

HISTOLOGICAL ALTERATIONS INDUCED BY *ROTYLENCHULUS RENIFORMIS* ALONE OR SIMULTANEOUSLY WITH *MELOIDOGYNE INCOGNITA* ON BANANA ROOTS.

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ABSTRACT

Vovlas, N., and H.M.R.K. Ekanayake. 1984. Histological alterations induced by *Rotylenchulus reniformis* alone or simultaneously with *Meloidogyne incognita* on banana roots. *Nematopica* 15:9-17.

A histopathological investigation of *Rotylenchulus reniformis* Linford and Oliveira on banana (*Musa acuminata* L.) roots showed that the nematode penetrates the cortex perpendicular to the stele, and establishes a permanent feeding site in the endodermis. Nematode feeding induced the fusion of endodermal, pericyclic, and vascular parenchymal cells to form a syncytium with hypertrophied nuclei and prominent nucleoli. Females of *R. reniformis* were also observed in a semiendoparasitic feeding position on galls induced by *Meloidogyne incognita* (Kofoid and White) Chitwood. Sections of banana roots infected concomitantly with the two nematode species showed *R. reniformis* syncytia formed by endodermal and pericyclic cells at the periphery of the stele, and *M. incognita* giant cells derived by vascular parenchyma or differentiating vascular cells in the central part of the stele.

Additional key words: reniform nematode, root-knot nematode, *Musa acuminata*, concomitant infection, histopathology.

RESUMEN

Vovlas, N. y H.M.R.K. Ekanayake. 1984. Alteraciones histológicas inducidas por *Rotylenchulus reniformis* solo o simultáneamente con *Meloidogyne incognita* en raíces de banano. *Nematopica* 15:9-17.

Una investigación histopatológica de *Rotylenchulus reniformis* Linford y Oliveira sobre raíces de banano (*Musa acuminata* L.) demostro que el nematodo penetra en la corteza en forma perpendicular al cilindro central y establece un lugar de alimentación permanente en la endodermis. La alimentación del nematodo induce a una fusión de células de la endodermis, periciclo y parénquima vascular para formar un sincitio con núcleo hipertrofiado y nucléolo prominente. Se observaron también hembras de *Rotylenchulus reniformis* en agallas inducidas por *Meloidogyne incognita* (Kofoid et White) Chitwood en posición de alimentación semiendoparásita. Secciones de las raíces de banano infectadas simultáneamente con las dos especies de nematodos demostraron un sincitio en *Rotylenchulus reniformis* formado por células de la endodermis y periciclo en la periferia del cilindro central y células gigantes en *Meloidogyne incognita* derivadas de un parénquima vascular o diferenciación de células vasculares en la zona central del cilindro.

Palabras claves adicionales: nematodo reniforme, nematodo nodulador, Musa acuminata, infección concomitante, histopatología.

INTRODUCTION

The reniform nematode, *Rotylenchulus reniformis* Linford and Oliveira, and the root-knot nematode, *Meloidogyne incognita* (Kofoid and White) Chitwood, are common parasites of a large number of cultivated plants, including banana (*Musa acuminata* L.), in tropical and subtropical countries (6, 11, 12). The damage caused by these two nematode species on bananas appears to be less serious than that induced by *Helicotylenchus multicinctus* (Cobb) Golden and *Radopholus similis* (Cobb) Thorne (2, 13). However, loss of yield caused by *Meloidogyne* spp. and *R. reniformis* has not been assessed experimentally on bananas.

There are several reports on the biology and host-parasite relationships of *R. reniformis* and *M. incognita* on banana (3, 6, 11) but information is lacking on the anatomical alterations induced on this host plant by either parasite alone, or in combination with one another.

Infections of banana by *R. reniformis* are common in Sri Lanka, and often associated with those of *M. incognita*. Mature females of *R. reniformis* are often observed on the roots galled by *M. incognita*. This study reports on the anatomical changes induced by *R. reniformis* alone and in association with *M. incognita* on banana roots.

MATERIALS AND METHODS

Banana roots infected with *R. reniformis* alone or concomitantly with *M. incognita* were collected from a banana orchard in Sri Lanka. Roots were washed free of soil, cut into 4-5 mm long segments, fixed in FAA (formalin, acetic acid, ethanol), dehydrated in tertiary butyl alcohol, and embedded in paraffin. Fixed tissue samples were then sectioned at 10-15 μm , stained in safranin and fast green, mounted in Dammar xylene, and examined with a compound microscope (5).

Root pieces infected with *R. reniformis* alone were also observed with a scanning electron microscope (SEM). Infected root pieces fixed in formalin were rinsed in distilled water, dehydrated through a series to 96% of ethanol and critical point dried (CP), using carbon dioxide. CP-dried specimens, after gold coating in a vacuum, were photomicrographed by SEM operating at an accelerating voltage of 10 Kv.

