THE *FUSARIUM OXYSPORUM* F. SP. *COFFEAE–MELOIDOGYNE INCOGNITA* COMPLEX IN 'BOURBON' COFFEE

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ABSTRACT

Negrón, J. A., and N. Acosta. 1989. The *Fusarium oxysporum* f. sp. *coffeae–Meloidogyne incognita* complex in 'Bourbón' coffee. Nematrópica 19:161–168.

The interaction of *Fusarium oxysporum* f. sp. *coffeae* with *Meloidogyne incognita* on *Coffea arabica* was studied in the greenhouse. Coffee seedlings were either not inoculated, inoculated with the fungus alone, the fungus and the nematode simultaneously, or the fungus 2 or 4 weeks after the nematode. Chlorosis, root necrosis, wilting, and stunting were greater in plants inoculated with the fungus 4 weeks after the nematode. Similar but less severe symptoms were observed in plants to which the fungus was added 2 weeks after the nematode. Significant differences among treatments were found in height and dry weight of roots and shoots. Histological studies of root sections revealed giant cell development and hyphal penetration of giant cells, xylem vessels, and the female nematode.

Key words: Coffea arabica, coffee, disease complex, *Fusarium oxysporum,* histopatology, *Meloidogyne incognita,* root-knot nematode.

RESUMEN

Negrón, J. A., y N. Acosta. 1989. El complejo *Fusarium oxysporum* f. sp. *coffeae–Meloidogyne incognita* en café 'Bourbón'. Nematrópica 19:161–168.

La asociación entre *Fusarium oxysporum* f. sp. *coffeae* y *Meloidogyne incognita* en *Coffea arabica* fue estudiada en el invernadero. Plántulas de café fueron inoculadas con el hongo solo, el hongo y el nematodo añadidos simultaneamente, el hongo añadido 2 o 4 semanas después del nematodo. Plantas no inoculadas se utilizaron como testigos. Los síntomas de clorosis, necrosis radical, marchitez y enanismo fueron más evidentes en las plantas inoculadas con el hongo 4 semanas despúes de la inoculación con el nematodo. Síntomas similares pero no tan marcados fueron observados en las plantas inoculadas con el hongo 2 semanas despúes del nematodo. Entre los tratamientos, se detectaron diferencias significativas en la altura de plantas sobre el nivel del suelo, en el peso seco de raíces y partes aéreas. Estudios anatómicos de secciónes histológicas mostraron el desarrollo de células gigantes, hifas del hongo invadiendo células gigantes, los vasos del xilema y las hembras del nematodo.

Palabras claves: café, *Coffea arabica,* complejo de enfermedad, *Fusarium oxysporum,* histopatología, *Meloidogyne incognita,* nematodo nodulador.

INTRODUCTION

In 1892, Atkinson (2) was the first to report a nematode–fungus complex. He observed that *Fusarium* wilt in cotton was always severe in fields infested by root–knot nematodes. Since this report, disease complexes involving root–knot nematodes and *Fusarium* have been studied extensively on several crops (3,9,11,14,16).

Nematode-fungal associations have been studied in different crops in Puerto Rico. The severity of sugar cane root rot caused by *Pythium graminicola* Subr. was more severe in the presence of *Pratylenchus zeae* Graham (18). Similarly, the severity of *Fusarium* wilt of *Dioscorea rotundata* Poir. (12) and *Fusarium* root rot of *Capsicum annuum* L. (19) were increased by *Meloidogyne incognita* (Kofoid & White) Chitwood.

Fusarium wilt is a serious disease commonly found on coffee plantations in Puerto Rico. This disease, caused by the fungus *F. oxysporum* f. sp. *coffeae* Alvarez, is characterized by reduction in vigor and production resulting in death of infected trees several years after appearence of the first symptoms. The existence of a disease complex involving *M. incognita* and *F. oxysporum* f. sp. *coffeae* has been suspected for some time since high population densities of *M. incognita* were associated with wilted coffee trees. The objectives of this study were to determine whether *M. incognita* increases the severity of *Fusarium* wilt in coffee grown in Puerto Rico and to elucidate the interrelationship of *M. incognita* and *F. oxysporum* f. sp. *coffeae*.

