICA* CV. BORBON

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ABSTRACT

Negrón, J. A. and N. Acosta. 1987. Studies on host-parasite relationships of *Meloidogyne incognita* and *Coffea arabica* cv. Borbon. Nematropica 17:71-78.

A greenhouse study was conducted to determine the host-parasite relationship of *Meloidogyne incognita* and *Coffea arabica* cv. Borbon. Two-month-old coffee seedlings were inoculated separately with 4000, 8000, 1600, and 32000 eggs and second-stage juveniles per plant. Uninoculated plants were used as controls. Six months after inoculation, chlorosis and dwarfing were significantly greater in plants inoculated with 32000 eggs and juveniles when compared to other treatments. Height and dry weight of roots and shoots were significantly reduced in inoculated plants. There were no significant differences among plants treated with any population level, but significant differences for all parameters were obtained between any inoculated plants and the control plants. Histological studies of root sections showed multinucleated, thick-walled giant cells surrounding the anterior portion of the females. Mechanical compression of parenchymatous and vascular tissue was observed. Adjacent non-giant cells were abnormally small and very numerous, suggesting the occurrence of hyperplasia.

Additional key words: root-knot nematode, coffee seedlings, inoculum densities, histopathology.

RESUMEN

Negrón, J. A. y N. Acosta. 1987. Estudio de la relación huésped-parásito de *Meloidogyne incognita* y *Coffea arabica* cv. Borbon. Nematropica 17:71-78.

Se realizó un estudio de invernadero para determinar la relación hospedero-parásito entre *Meloidogyne incognita* y *Coffea arabica* cultivar Borbón. Plantas de dos meses de edad fueron inoculadas con 4000, 8000, 16000 y 32000 huevos y segundos estadios juveniles por planta. Se utilizaron plantas sin inocular como testigos. Seis meses después de la inoculación las plantas tratadas con 32000 huevos y estadios juveniles mostrarón más clorosis y enanismo que los demás tratamientos. La altura y el peso seco de las raíces y de las partes aéreas se redujeron significativamente en las plantas inoculadas. No se obtuvieron diferencias significativas entre las plantas tratadas, pero si se obtuvieron diferencias significativas en todos los parámetros entre las plantas inoculadas y las testigo. Observaciones de las secciones histológicas evidencian la presencia de células gigantes multinucleadas con paredes engrosadas rodeando la parte anterior de la hembra en el estado adulto. Se observó daño mecánico en los tejidos parenquimatosos y vasculares. Las células adjacentes a las células gigantes eran más pequeñas que las normales y muy numerosas, lo que sugiere la ocurrencia de hiperplasia.

Palabras claves adicionales: nematodo nodulador, plántulas de caféto, densidades de inoculo, histopatología.

INTRODUCTION

Coffee (*Coffea arabica* L.) is a very important world crop. Brazil, Colombia, Mexico, some countries of Africa, India, El Salvador, Guatemala, and Costa Rica are the major coffee producers. Coffee is grown on some 9,085,000 ha with an annual production of 4,430,000 metric tons (1). In Puerto Rico coffee is grown on approximately 3841 ha, producing nearly 1556 metric tons that generate income for nearly 12,000 persons during the harvest season (2).

Coffee is attacked by various fungi, insects, and nematodes. The first available report on the presence of the root-knot nematode on coffee roots was published by Jobert in 1878 (10). This species was described by Goeldi as *Meloidogyne exigua* and reported as associated with coffee roots. In Puerto Rico, Ayala (3) and Ayala and Román (4) reported *Radopholus similis* (Cobb) Thorne, *M. incognita* (Kofoid and White) Chitwood, *Pratylenchus coffeae* (Zimmermann) Filipjev and Schuurmans Stekhoven, and species of *Xiphinema, Helicotylenchus,* and *Rotylenchulus* associated with coffee plants. Monterroso (15) first reported the presence of *M. exigua* attacking coffee roots in Puerto Rico. Echávez-Badel (6) concluded that *M. incognita* and *P. coffeae* are the most damaging nematodes in the coffee-growing regions of the Island.