RESULTS AND DISCUSSION

In banana roots infected only with *R. reniformis*, females with the posterior portion of the body protruding from the root surface were

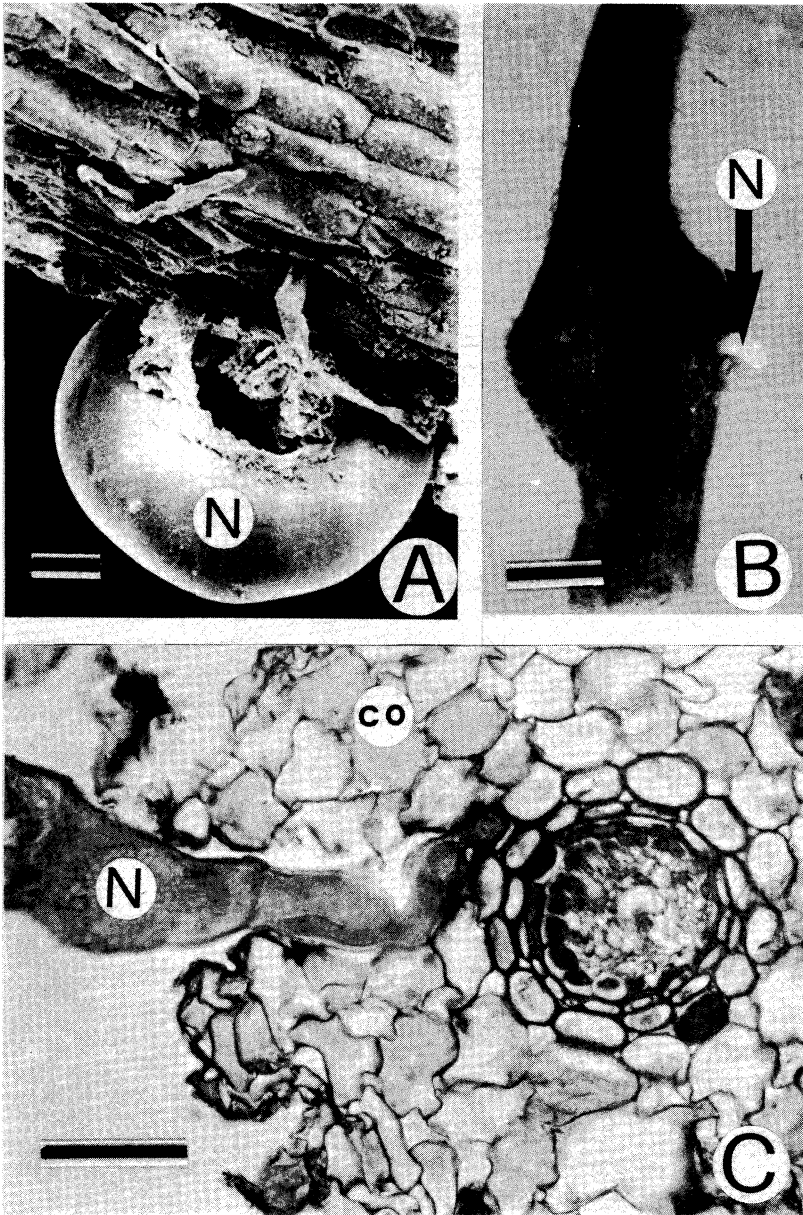


Fig. 1. Banana roots infected with *Rotylenchulus reniformis* alone (A) or concomitantly with *Meloidogyne incognita* (B); N = *R. reniformis*. C. Female (N) of *R. reniformis* partially penetrated into the cortex (co). (Scale bar = 50 μ m).