MATERIALS AND METHODS

Cultures of *M. incognita* and *F. oxysporum* f. sp. *coffeae* were obtained from diseased coffee trees growing in a commercial plantation. A monospecific population of *M. incognita* was increased on tomato (*Lycopersicon esculentum* Mill.) cvs. Rutgers and Homestead 94. Fungi were isolated from roots and stems of diseased plants obtained from a commercial coffee plantation and maintained on potato dextrose agar (PDA). After purification, those isolates showing the characteristics of *F. oxysporum* were sent to Dr. Shirley Smith at the *Fusarium* Research Center, Pennsylvania State University, who confirmed our identification.

Two-month-old plants of coffee cv. Buorbón free of nematodes and insects were obtained from a commercial nursery and planted in 15-cm-diam plastic pots containing 1 500 cm^3 of a methyl bromide-treated soil mixture (64% sand, 10% clay, and 26% silt) with a pH 6.85. At transplanting, three inoculation tubes, 15 cm long × 1 cm diam (Fig. 1) were placed equidistantly around the plant. Plants were kept in the greenhouse for 2 weeks prior to inoculation (10).

Nematode inoculum was prepared by extracting eggs and second-stage juveniles (J2) from roots using NaOCl (7). Fungal inoculum was

produced by removing discs with a No. 4 cork borer from the periphery of 7-day-old colonies of *F. oxysporum* f. sp. *coffeae* growing on PDA at 28 C and placing the disks in 500 ml flasks containing 250 ml of a previously sterilized nutrient solution (10). The flasks were placed on a rotary shaker at 28 C and 100 rpm for 7 days.

The experiment was conducted in a greenhouse for 6 months. Treatments consisting of the fungus alone, the fungus and the nematode added simultaneously, the fungus 2 and 4 weeks after the nematode and noninoculated plants, were replicated 15 times in a completely randomized design. Nematode inoculum consisting of 16 000 eggs and J2 was added to each pot using the inoculation tubes. Thirty ml, containing 200 000 spores/ml (6), of a 7-day-old culture of *F. oxysporum* f. sp. *coffeae* was added to the rhizosphere of coffee seedlings through the inoculation tubes.

Six months after inoculation, data on plant height (from soil level to the apex of the seedling) and dry weight of shoots and roots were recorded. Root-knot severity was rated on a 0–5 scale where 0 = 0 galls; 1 = 1–2 galls; 2 = 3–10 galls; 3 = 11–30 galls; 4 = 31–100 galls; and 5 = more than 100 galls per root system. Portions of the root system from all treatments were cut, washed free of soil, and fixed in FAA (8). Samples (1 cm long) were dehydrated in tert-butyl-alcohol, and embedded in Paraplast® (Sherwood Medical, St. Louis, Missouri, U.S.A.) (8). Longitudinal sections (12 μm thick) were cut with a rotary microtome, mounted on glass slides previously coated with Haupt's adhesive and stained with safranin for 7 minutes at 48 C and fast green for 5 seconds.

RESULTS AND DISCUSSION

Typical symptoms of *Fusarium* wilt diseases such as chlorosis, root necrosis, and wilting were observed to a greater extent in plants inoculated with the fungus 2 and 4 weeks after the nematode than in plants with both organisms added simultaneously, or in those with the fungus alone.

Vascular pathogens such as *F. oxysporum* f. sp. *coffeae*, alter the normal translocation of water in the plant by clogging the vessels with fungal structures, by accumulation of metabolic products from the pathogen, by activity of toxins produced by the pathogen or by production of tyloses by the plant (4,5). These inhibitions to water transport may result in the expression of chlorosis and wilting as observed in the present study.

Significant differences ($P = 0.05$) in height (Fig. 1 and Table 1) were obtained in plants inoculated with the *F. oxysporum* f. sp. *coffeae* 4 weeks after *M. incognita* and those inoculated with the fungus 2 weeks after the nematode when compared to plants inoculated with both pathogens simultaneously, plants inoculated with the fungus only, and the controls.