Many other studies on the effect of different nematodes on coffee plantations have been conducted and it is known that various species of *Meloidogyne* are among the most harmful and important (6,11,12,13,15, 16,17,19). The study herein reported was conducted to determine the density levels of *M. incognita* necessary to cause significant damage to coffee seedlings in Puerto Rico.

MATERIALS AND METHODS

Two-month-old coffee seedlings (cv. Borbón) free of nematodes and insects were obtained from commercial nurseries. Seedlings were planted in 15-cm-diam plastic pots containing 1500 cm^3 of a methyl-bromide treated soil mixture (64% sand, 10% clay, and 26% silt) at pH 6.8. When transplanting, three inoculation tubes, 15-cm long x 1-cm diam were placed equidistantly around the plant. One end of each tube came in

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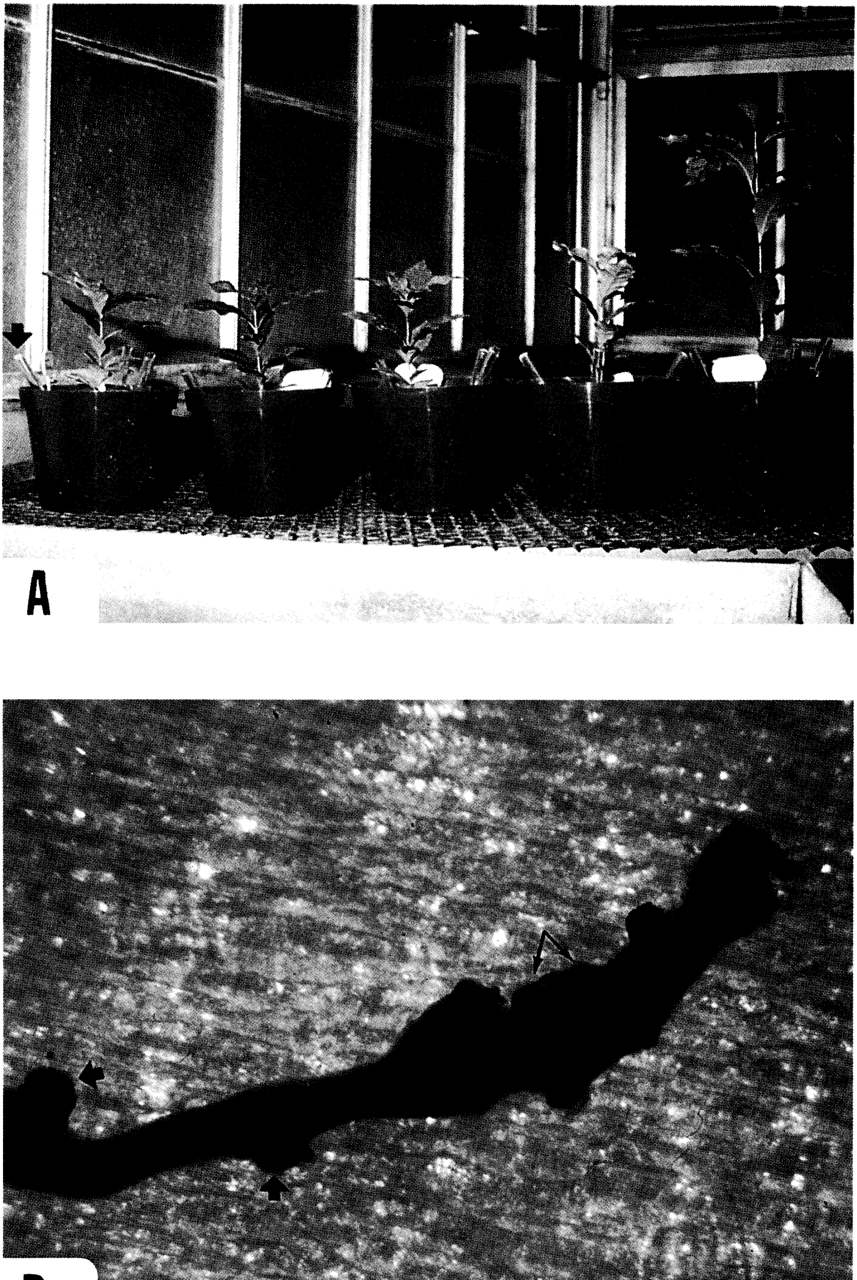
Fig. 1. A. Coffee seedlings six months after inoculation with *Meloidogyne incognita* eggs and second-stage juveniles. From left to right: 4000, 8000, 16000, 32000, and control showing glass tubes (arrows) through which water and/or inoculum was added; B. A root section with female nematodes and egg masses (arrow) protruding through cracks in the cortex and epidermis.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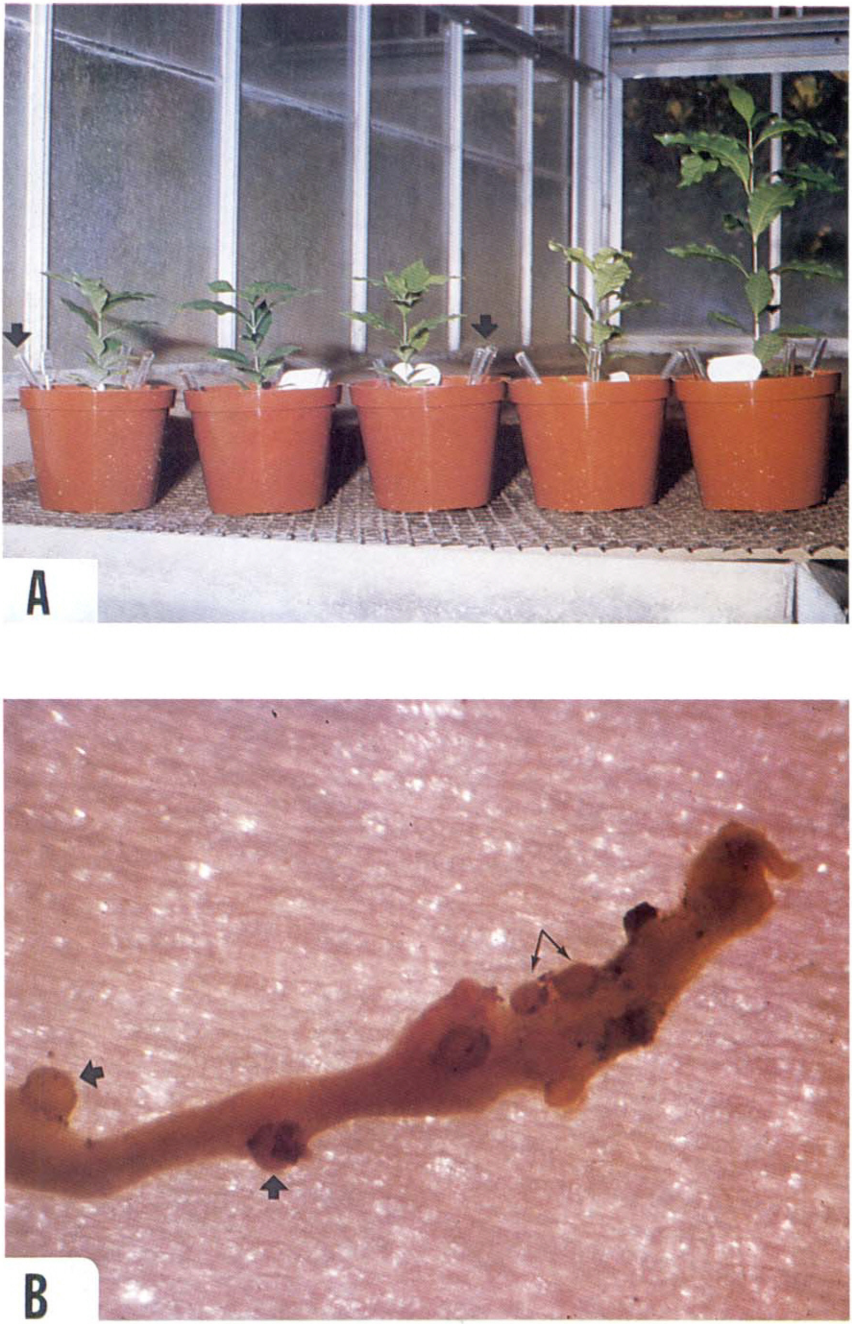