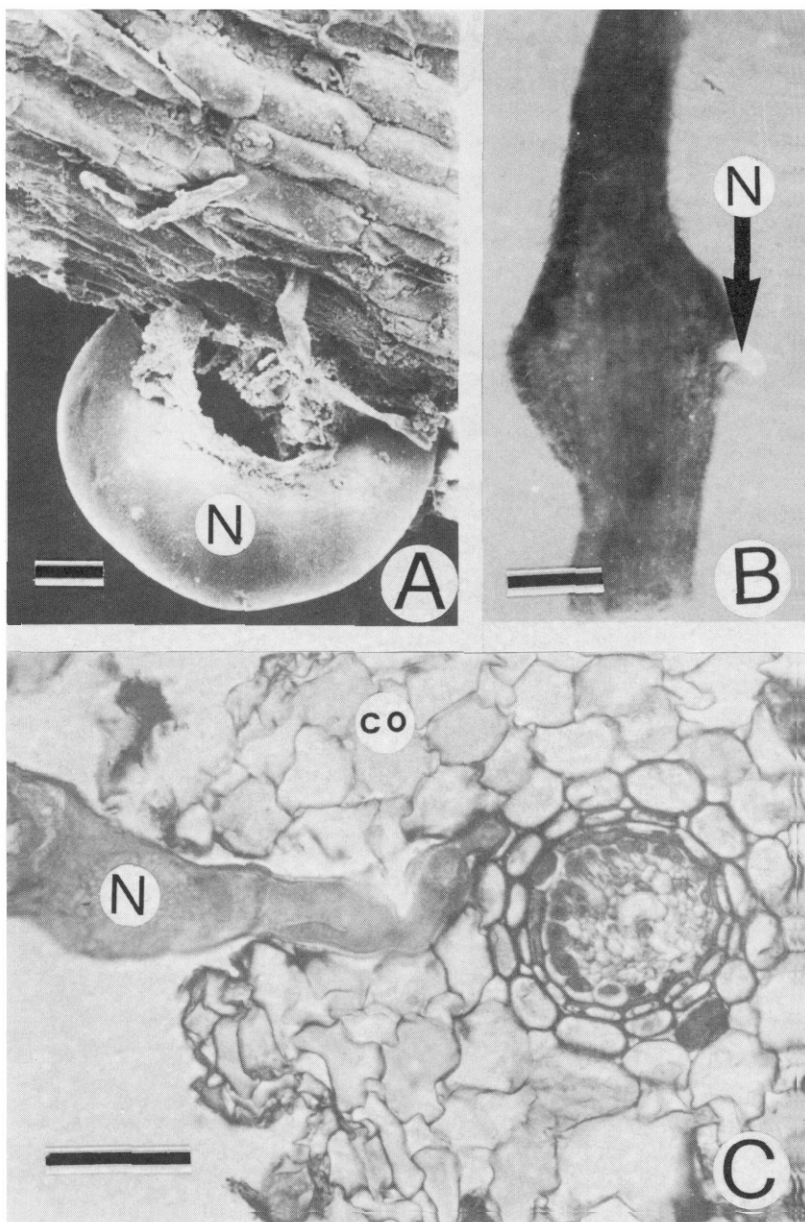


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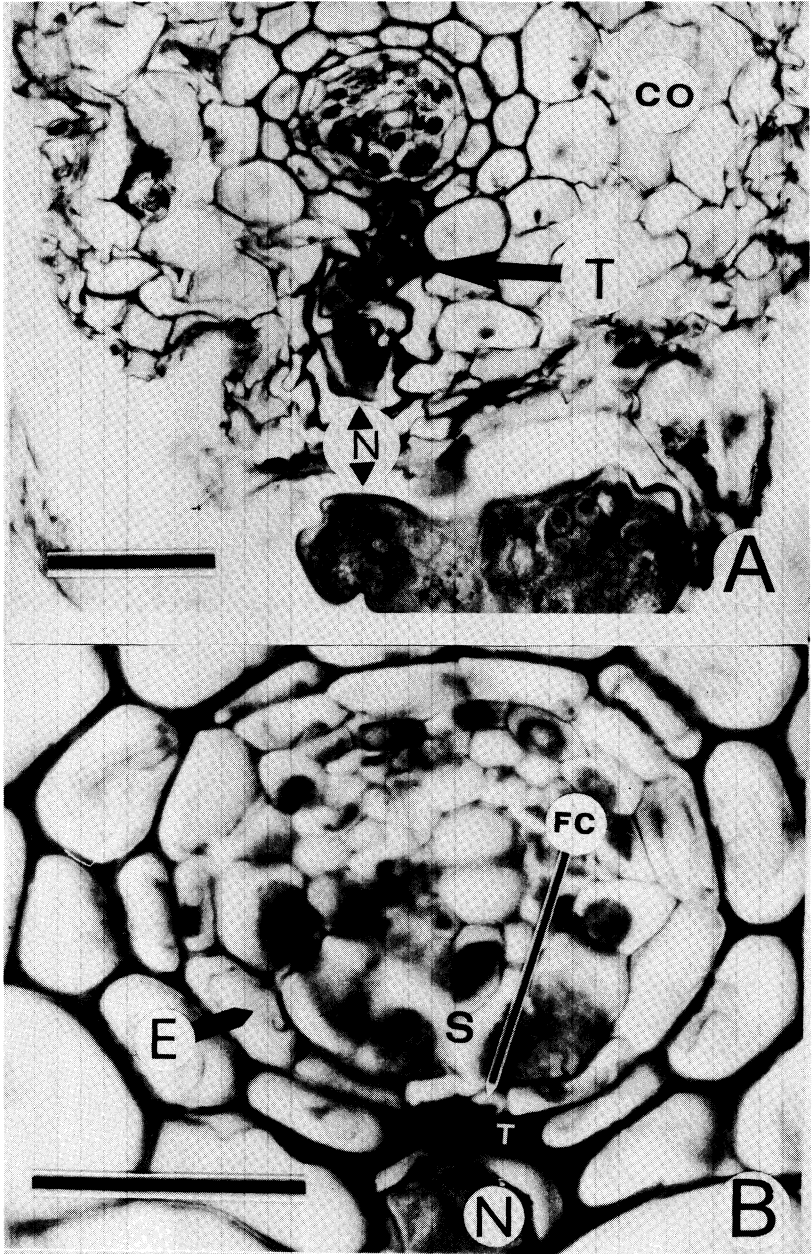


Fig. 2. Cross sections of banana roots infected with *Rotylenchulus reniformis* alone. A. Female (N) partially penetrated into the cortex (co) and feeding in an endodermal cell. Cortical cells adjacent to the nematode body show thickened (T) walls. B. Syncytium (S) induced by nematode (N) feeding in an endodermal cell. Note the thickened (T) wall in the outer portion of the feeding cell (FC). The wall thickening of the feeding cell in contact to pericycle is degraded; E = endodermis. (Scale bar = 50 μ m).