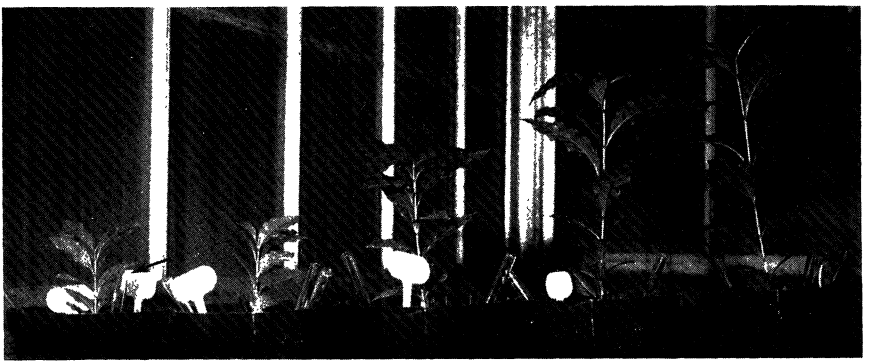

Fig. 1. Differences in height of coffee seedlings (cv. Bourbón) 6 months after inoculation with *Meloidogyne incognita* eggs and second-stage juveniles and *Fusarium oxysporum* f. sp. *coffeae*. From left to right: A) Fungus added 4 weeks after the nematode. B) Fungus added 2 weeks after the nematode. C) Fungus and nematode added simultaneously. D) Fungus added alone. E) Control.

Significant differences in height also were obtained between plants inoculated with the fungus and the nematode simultaneously and those inoculated with the fungus alone when compared to the noninoculated plants.

Significant reductions ($P = 0.05$) were obtained in dry weight of roots and shoots of inoculated plants when compared to the controls (Table 1). Significant differences in root gall indices also were detected among nematode inoculated plants, suggesting an effect on the nematode that was dependent upon the time at which the fungus was added (Table 1 and Fig. 2 A–E). The highest gall index (4.7) was obtained from plants inoculated with the fungus and the nematode simultaneously. The index was lower when the fungus was added 2 or 4

Table 1. Effects of single and combined inoculations of *Meloidogyne incognita* and *Fusarium oxysporum* f. sp. *coffeae* on growth of coffee cv. Bourbón and nematode infection.

Treatment	Height (cm)	Dry weight (g)		Gall index[z]
		Root	Shoot	
F. oxysporum added 4 weeks after *M. incognita*	15.4 a	0.4 a	1.1 a	3.9 a
F. oxysporum added 2 weeks after *M. incognita*	16.6 a	0.4 a	1.3 a	4.3 b
F. oxysporum and *M. incognita* added simultaneously	20.9 b	0.6 b	1.9 b	4.7 c
Fungus alone	21.2 b	0.7 b	2.0 b	0.0 d
Control	23.5 c	0.8 c	2.3 c	0.0 d

Data are means of 15 replications per treatment. Means in a column followed by the same letter do not differ significantly ($P = 0.05$) according to Duncan's multiple range test.
[z]Index based on a 0–5 scale where 0 = 0 galls; 1 = 1–2 galls; 2 = 3–10 galls; 3 = 11–30 galls; 4 = 31–100 galls; and 5 = > 100 galls per root system.