Fig. 1. A. Coffee seedlings six months after inoculation with *Meloidogyne incognita* eggs and second-stage juveniles. From left to right: 4000, 8000, 16000, 32000, and control showing glass tubes (arrows) through which water and/or inoculum was added; B. A root section with female nematodes and egg masses (arrow) protruding through cracks in the cortex and epidermis.

contact with the roots (Fig. lA). Nematode eggs, second-stage juveniles, water, and nutrients were added to plants through these tubes, as described by Meléndez (14). A monospecific population of *M. incognita* obtained from a commercial coffee plantation was increased in the greenhouse on tomato cultivars Rutgers and Homestead 94. Nematode eggs and second-stage juveniles were extracted from tomato roots according to the method described by Hussey and Barker (9). The inoculum densities used were 4000, 8000, 16000, and 32000 eggs and juveniles per plant. Control plants were inoculated with supernatant water containing microorganisms but no nematodes. All treatments were replicated 15 times in a completely randomized design. Plants were kept in a greenhouse for six months, and received enough water to maintain adequate moisture and vigor throughout the experiment. Data on height, gall index, and dry weight of roots and shoots were recorded at termination of the experiment.

Six months after inoculation, root samples were obtained, washed free of soil and fixed in FAA according to Johansen's method (11). Root sections 1-cm long were dehydrated in tertiary butyl alcohol and embedded in Paraplast® (Sherwood Medical, St. Louis), a compound of purified parafin and plastic polymers of a regulated molecular weight. Longitudinal sections for microscope examination were cut to a thickness of 10 to 12 μm with a rotary microtome, mounted in glass slides previously coated with Haupt's adhesive, and stained with safranine for 7 min at 48 C and fast green for 5 sec (11).

RESULTS AND DISCUSSION

Symptoms observed on inoculated plants included dwarfing, lack of vigor, vein discoloration, root galls, and a reduced root system. These symptoms were similar to those reported by Chitwood (5), Lordello (13), Echávez-Badel (6), and others (16,17,19) in coffee seedlings infected with different species of root-knot nematodes. The reduced root system had numerous terminal galls and female nematodes with their posterior ends protruding through cracks in the cortex and epidermis (Fig. 1B). This condition has been previously reported by Chitwood and Berger (5) and by Lordello (11) as a symptom of the damage caused by *M. exigua*.

Significant differences in all parameters evaluated were observed between control plants and those inoculated with either 4000, 8000, 16000, and 32000 eggs and juveniles (Table 1). No significant differences were obtained among inoculated plants. Plants treated with 32000 and 16000 eggs and juveniles suffered a 20% and a 19% height reduction, while those treated with 8000 and 4000 eggs and juveniles showed reductions of 16% and 15% respectively, compared to controls (Fig.

Table 1. Gall index, height, and dry weight of roots and shoots of 'Borbón' coffee seedlings inoculated with different population densities of *M. incognita.*

Inoculum density	Gall Index[y] (0-5)	Height (cm)	Dry weight (g)	
			Roots	Shoots
0	0.00 a[z]	23.60 a	0.85 a	2.35 a
4000	4.40 b	20.07 b	0.52 b	1.75 b
8000	4.64 b	19.71 b	0.52 b	1.62 b
16000	4.67 b	19.14 b	0.51 b	1.49 b
32000	4.60 b	18.79 b	0.58 b	1.56 b