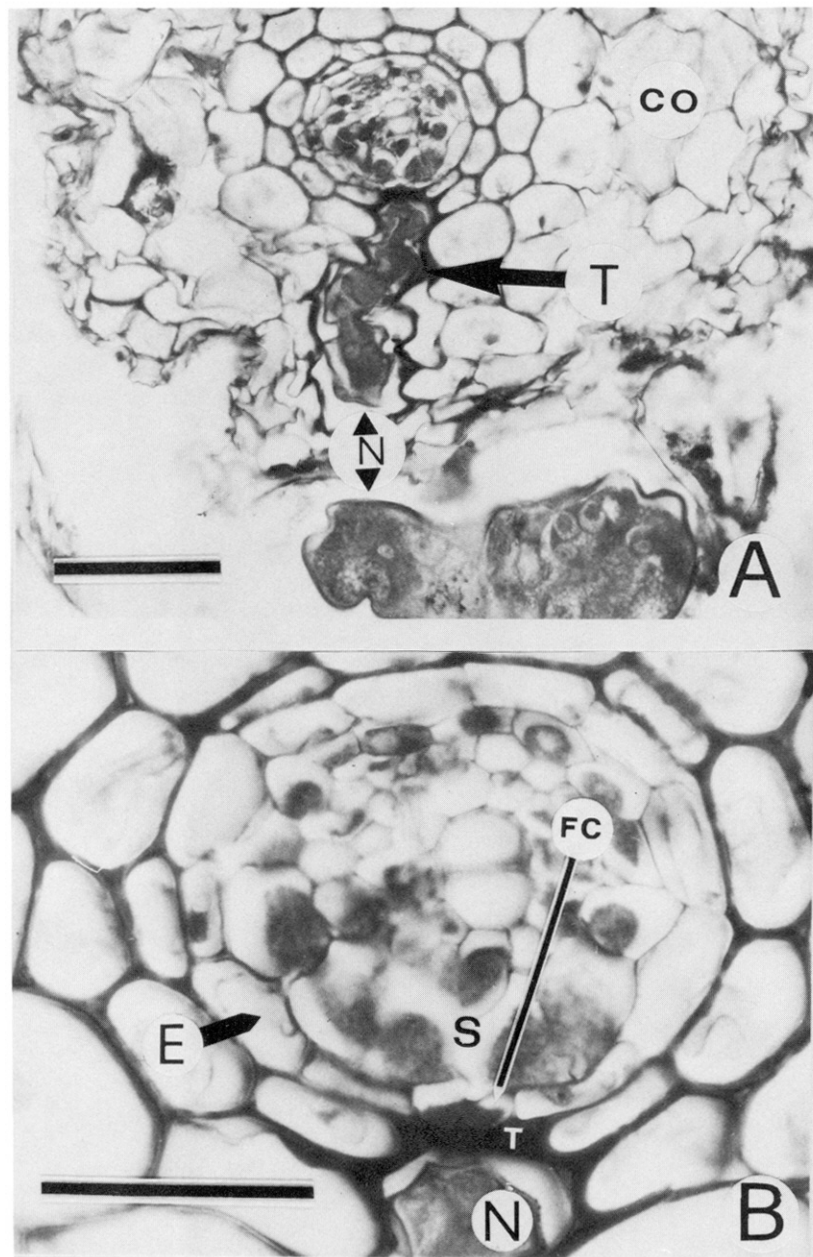


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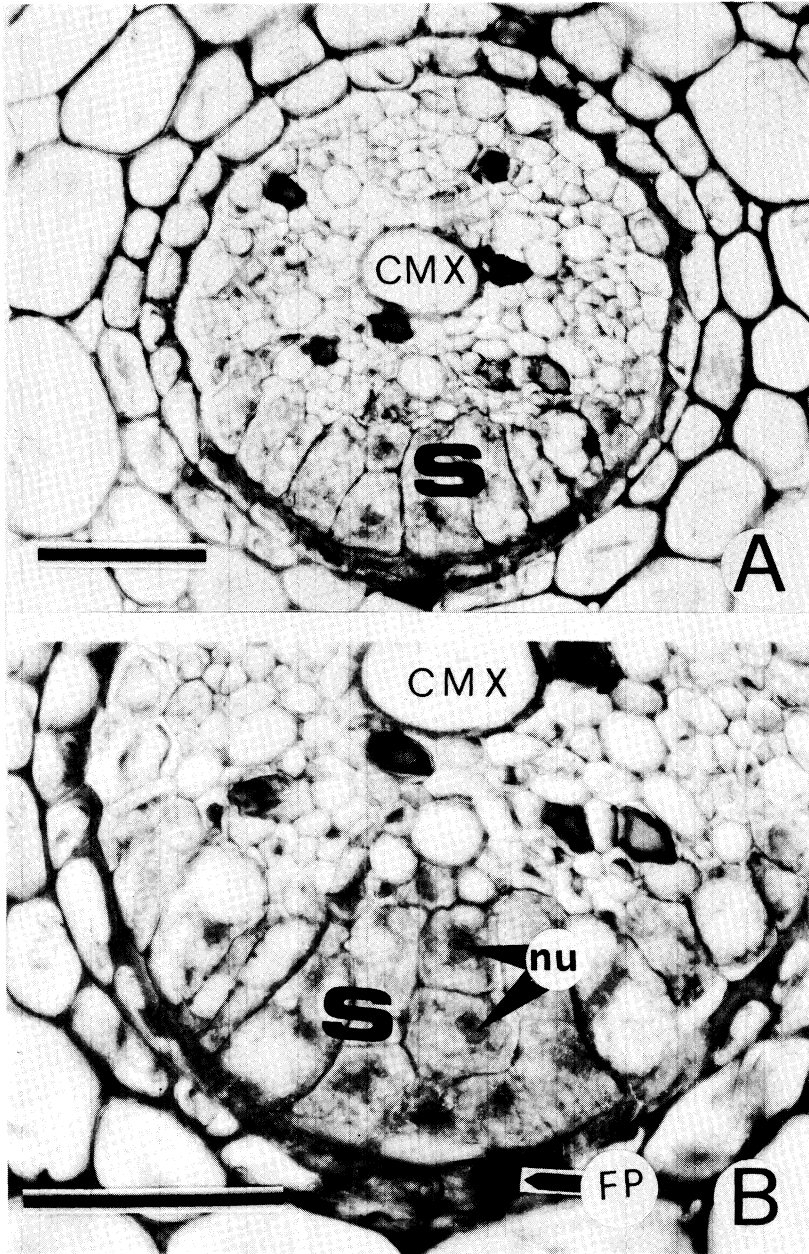


