Biology of *Meloidogyne platani* Hirschmann Parasitic on Sycamore, *Platanus occidentalis*[1]

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Abstract: The development of *Meloidogyne platani* on sycamore was followed for 40 days (22–28 C). Juveniles penetrated the feeder roots behind the root cap and invaded the vascular cylinder within 3 days after inoculation. All subsequent development of the nematodes and host effects occurred only within the stele. The second juvenile molt and sex differentiation occurred by the 17th day. Young females were observed by the 26th day. Eggs were observed inside the roots by the 35th day and were exposed to the surface of galls by the 40th day. In pathogenicity studies, a significant negative correlation was shown to exist between fresh shoot and root weights and inoculum density. Besides sycamore, white ash was the only hardwood species tested to become infected. Of the herbacious plants tested, tobacco was heavily galled, tomato and watermelon moderately galled, and pepper only slightly galled. Egg production was moderate on tobacco, slight on tomato and watermelon, and absent on pepper. *Key words:* root-knot nematodes, *Meloidogyne* spp., Sycamore, histopathology, pathogenicity.

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American sycamore, *Plantanus occidentalis* L. (plane tree), one of the largest deciduous trees of eastern North America, occurs most frequently and reaches its largest size in alluvial soils along streams and bottomland (7). Information on nematode pathogens of sycamore is limited, and most reports have concerned only association of plant-parasitic nematodes (3,19,23). In a greenhouse study, Ruehle (20) has shown that *Meloidogyne arenaria* (Neal) Chitwood, *M. hapla* Chitwood, *M. incognita* (Kofoid and White) Chitwood, and *M. javanica* (Treub) Chitwood were not parasites of sycamore. However, in 1971 Clemons and Krusberg (4) found that roots of 10 of 20 London plane trees, *Platanus acerifolia* (Ait.) Willd., in Washington, D.C., were infected with *Meloidogyne* spp.

The present study was undertaken to determine the pathogenicity, host range, and histopathology of *Meloidogyne platani* Hirschmann (9) on sycamore seedlings. A preliminary report has been made (1).

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MATERIALS AND METHODS

The population of *Meloidogyne platani* used in these studies was obtained from infected sycamore seedlings collected from a nursery in Franklin, Virginia. The nematode was increased on one-year-old sycamore seedlings from a local nursery and later maintained on young seedlings grown from seed in the greenhouse.

To obtain sycamore seed for production of seedlings, intact fruits were picked directly from a tree on the North Carolina State University campus, Raleigh, North Carolina, in December 1976. The fruit (a spherical aggregate of achenes) was separated into individual achenes; the tawny hairs were removed; and the achene surface was sterilized with 1.25% sodium hypochlorite (25% commercial bleach) for 10 minutes, rinsed with water three to four times, and allowed to dry (Dr. R. C. Kellison, personal communication). The achenes were germinated in a wooden flat containing steam-sterilized peat moss in the greenhouse. The seedlings were transplanted at the two-leaf stage into 4-cm clay pots containing a steam-sterilized mixture of equal parts of sand and soil and grown at approximately 25 C. Eggs for inoculation were extracted with 0.5% sodium hypochlorite for 4 min (11) from infected roots of sycamore seedlings grown in the greenhouse. Unless otherwise indicated, inoculations were made by pouring a water suspension of eggs over and around the root system. Soil used in

these studies was a steam-sterilized mixture of equal parts of sand and Congaree loam soil.

Pathogenicity studies on sycamore: Seedlings 15-cm tall were transplanted into 15-cm clay pots containing sterilized soil. At the time of transplanting, 0, 1,000, 10,000, or 100,000 eggs of *M. platani* were added to each pot. All treatments were replicated five times and arranged in a completely randomized design. After 10 wk of growth at 22–28 C, plants were harvested and shoot and root fresh weights were determined.

Differential hosts studies: Tests were conducted to determine the ability of *M. platani* to infect and reproduce on differential hosts commonly used at North Carolina State University for root-knot nematode species identification and detection of variability. These included *Arachis hypogaea* L. 'Florunner' (peanut), *Capsicum frutescens* L. 'California Wonder' (pepper), *Citrullus vulgaris* Schard 'Charleston Grey' watermelon, *Fragaria chiloensis* Duchesne 'Allbritton' (strawberry), *Gossypium hirsutum* L. 'Deltapine 16' (cotton), *Ipomoea batatas* (L.) Lam. 'All Gold' and 'Porto Rico' (sweet potato), *Lycopersicon esculentum* Mill. 'Rutgers' (tomato), *Nicotiana tabacum* L. 'N.C. 95' (tobacco), and *Zea mays* L. 'A-401 Minn.' (corn). Sycamore was also included.