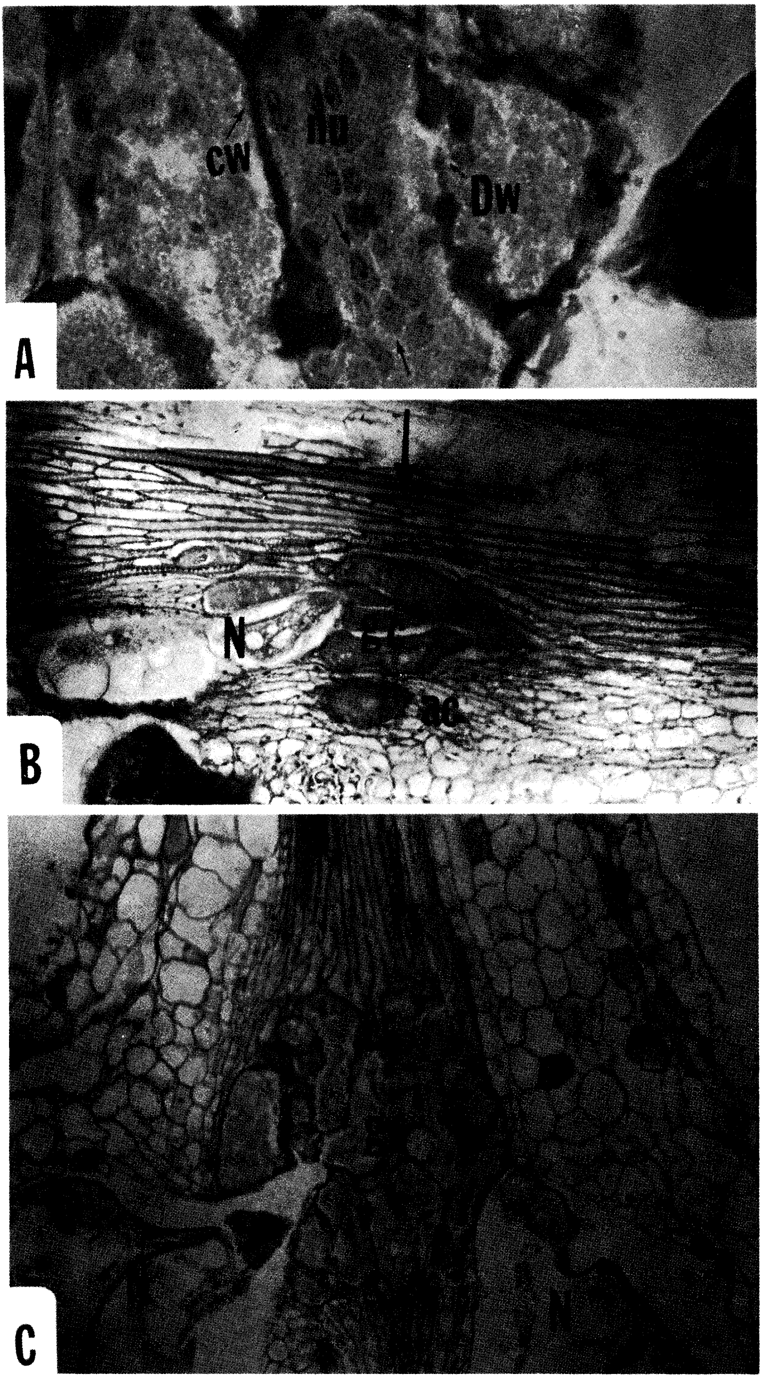
[y]Gall index based on a scale from 0 to 5: 0 = zero galls; 1 = 1-2 galls; 2 = 3-10 galls; 3 = 11-30 galls; 4 = 31-100 galls; and 5 = more than 100 galls.
[z]Means of 15 replicates per treatment. Numbers in columns followed by the same letters do not differ significantly (P = 0.05), according to Duncan's multiple range test.

1A). Results obtained show that population densities of 4000 eggs and juveniles are sufficient to cause significant reductions in the development of two-month-old coffee seedlings.

Histological sections revealed females whose anterior end was surrounded by giant cells (Fig. 2A). These cells had thickened walls and numerous nuclei, many of which appeared deformed. The cytoplasm was dense and of granular appearance, and cell wall dissolution was taking place (Fig. 2A). According to Endo (7) the granular condition of the cytoplasm is due to the high content of proteins, carbohydrates, lipids, nucleic acids, endoplasmic reticulum, and enzymes that occur during the cell wall dissolution and the coalescense of nuclear contents. Adjacent cells were numerous and smaller than normal, which suggests the occurrence of accelerated multiplication (Fig. 2B). These findings differ from the studies of Huang and Maggenti (8), who concluded that giant cells are formed solely by consecutive mitotic divisions without cytokinesis.

Mechanical damage (Fig. 2B) such as compressed layers of parenchymatous and vascular tissue around the female nematode probably results from growth of the female. Adult females were found next to the vascular bundles, with giant cells around their anterior portion. These giant cells were either covering the vascular bundles or had developed from undifferentiated cells that would normally be part of the vascular bundle (Fig. 2C).