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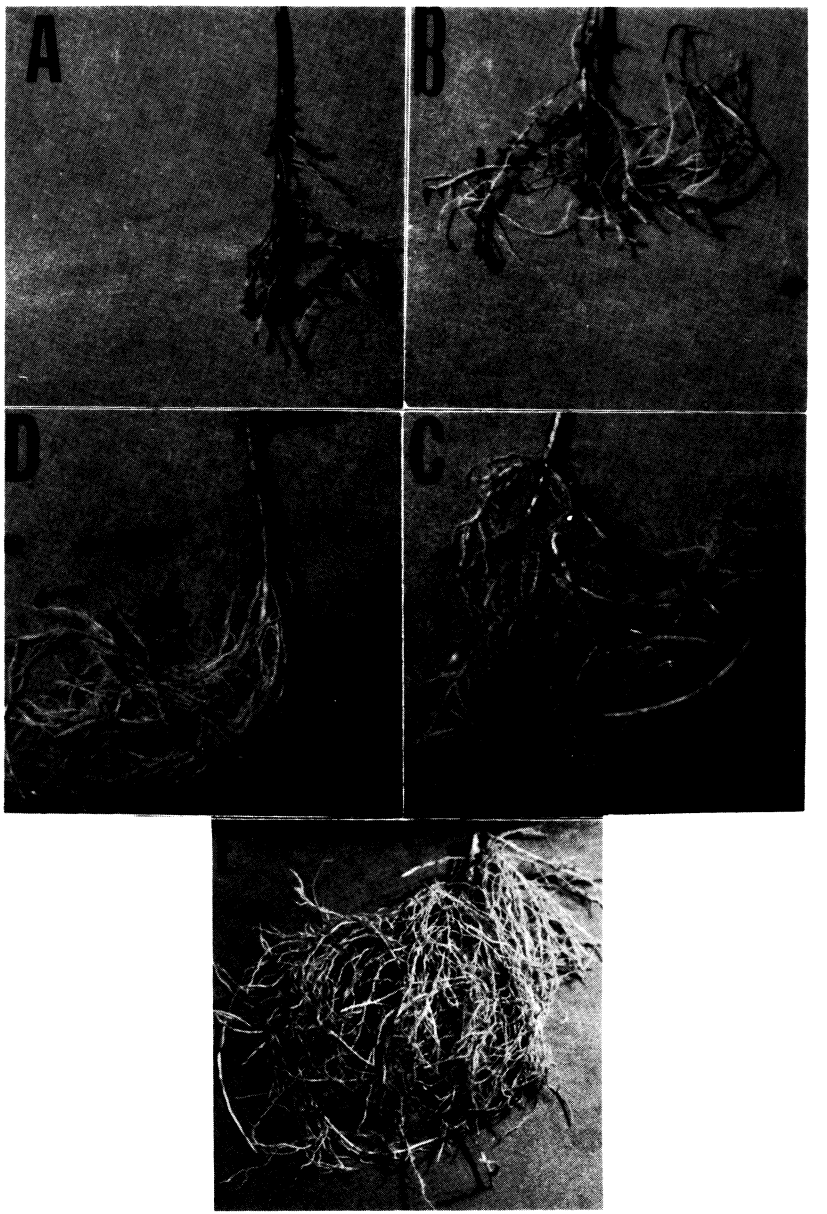
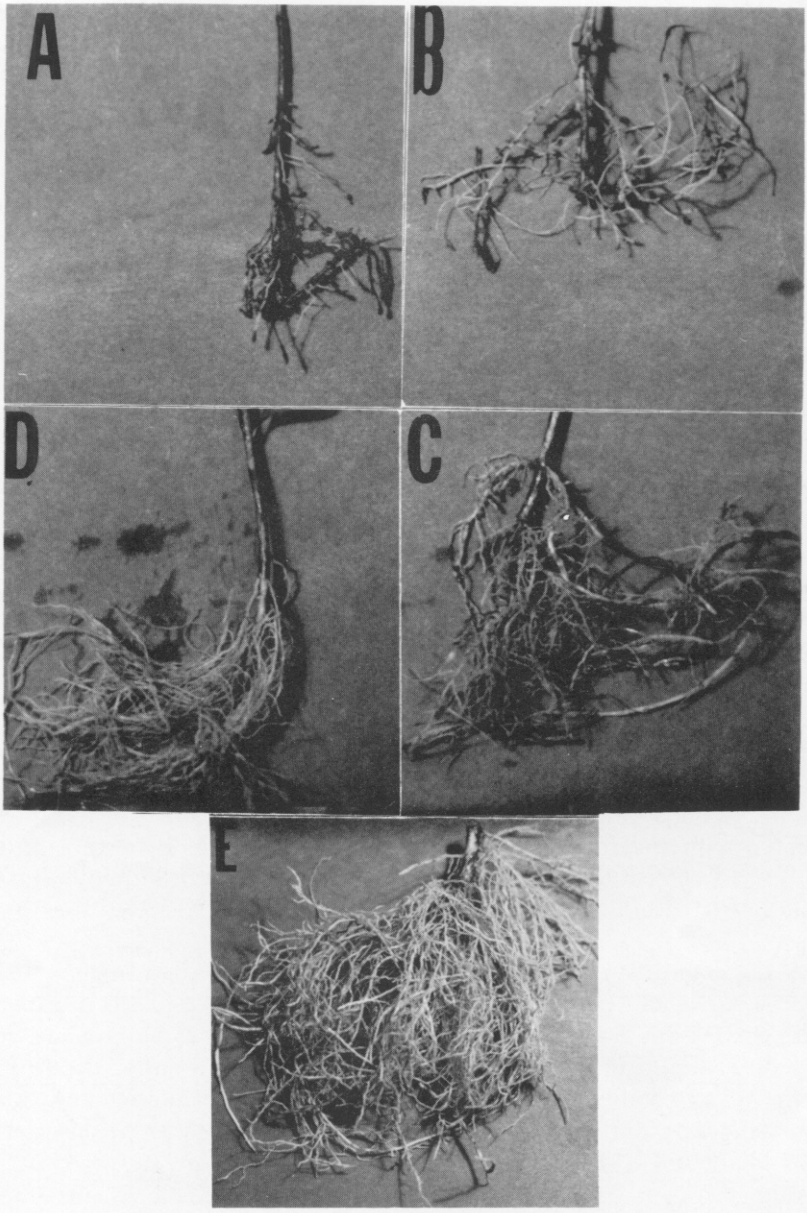