Three-week-old plants were transplanted into 10-cm clay pots containing sterilized soil, and 5,000 *M. platani* eggs were added. The plants, grown at 25–28 C for 9 wk, were watered and fertilized as needed. Each treatment was replicated four times. After 9 wk, the roots of each plant were gently washed free of soil and rated for number of galls and egg masses using the following scale: 0 = none, 1 = 1–2, 2 = 3–10, 3 = 11–30, 4 = 31–100, 5 = greater than 100. Root systems with no apparent galls were stained with phloxine B (.15 gm/liter tap water) for 15 min to detect egg masses.

Susceptibility of hardwood seedlings: The susceptibility of five hardwood species native to the southeastern United States to *M. platani* from sycamore was tested. The hardwood species were *Acer rubrum* L. (red maple), *Liriodendron tulipifera* L. (yellow popular), *Liquidambar styraciflua* L. (sweet gum), *Fraxinus americana* L. (white ash), and *Cornus florida* L. (dogwood). *Platanus occidentalis* L. (sycamore) was also included. One-year-old seedlings were obtained from a methyl bromide-fumigated plant bed at the Clayton Forestry Center in Clayton, North Carolina. All seedlings were washed free of soil, transplanted into 20-cm clay pots containing nematode-free soil, and then grown for 2 months before inoculation in order to establish a good root system. A water suspension of 10,000 eggs of *M. platani* was poured into five holes 10–15 cm deep around the seedlings. The plants were then grown at 25–28 C for 4 months and were watered, weeded, and fertilized as needed. Each treatment was replicated four times. After 4 months the seedlings were removed from the pots, washed free of soil, examined, and rated for galls and egg mass production.

Histopathological studies: Sycamore seedlings approximately 12 cm high were used in this study. The inoculum (4,000 eggs suspended in 20 ml water) was poured in a depression in the soil in 10-cm clay pots 3 days prior to placing the plant in the pot. The placement of plants in the infested soil was considered to be the time of inoculation. The study was conducted under greenhouse conditions at approximately 22–28 C. At 3–5-day intervals after inoculation, roots from one plant were harvested, gently washed with water, and allowed to drain. The roots were fixed in FAA (40% formaldehyde, 6.5 ml; glacial acetic acid, 2.5 ml; and 50% ethanol, 100 ml) for at least 3 wk, dehydrated in a tertiary butyl alcohol series, and embedded in Tissuemat (melting point 56–58 C). Root galls were sectioned transversely or longitudinally at 12 μm with a rotary microtome, then stained with Johansen's Quadruple Stain (14), and examined microscopically.

RESULTS

Pathogenicity studies on sycamore: The shoots of inoculated plants, especially those inoculated with the highest inoculum level (100,000 eggs/pot), were stunted and the leaves were light green (Fig. 1A). A significant negative correlation ($r = -0.99$) developed between fresh shoot and root weights and inoculum density (Fig. 2). The