Based on the study of the histological sections, it is evident that damage caused by the nematode can cause atrophy not only of the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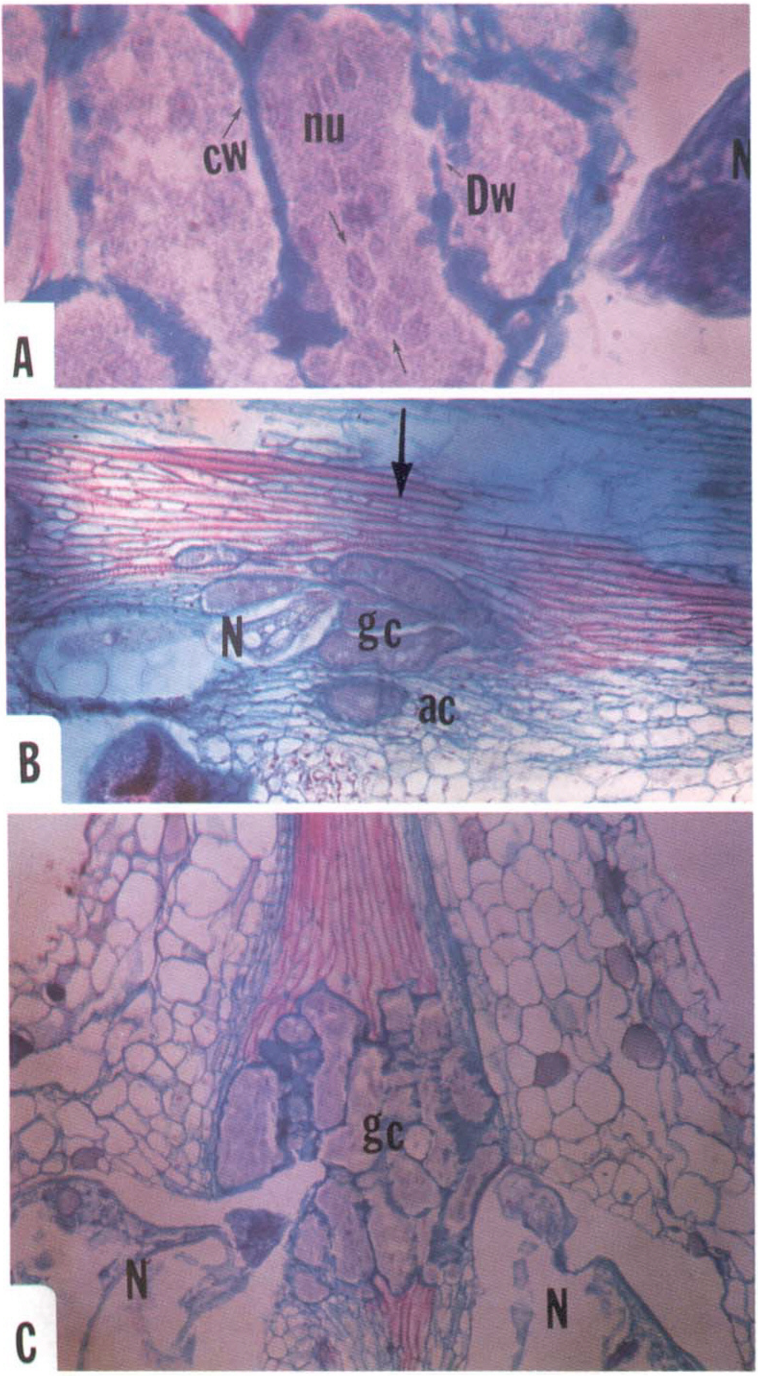

Fig. 2. Histological sections of coffee roots infected with the root-knot nematodes. A. Female nematode (N) with the anterior portion surrounded by giant cells, thickened cell walls (cw), dissolved cell walls (Dw), and nuclei (nu); B. Compression of parenchymatous and vascular tissues (arrow), giant cells (gc), nematode (N), and abnormal cells (ac); C. Female nematodes (N) in feeding position with giant cells (gc) inside the vascular tissue.

affected sections but of the whole plant. This condition can be attributed to the disfunction of affected cells that would otherwise develop in normal root sections. This explains the reductions in the heights and in dry weight of roots and aerial parts of the inoculated plants. The mechanical compression of xylem vessels and of cells surrounding the females probably occluded the movement of water and nutrients. These blocked vessels became insufficient for the normal development of the plant, which showed the symptoms previously described as lack of vigor.