Fig. 2. Coffee roots from 6-month-old coffee seedlings inoculated with *Meloidogyne incognita* and *Fusarium oxysporum* f. sp. *coffeae* showing root rot and reduction in size compared to the control. A) Fungus added 4 weeks after the nematode. B) Fungus added 2 weeks after the nematode. C) Fungus and nematode added simultaneously. D) Fungus alone. E) Control.



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weeks after the nematode (4.3 and 3.9, respectively). Apparently, an antagonistic effect against *M. incognita* occurred when the plants were inoculated with the fungus 2 or 4 weeks after the nematode. Powell (17) has stated that populations of sedentary nematodes are generally reduced as a result of interactions with fungi. Negrón and Acosta (13) reported no significant differences in the gall index of coffee seedlings inoculated with different population densities of *M. incognita*. Fungal penetration and colonization of the root system enhanced by the previous establishment of the nematode may account for reductions in growth and development of the host as well as for differences in the gall index of nematode-infected plants.

Previous authors (10,14–16) reported that tobacco roots with root-knot galls were more susceptible to invasion by fungi than roots without galls. They suggested that the nematode acts as an aggravator, modifying the internal physiology of the plant allowing a better establishment of the fungus in addition to causing mechanical injury in the roots. Antagonistic relationships between fungi and nematodes when the plants are inoculated with the fungus either 2 or 4 weeks after the nematode were reported previously (15). The physiological changes induced by *M. incognita* in tobacco are responsible for the profuse colonization by several different fungi and the predisposition of the host to penetration and colonization by the fungi. This process reached its maximum level 2 to 4 weeks after nematode infection or after the nematode initiated giant cell formation. Those observations of diseased tobacco are consistent with those reported herein for coffee.

Reductions in values of the different parameters measured in plants treated with the fungus alone confirms the findings of other authors (9–11,16) who demonstrated that fungal penetration and colonization may occur without predispostion caused by nematodes or other incitants.