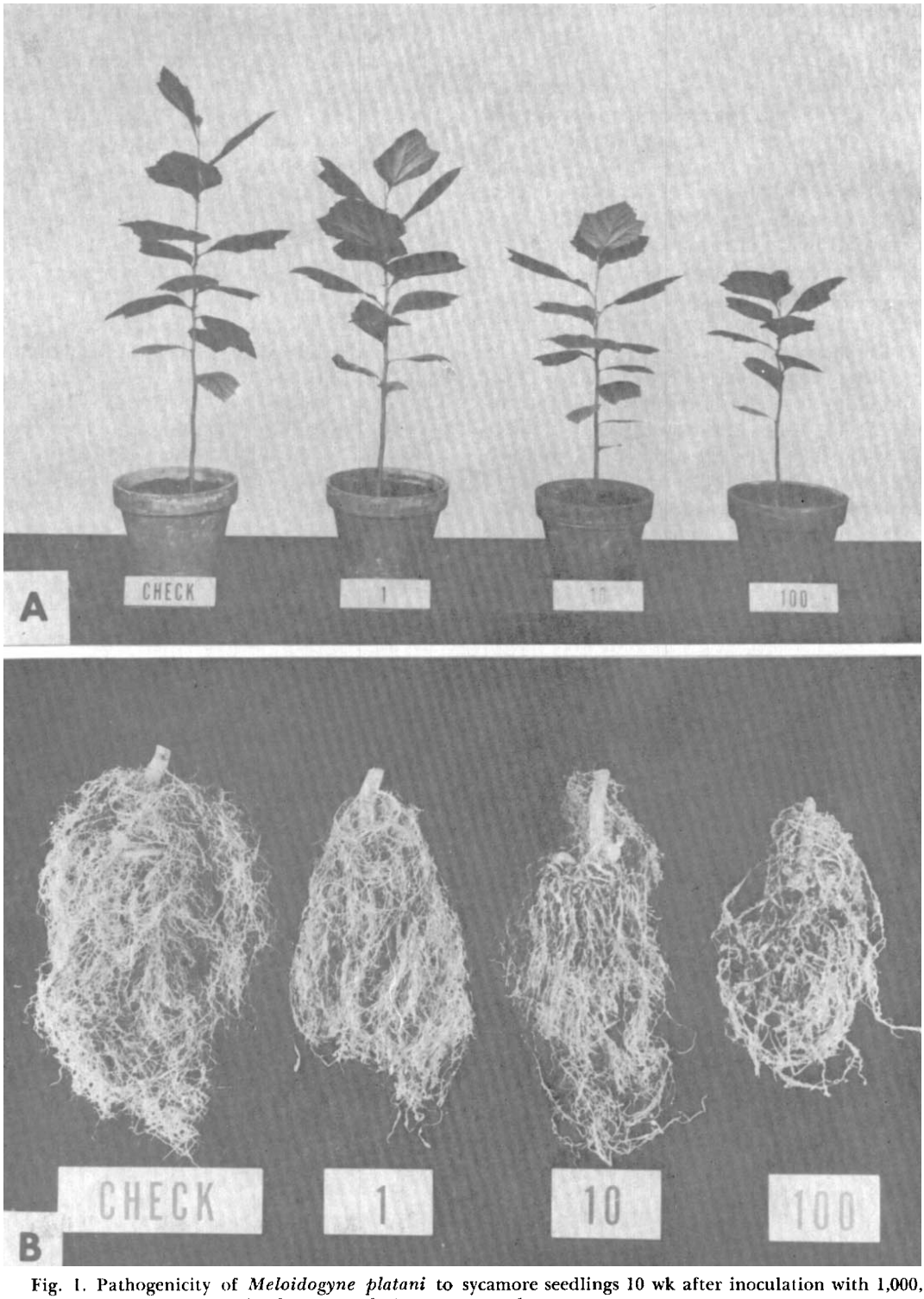

Fig. 1. Pathogenicity of *Meloidogyne platani* to sycamore seedlings 10 wk after inoculation with 1,000, 10,000, or 100,000 eggs/pot. A) Shoot growth. B) Root growth.