Fig. 3. Cross sections of banana roots infected with *Rotylenchulus reniformis* alone. A. Syncytium (S) induced by nematode feeding activity and formed by fusion of endodermal, pericyclic, and vascular parenchyma cells. B. The syncytium (S) of fig. 3A at more magnification showing hypertrophied nuclei (nu). CMX = central metaxylema; FP = feeding point. (Scale bar = 50 μ m).

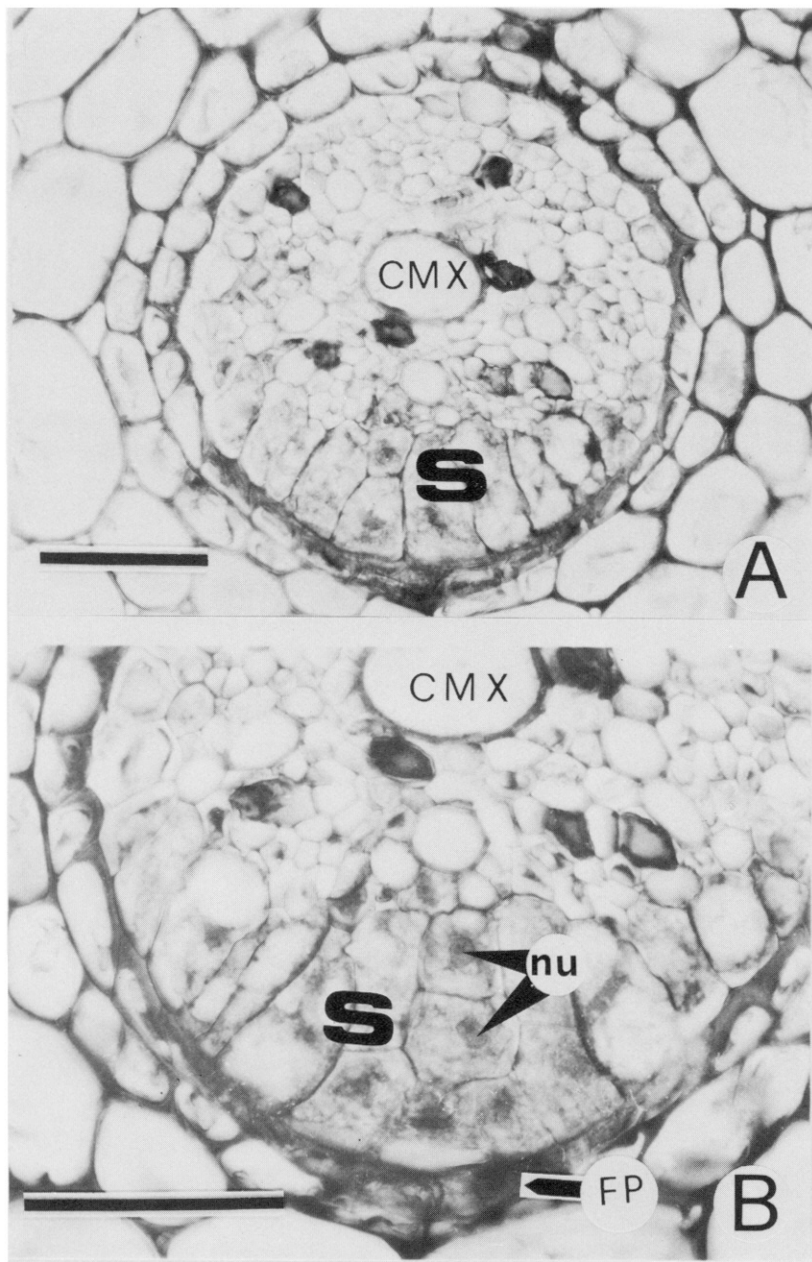


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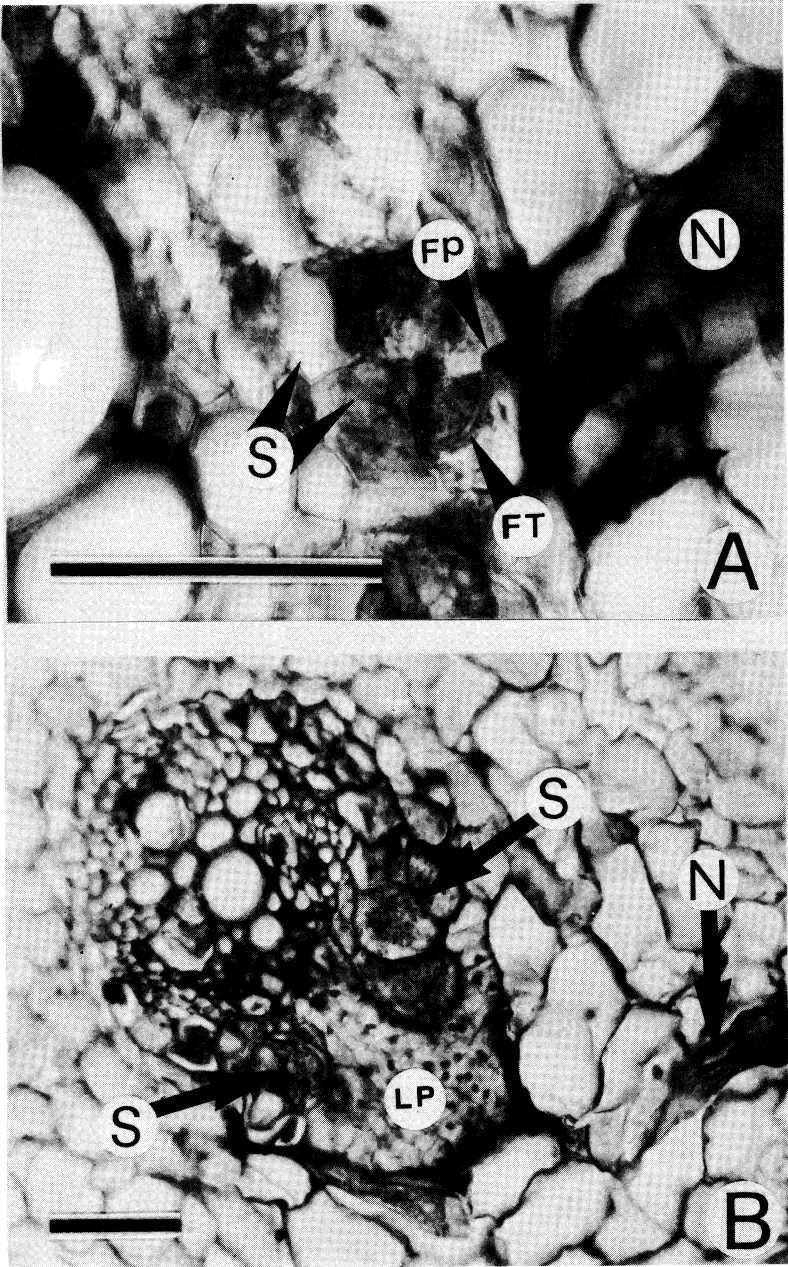