Histological studies of infected root sections revealed hyphae inside the xylem vessels, giant cells and female nematodes of plants inoculated with the fungus 2 and 4 weeks after *M. incognita* (Fig. 3A). Hyphae were not observed in the xylem of plants inoculated with either the fungus alone or when inoculated with both organisms simultaneously. Some females and giant cells were colonized extensively by the fungus (Fig. 3C). Giant cells colonized by the fungus were partially depleted of cell contents in some sections and were completly depleted in others. Hyphae in these cells were abundant (Fig. 3B). There was no evidence indicating that the colonization by the fungus of female nematodes and giant cells was associated directly with that of the xylem vessels. There were more adult females present in root sections from plants with the fungus and the nematode added simultaneously than in those inoculated with the fungus 2 and 4 weeks after the nematode which is consistent with the gall index findings.

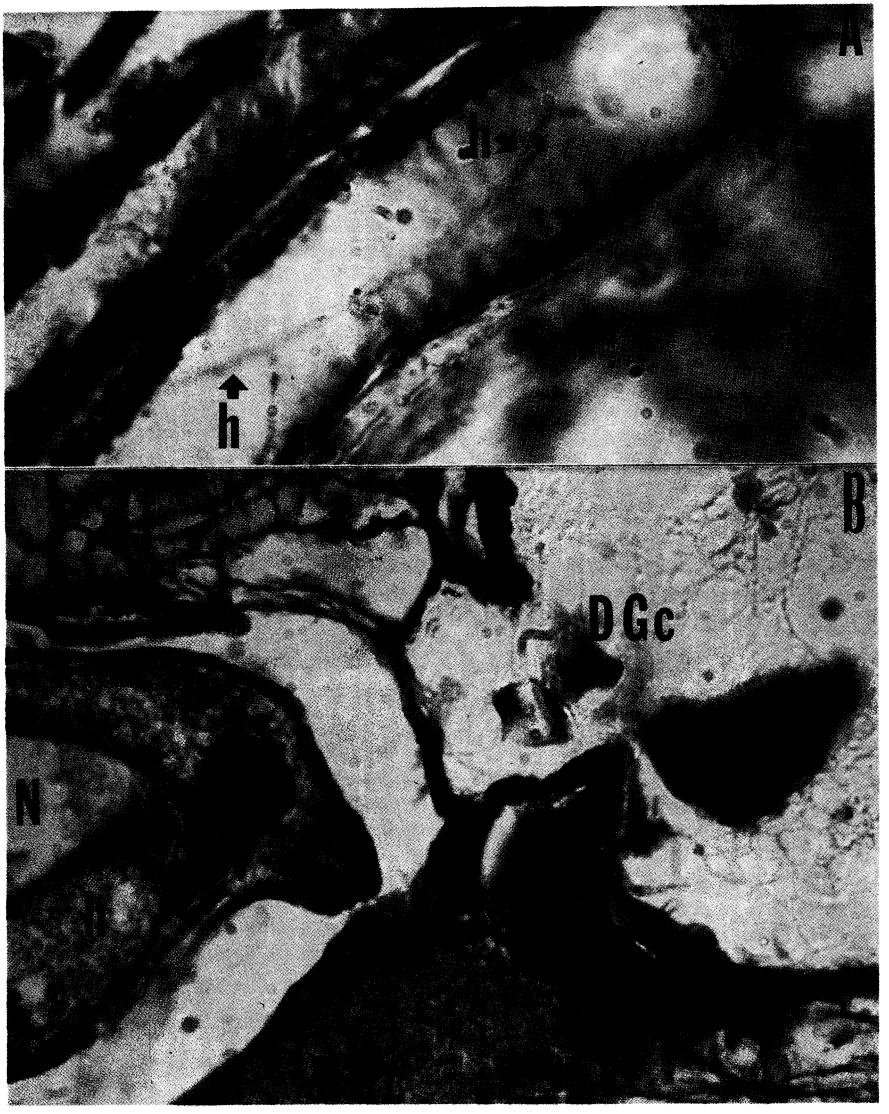
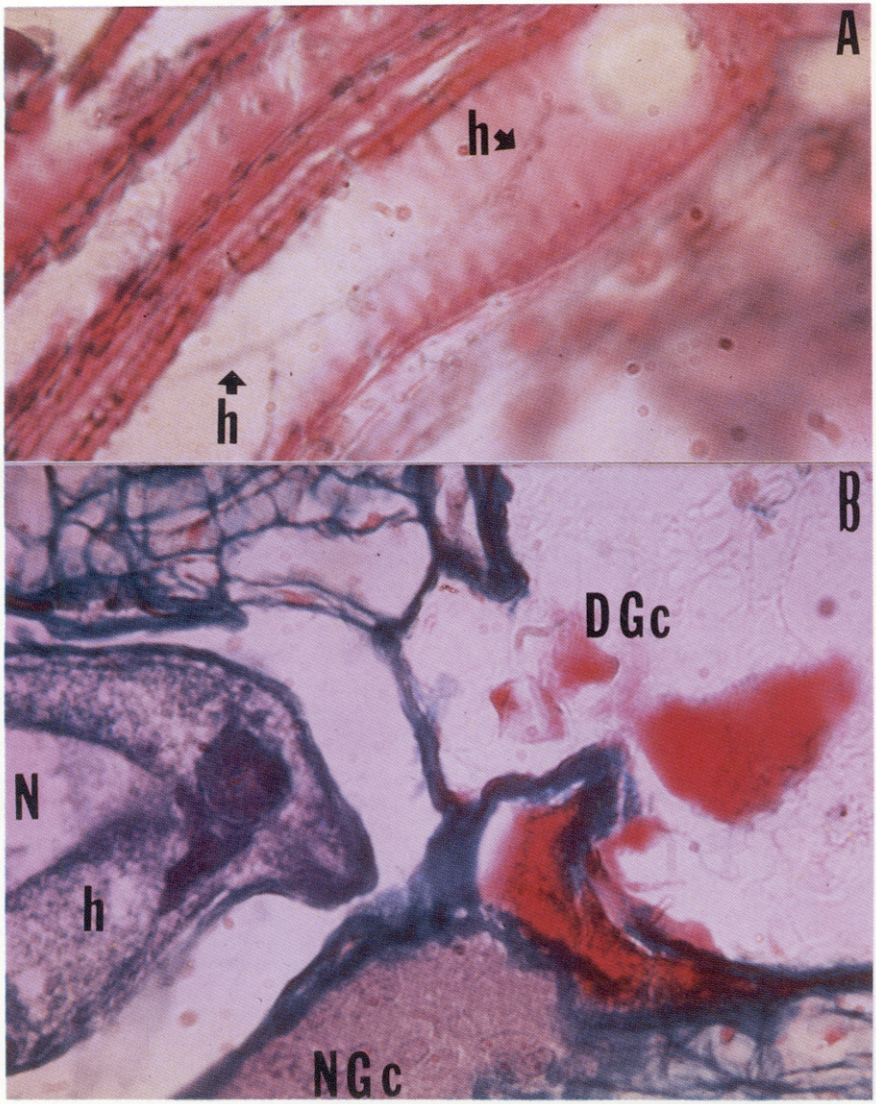


Fig. 3. Micrographs of coffee root infected with *Meloidogyne incognita* and *Fusarium oxysporum* f. sp. *coffeae*. A) Histological section showing xylem infected with hyphae (h). B) Female nematode (N) infected with hyphae (h) and depleted of contents with the anterior portion surrounded by giant cells. Normal giant cell (NGc) and depleted giant cell (DGc).

Minton and Minton (11) found that in cotton seedlings, *F. oxysporum* f. sp. *vasinfectum* (Atk.) Snyder & Hansen colonized giant cells induced by the root-knot nematode. Meléndez and Powell (10) reported that *F. oxysporum* f. sp. *nicotianae* (J. Johnson) Snyder & Hansen extensively colonized giant cells resulting in depletion of the cytoplasmic content.



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Results from these greenhouse studies have demonstrated the existance of an interaction between *M. incognita* and *F. oxysporum* f. sp. *coffeae* that is aggravated when the nematode is established in the plant at least 2 weeks before the fungus.

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