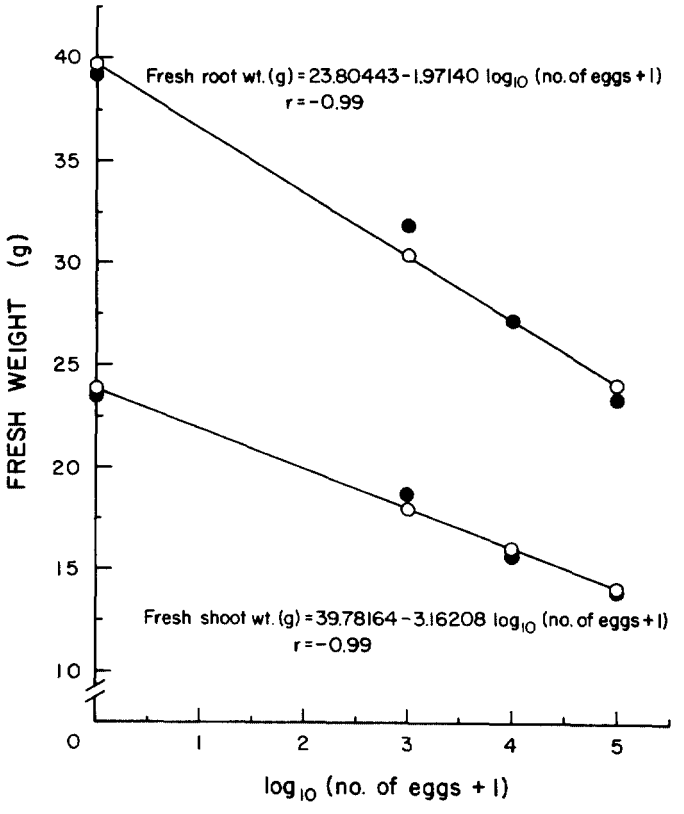

Fig. 2. Relationship between fresh weights of *Platanus occidentalis* L. and inoculum density (Pi) of *Meloidogyne platani* 10 wk after inoculation.

feeder roots were severely galled and contained abundant egg masses (Fig. 1B).

Differential hosts studies: Tobacco roots were heavily to severely galled (average rating = 4.5) and supported moderate egg mass production (average rating = 3). Tomato and watermelon roots were moderately galled (average ratings = 2.8 and 3.0, respectively) but supported very few egg masses (average rating = 1). Pepper roots were slightly galled (average rating = 0.5), and no egg masses were observed. The other host differentials were not infected. Sycamore roots, included as controls, were severely galled (average rating = 5) and egg masses were abundant (average rating = 4).

Susceptibility of hardwood seedlings: Besides sycamore, white ash was the only hardwood tested to become infected. The roots were moderately to heavily galled (average rating = 3.8) and supported moderate egg mass production.

Histopathological studies: Three days after inoculation, second-stage juveniles penetrated the feeder roots behind the root cap, moved through the cortex and settled in the vascular cylinder. No cortical hypertrophy was observed, but slight destruction of the cortical cells resulted from juveniles' migration. Many juveniles were observed to have established contact with the stele. Vascular cells in contact with the nematode head region were hypertrophied but not multinucleate.

Seven days after inoculation, second-stage juveniles within the vascular cylinder increased in size (Fig. 3C) and incipient giant cells were observed. The cell cytoplasm was more granular and more dense than that of the surrounding cells. The incipient giant cells measured 39–52 × 26–39 μm, whereas normal cells (Figs. 3A, B) in that area averaged 28 × 20 μm in cross section. One or two nuclei, 10–18 μm in diameter, were present in each incipient giant cell. Each nucleus contained 1–2 nucleoli measuring 2–4 μm in diameter. The juveniles' heads were in contact with the giant cells and median bulbs were larger than at earlier observations, indicating feeding activities. In some sections, adjoining giant