Fig. 4. Cross sections of banana roots infected with *Rotylenchulus reniformis* alone. A. Feeding peg (FP) induced by the nematode (N) corresponding to stylet penetration in an endodermal cell. A feeding tube (FT) is also visible in the syncytium (S). B. A syncytium (S) divided by a lateral root primordium (LP). N = nematode. (Scale bar = 50 μ m).

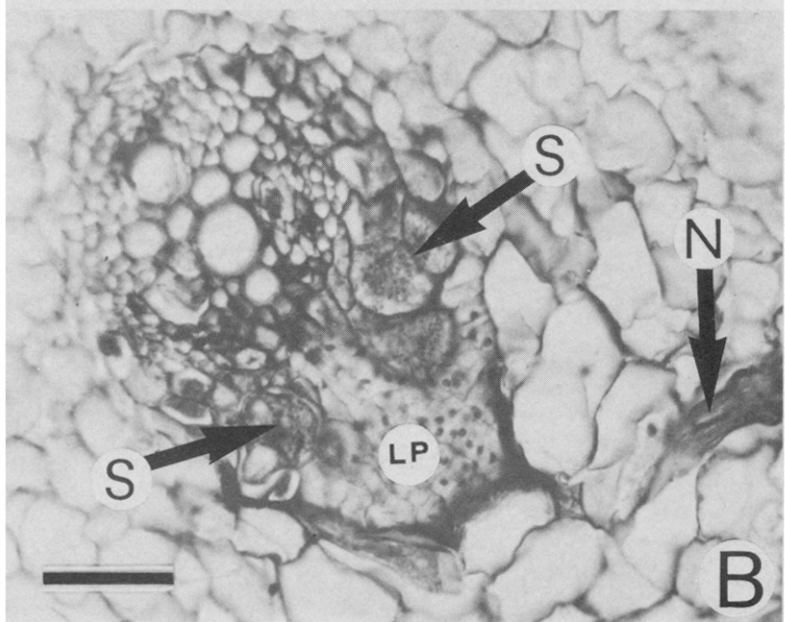
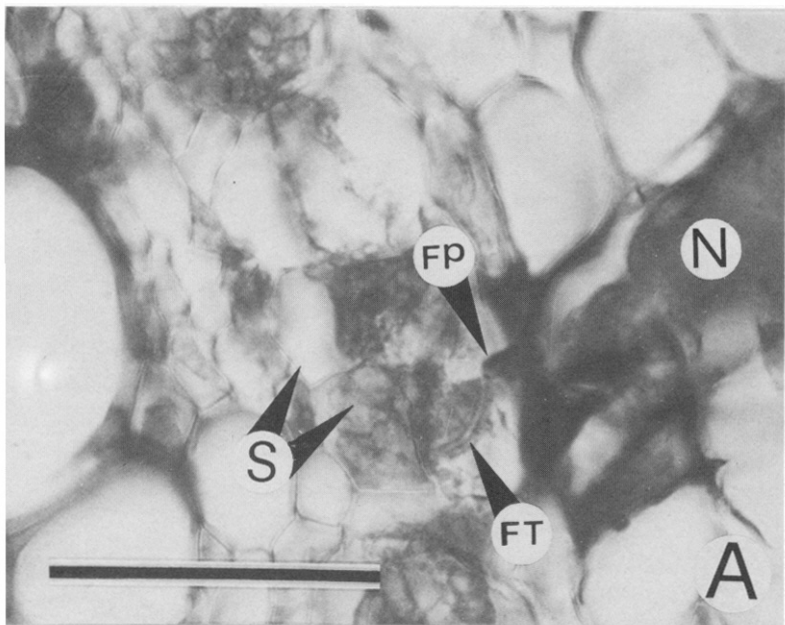