LITERATURE CITED

1. ANONYMOUS. 1976. Food and Agricultural Organization of the United Nations. Production Yearbook 30:178-179.
2. ANONYMOUS. 1983. Facts and figures on Puerto Rico's agriculture. 1981-82/1982-83. Commonwealth of Puerto Rico. Dept. of Agriculture. Office of Agricultural Statistics. Santurce, P.R. 115 pp.
3. AYALA, A. 1969. Nematode problems in Puerto Rican agriculture. Pp. 135-145 *in* Proceedings of the Symposium of Tropical Nematology. Agric. Exp. Sta. Univ. P.R., Mayagüez, P.R.
4. AYALA, A., AND J. ROMAN. 1963. Distribution and host range of the burrowing nematode in tropical soils. J. Agric. Univ. P.R. 47(1):28-37.
5. CHITWOOD, B. G., and C. BERGER. 1960. Nemic parasites of coffee in Guatemala. Phytopathology 50:361 (Abstr.).
6. ECHAVEZ-BADEL, R. J. 1977. Combate químico de los nemátodos en viveros de café (*Coffea arabica* L.). M.S. Thesis. University of Puerto Rico, Mayagüez, P.R. 60 pp.
7. ENDO, B. Y. 1971. Nematode-induced syncytia (giant cells). Host-parasite relationships of Heteroderidae. Pp. 91-115 *in* B. M. Zuckerman, W. F. Mai, and R. A. Ronde (eds.), Plant parasitic nematodes. Vol. 2. Academic Press, New York & London. 347 pp.
8. HUANG, C. S., and A. R. MAGGENTI. 1969. Mitotic aberrations and nuclear changes of developing giant cells in *Vicia fabae* caused by root knot nematode, *Meloidogyne javanica.* Phytopathology 59:447-455.

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9. HUSSEY, R. S., and K. R. BARKER. 1973. A comparison of methods of collecting inocula of *Meloidogyne* spp., including a new technique. Plant Dis. Reptr. 57:1025-1028.
10. JOBERT, C. 1878. Sur une maladie du cafeir observee lau Brasil. Compt. Rend. Acad. Sci. Paris 87:941-943.
11. JOHANSEN, D. A. 1940. Plant Microtechnique. McGraw Hill Book Co., Inc. New York. 523 pp.
12. LORDELLO, L. G. E. 1972. Nematode pests of coffee. Pp. 268-284 *in* J. M. Webster (ed.), Economic Nematology. Academic Press, London. 563 pp.
13. LORDELLO, L. G. E., O. A. PICCINEN, and R. A. LORDELLO. 1973. Susceptibility of coffee trees to a root knot nematode. Nematol. Medit. 1:36-57.
14. MELENDEZ P. L., and N. T. POWELL. 1967. Histological aspects of the *Fusarium* wilt-root knot complex in flue cured tobacco. Phytopathology 57:286-293.
15. MONTERROSO, D. 1973. Estudio de los nemátodos que atacan al café (*Coffea arabica* L.), su distribución en Puerto Rico y algunas alternativas de control. M.S. Thesis, University of Puerto Rico, Mayagüez, P.R. 112 pp.
16. SHIEBER, E. 1968. nematode problems of coffee in Guatemala. Plant Dis. Reptr. 44:722-723.
17. SCHMIDT, C. T. 1969. *Meloidogyne coffeicola,* a serious root nematode problem in Brazilian coffee. F.A.O. Plant Prot. Bull. 17(3):56-57.
18. VASCONCELOS, B., S. FERRAS, and C. SHIMOGA. 1976. Histopatología de raíces de caffeiro parasitadas por *Meloidogyne exigua.* 1. Formacao das células gigantes. Summa Phytopathologica 2(2):97-102.
19. WHITEHEAD, A. G. 1969. Nematodes attacking coffee, tea and cacao and their control Pp. 238-250 *in* J. E. Peachey (ed.), Nematodes of Tropical Crops. Tech. Commun. Commonw. Bur. Helminth. No. 40. St. Albans, Herts, England. 355 pp.