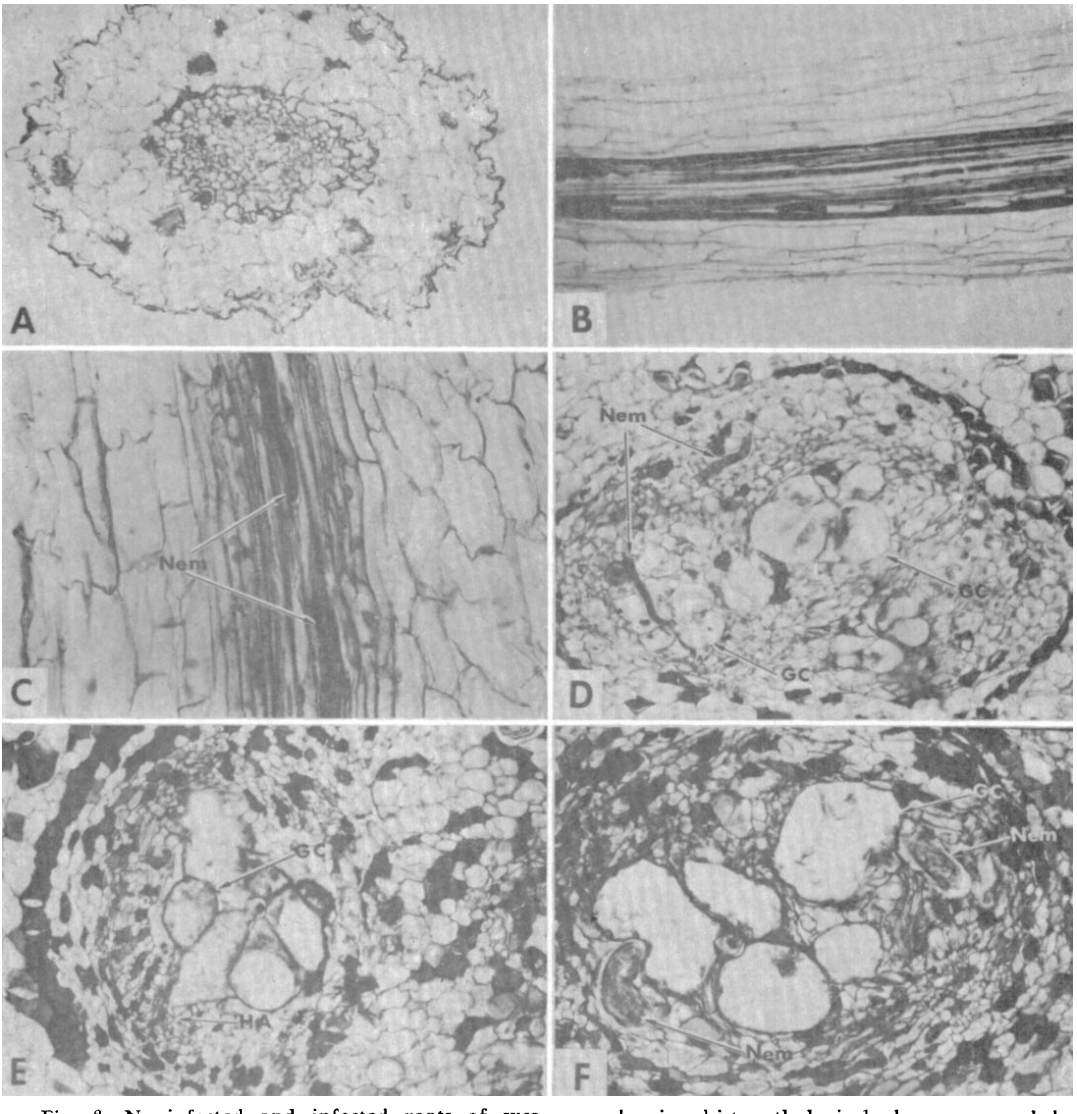

Fig. 3. Noninfected and infected roots of sycamore, showing histopathological changes caused by *Meloidogyne platani* 7–21 days after inoculation. A) Cross section of a noninfected feeder root (×100). B) Longitudinal section of a noninfected feeder root (×100). C) Longitudinal section showing two second-stage juveniles (Nem) embedded in the stele 7 days after inoculation (×250). D) Early stage of giant cell (GC) formation 10 days after inoculation (×100). E) Cross section showing development of giant cells and hyperplasia (HA) around the giant cells 17 days after inoculation (×100). F) Cross section showing giant cells at their maximum size 21 days after inoculation; note the giant cells are vacuolated with thick cell walls (×100).

cell walls appeared to have dissolved. Atypical tissues designated "abnormal xylem" were observed for the first time around the giant cells. The xylem cells were shorter, narrower, and misshapen with less lignification in the cell walls.

Ten days after inoculation, the giant cells had enlarged, but little or no secondary thickening of the cell walls was observed (Fig. 3D). Five to seven giant cells were present around the nematode head. Each giant cell contained 2–5 hypertrophied nuclei. One to two nucleoli were present in each nucleus. Juveniles associated with the giant cells showed an increase in body size, but were still in the second juvenile stage. Hyperplastic parenchyma was observed around the giant cells.

Seventeen days after inoculation, the second molt and sex differentiation had oc-

curred. The giant cells were larger and measured 122–130 μm × 78–130 μm in cross section. A slight thickening of giant cell walls was evident. The cytoplasm in giant cells became more granular and dense. Each giant cell contained 5–14 severely hypertrophied nuclei, 13 × 16 μm. In some sections the nuclei were arranged in clumps inside the giant cells. Hyperplasia was observed around the giant cells (Fig. 3E).

Twenty-one days after inoculation, giant cells increased to 156–234 μm × 104–130 μm in cross section. The cytoplasm was less dense and vacuoles were observed in the giant cell cytoplasm (Fig. 3F). The giant cell walls became irregularly thickened. Some nuclei degenerated and their contents were diffused into cytoplasm. Hyperplasia and hypertrophy were observed around the giant cells. In some sections almost the whole stele was filled by giant cells.