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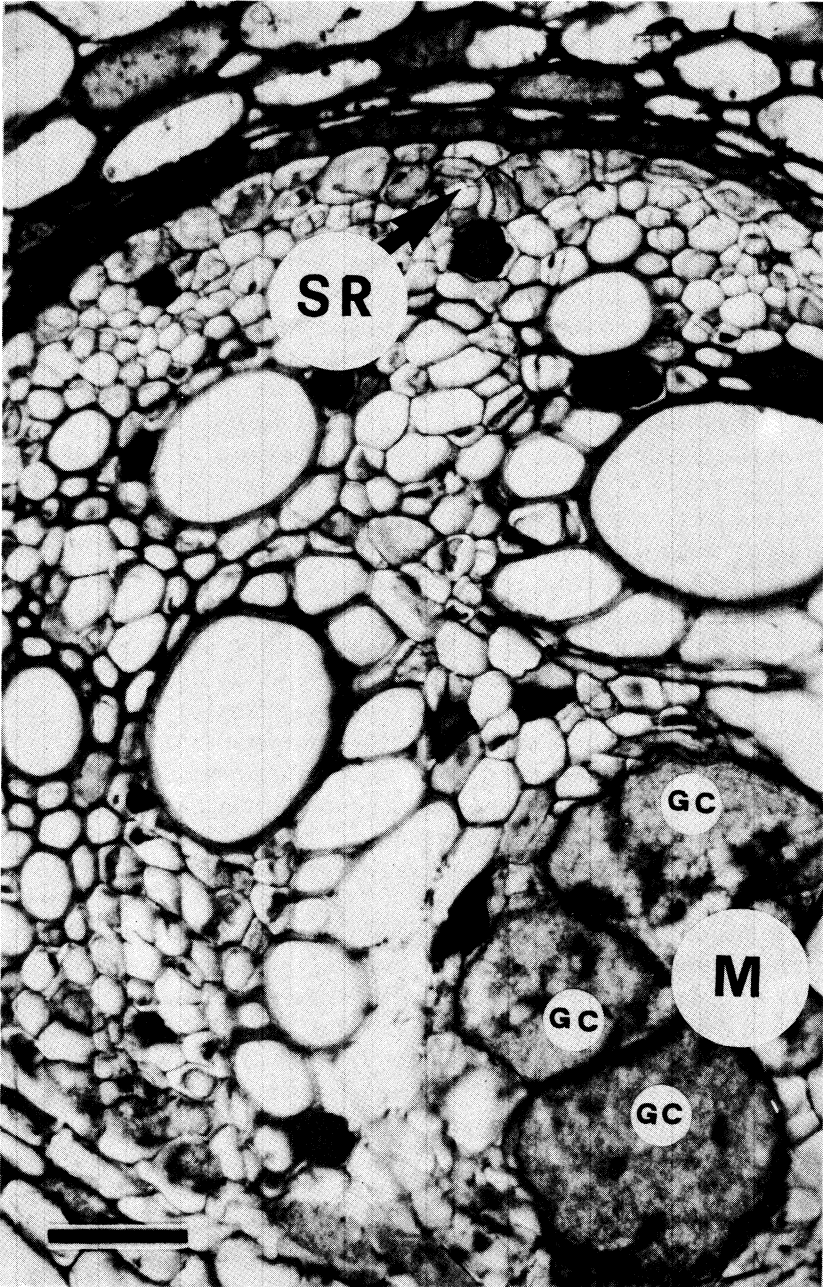


Fig. 5. Cross section of banana root gall concomitantly infected by *Rotylenchulus reniformis* and *Meloidogyne incognita*. The *R. reniformis* syncytium (SR) is formed by fusion of endodermal and pericyclic cells, whereas the *M. incognita* (M) giant cells (GC) are formed by differentiating vascular elements and are located in the center of the stele. (Scale bar = 50 μ m).