Twenty-six days after inoculation, no further increase in giant cell size was observed and more nuclei had degenerated. The vascular tissues around young females were either compressed, because of the pressure applied by the enlarged nematode body, or were collapsed (Fig. 4A).

Thirty-one days after inoculation, the giant cells were partially vacuolated with two areas of cytoplasm concentrated around the nuclei and along the walls and connected by fine cytoplasmic strands (Fig. 4B). Nuclei continued to degenerate and their contents diffused into cytoplasm. The walls between the giant cells appeared to be dissolved. Hyperplastic and hypertrophied vascular tissues, as well as abnormal xylem, were located around the giant cells.

Thirty-five days after inoculation, more giant cells were highly or completely vacuolated or degenerated. The enlargement of the nematode body and the formation of egg masses caused disruption of the vascular and cortical cells (Fig. 4C). Some vascular tissue was still intact.

Forty days after inoculation, the giant cells were more vacuolated. The nematode body and egg masses, as they protruded into the cortical tissue, caused severe rupture to the cortex (Figs. 4D, E). Hypertrophied nuclei and abnormal xylem were still apparent (Fig. 4F).

DISCUSSION AND CONCLUSIONS

These investigations constitute the first extensive report on the biology of *Meloidogyne platani,* a primary pathogen on sycamore. This nematode also causes galls and reproduces well on tobacco and, to a lesser degree, on watermelon, white ash, and tomato. Although the perineal pattern morphology is similar to *M. arenaria,* other morphological characters (9), host response differences, and apparent limited distribution definitely set this species apart from *M. arenaria.*

Second-stage juveniles penetrated the feeder roots behind the root cap and invaded the vascular cylinder within 3 days after inoculation. All subsequent development of the nematodes and host effects occurred only within the stele.

Total development of root-knot nematode solely within the stele is uncommon and has been observed only in some ornamentals and forest trees. Davis and Jenkins (5) observed the phenomenon in gardenia infected with three species of *Meloidogyne.* It was also observed by Wang *et al.* (24) in *M. incognita*-infected China fir (*Cunninghamia lanceolata* Hook) and Scotch pine (*Pinus sylvestris* L.). Hyperplasia and hypertrophy were observed in the vascular tissues around the giant cells, but not in the cortex as is common in other hosts infected by different *Meloidogyne* species (5,17,24). The abnormal xylem reported by other investigators (5,13,15,22) was noticed in this study within a week after inoculation. Abnormal xylem became prevalent and developed in later stages of giant cell formation. Rod-like projections (a part of the secondary thickening of the giant cell walls), as reported by Krusberg and Nielsen (15), Huang and Maggenti (10), and Jatala and Jensen (13), were not observed in this study.

Based on current information, *M. platani* can be grouped with several other described species of *Meloidogyne* which naturally occur on only one species of tree or other woody perennial. These include *M. megatyla* Baldwin and Sasser, 1979 (2) on loblolly pine (*Pinus taeda* L.); *M. brevicauda* Loos, 1953 (16) on tea (*Camellia sinensis* L.); *M. mali* Itoh, Ohshima and Ichinohe, 1969 (12) on apple (*Malus*

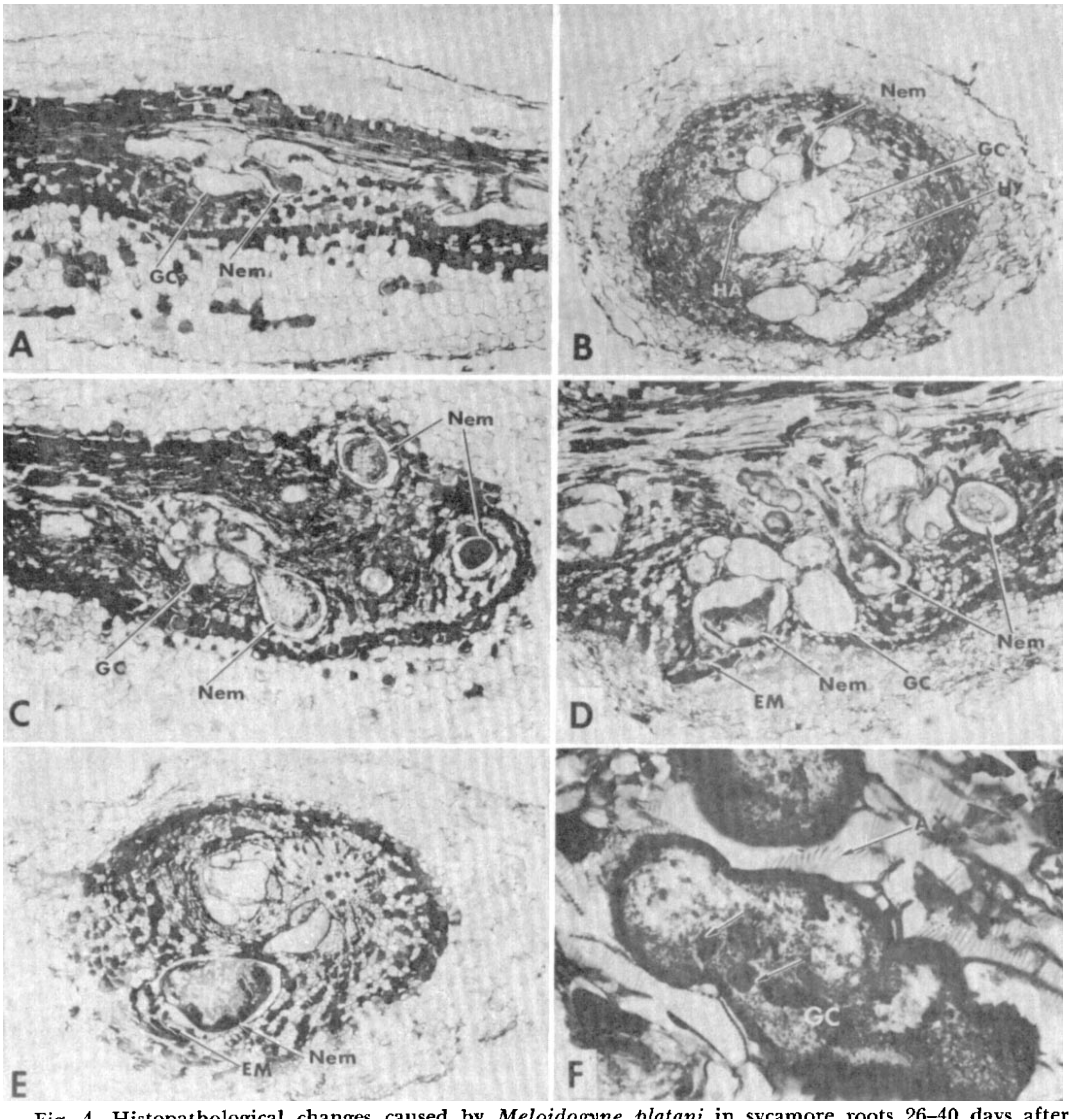

Fig. 4. Histopathological changes caused by *Meloidogyne platani* in sycamore roots 26–40 days after inoculation. A) Longitudinal section showing a young female associated with four giant cells in the stele 26 days after inoculation; note the compressed vascular tissues around the nematode body (×40). B) Cross section of giant cells surrounded by hyperplasia (HA) and hypertrophy (Hy) 31 days after inoculation; note diffused giant cell walls (×40). C) Longitudinal section showing an adult female associated with giant cells within the stele 35 days after inoculation (×40). D) Stele completely disorganized 40 days after inoculation; egg mass (EM) deposited through a rupture in the cortex (×40). E) Cross section showing female with egg mass as they protruded into the cortex causing severe rupture to the cortical tissues 40 days after inoculation (×40). F) Enlarged view of a giant cell with hypertrophied nuclei (N) and nucleoli (Nu) surrounded by abnormal xylem (AX) 40 days after inoculation (×250).

pumila Mill.); *M. ovalis* Riffle, 1963 (18) on sugar maple (*Acer saccharum* Marshall); *M. ardenensis* Santos, 1968 (21) on periwinkle (*Vinca minor* L.); *M. deconincki* Elmiligy, 1968 (6) on ash (*Fraxinus excelsior* L.); *M. litoralis* Elmiligy, 1968 (6) on *Ligustrum* sp.; and an undescribed species (8) on blueberry (*Vaccinium corymbosum* L.).

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