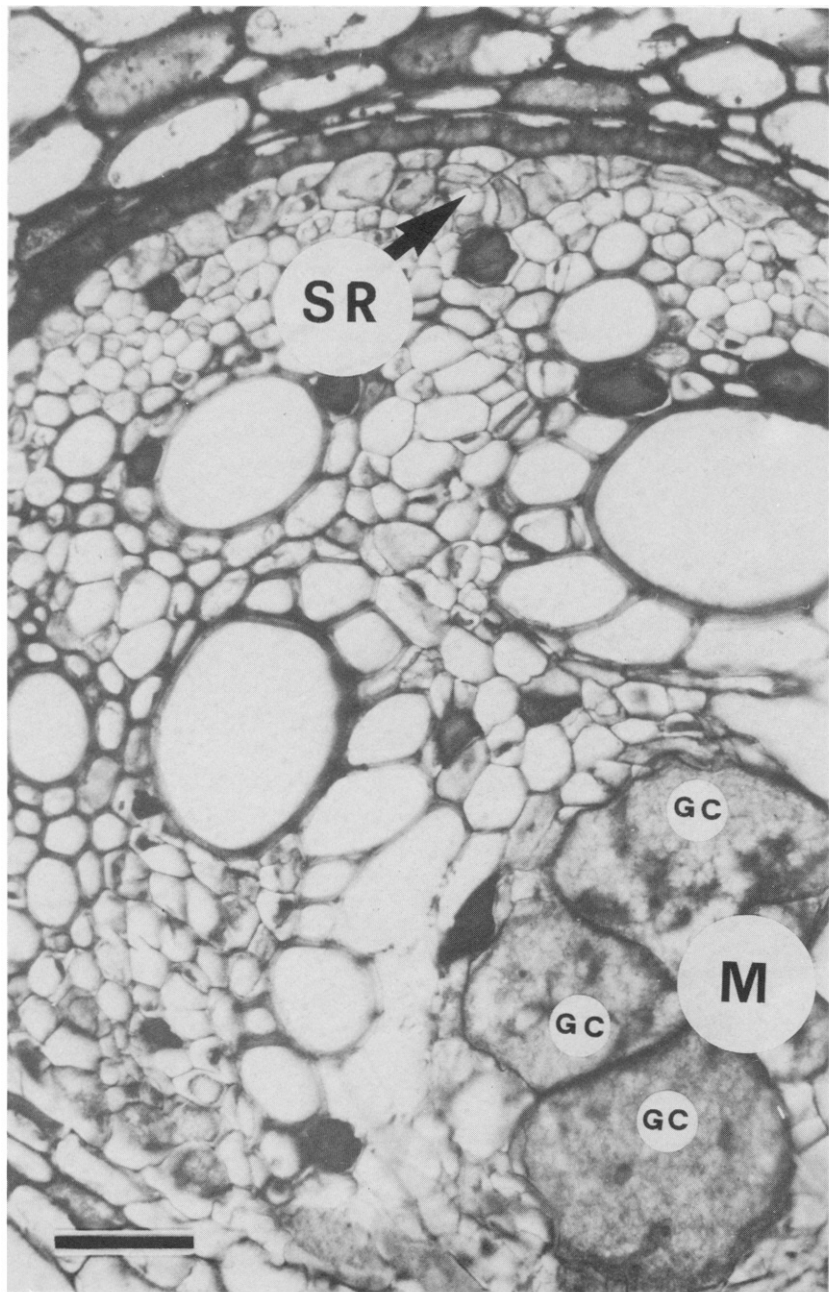


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detected on the tip and along the axis of the root (Fig. 1A). Often adult females of the nematode were observed protruding from the surface of galls induced by *M. incognita* (Fig. 1B). Females penetrated partially into the cortex and damaged cortical cells. They established a permanent feeding site in the endodermis by modifying an endodermal cell (Figs. 1C, 2A, 2B). This endodermal cell, referred by Rebois (9) as a prosyncyte and as a feeding cell by Razak and Evans (7), fused with adjacent endodermal and pericyclic cells forming a syncytium. The syncytial cells were enlarged with hypertrophied nuclei, prominent red-stained nucleoli, and darkly stained cytoplasm (Figs. 3A, 3B). Also vascular parenchymal cells were in some cases involved in the expansion of the syncytium that occupied 1/4 of the stelar area with consequent disorganization of the root structure (Figs. 3A, 3B).

The endodermal cell wall, at the point of stylet insertion, was thicker and more deeply stained by safranin than normal endodermal cells (Figs. 2B, 4A).

At the point of stylet penetration, a peg-like structure containing the nematode stylet was observed on a cell wall (Fig. 4A). The feeding peg, originating probably from cell wall material, was projected about 5 μm into the cytoplasm (Fig. 4A). Characteristic hyaline tubular secretions observed in other reniform-nematode-infected host plants by Rebois et al. (8) and Razak and Evans (7) were also detected in the feeding cell of banana roots (Fig. 4A). The feeding cell was situated at about the midpoint of the syncytium composed of 10-18 endodermal and pericyclic cells. Root age and size seemed to influence the number of cells forming the syncytium. In young roots, syncytial endodermic and pericyclic cells were observed along the circumference of the entire root section (Fig. 2B). In heavily infested roots, two nematodes were sometimes observed feeding on the same syncytium.

The histological alterations induced by *R. reniformis* on banana roots, described here, are similar to those induced by this species on roots of cantaloupe, cotton, soybean, and sunflower (1, 4, 9, 10).

Adult females of *R. reniformis* were also observed in *M. incognita* induced galls (Fig. 1B). Cross sections through the galls and with the two nematode species showed *R. reniformis* induced syncytia at the periphery of the stelar area and *M. incognita* giant cells in the central part of the root (Fig. 5). Although the two species concomitantly infect the same root, the induced cellular alterations occurred in different tissues; *R. reniformis* in endodermal and pericyclic cells, *M. incognita* in vascular parenchyma or differentiating vascular cells. Connections between the specialized cells induced by these two nematodes were not observed.

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