Histopathogenesis of Galls Induced by *Meloidogyne naasi* in Wheat Roots[1]

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Abstract: Histopathogenesis of galls induced by *Meloidogyne naasi* in wheat roots was studied. Large numbers of larvae penetrated wheat root tips within 24 hr; larvae migrated both inter- and intracellularly, causing cortical hypertrophy. Giant cells were formed in the stele around the head of each nematode within 4 to 5 days. Initial pathological alterations in giant cell formation consisted of hypertrophy of protophloem and protoxylem cells, their nuclei and nucleoli. Giant cells contained 2 to 8 agglomerated multinucleolate nuclei. Synchronous mitotic divisions were first observed 9 days after inoculation. After 21 days, giant cells became highly vacuolate. Observations 40 days after inoculation revealed a complete degeneration of cell contents in many giant cells but their thick walls remained intact. Abnormal xylem completely surrounded the degenerated or partially degenerated giant cells. *Key Words: Meloidogyne naasi*, Barley root-knot nematode, Histology, Cytology, Pathogenesis, Host-parasite relationships, Galls, Wheat.

Root-knot nematodes induce galls on the roots of many plants. The following histopathological changes contribute to the formation of galls: (i) hypertrophy of the cortex, xylem parenchyma and metaxylem; (ii) hyperplasia of the pericycle and xylem parenchyma; and (iii) production of giant cells (1, 2, 3, 4, 10, 11). The enlargement of the nematode body and production of eggs inside the host also contribute significantly to gall size (6).

Hodges & Taylor (6) investigated the histopathology of *Meloidogyne naasi* on creeping bentgrass (*Agrostis palustris*) and tentatively identified the nematode as a member of the *M. incognita* group. They reported two types of abnormal changes in the cortex of creeping bentgrass infected with *M. naasi*: (i) the number of cell layers in the cortex increased from 3 to 4–5 layers, and (ii) hyperplasia of inner cortex in old infected roots. They further reported that giant cells were always found within the stele. This paper reports information on the histopathological and cytological alterations caused in wheat roots by *M. naasi*.

Materials and Methods

Nematodes used in this study were from greenhouse subcultures of a population originally collected from Du Page County, Illinois, on creeping bentgrass (12). *M. naasi* eggs were separated from galled roots by treating the roots with 1% pectinase solution (5). Eggs were surface sterilized with 0.26% sodium hypochlorite and incubated at 27 C; larvae hatched within 3–4 days. The larvae were surface sterilized with 100 ppm phenyl mercuric acetate (made from the commercial fungicide Phix, active ingredient 22% phenyl mercuric acetate) for 15 min and rinsed three times in sterile distilled water before being inoculated into wheat seedlings.

Wheat seeds (var. 'Pawnee') were surface sterilized 15 min in 2% sodium hypochlorite, rinsed three times in sterile distilled water and germinated on proteose agar (13) petri dishes. Three-day-old seedlings were transferred one per dish to a 60 × 15-mm plastic petri dish containing 2-mm layer of moist,

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sterile, white sand and the root system of each seedling was covered by an 8–10-mm layer of sand. Approximately 1000 surface-sterilized larvae were introduced into the center of each dish with a hypodermic syringe. After 24 hr the roots of each seedling were washed thus limiting the exposure time to 24 hr. All steps were carried out axenically until the infection was established. Each seedling was then transplanted into a sterilized micropot (a 150-ml polyethylene beaker with a hole drilled in the bottom) filled with sand, and placed in a growth chamber at 26 C day and 20 C night temperature, and with a 16-hr photoperiod. Four seedlings daily were harvested and the roots were fixed in FAA from the first until the sixth day, then every 3 days until the 30th day and finally on the 40th day. Galled roots were embedded in paraffin, sectioned transversely and longitudinally 10–12μ thick and stained with safranin and fast-green (9).

Results

Figure 1 A shows a transverse section of a non-infected root of wheat (var. 'Pawnee'), 20 days after germination. The center of the root is occupied by a single, large metaxylem element.

ONE DAY AFTER INOCULATION: Large numbers of nematode larvae had penetrated within 24 hr after inoculation. Most penetration occurred in the region of cell differentiation and elongation. A slight cortical hypertrophy was evident and larval migration was both inter- and intracellular (Fig. 1 B).

TWO DAYS AFTER INOCULATION: Larvae migrated proximally through the cortex and settled near the newly developing secondary root primordia, many near the innermost layer of cortex bordering the endodermis. No evidence of giant cell initiation was seen but considerable destruction of the cortical cells resulted from the migration of larvae (Fig. 1 C).

THREE DAYS AFTER INOCULATION: By the third day many larvae had entered the stele. However, there was no evidence of cellular, nuclear or nucleolar hypertrophy in the cells around the lips of the parasite (Fig. 1 D).

FOUR DAYS AFTER INOCULATION: Hypertrophy in the cortex and rapid proliferation of pericycle cells in infected root tips caused swellings typical of *Meloidogyne* spp. In heavily infected roots the linear growth ceased. Many larvae were observed to have established contact with the stele, and protophloem cells in contact with the nematode lips were hypertrophied but not multinucleate.

FIVE DAYS AFTER INOCULATION: Incipient giant cells were first observed. The protophloem and protoxylem cells close to nematode heads became enlarged and their nuclei and nucleoli were hypertrophied (Fig. 2 A). The cell cytoplasm was more granular and dense than that of the surrounding cells. The incipient giant cells measured 30–48μ × 20–25μ, whereas the normal cells in that area averaged 15–20μ × 8–10μ in cross section. One or two nuclei 10–20μ in diameter were present in each incipient giant cell. Each nucleus contained 2–6 nucleoli measuring 2–3μ diameter. The larvae still in the cortex failed to induce giant cells or a local hypertrophy of the cortex, suggesting that the cortical hypertrophy, which was responsible for the initial galling of infected roots, was the host's response to infection rather than induced by the larvae.

SIX DAYS AFTER INOCULATION: Little or no secondary thickening of giant cell walls was evident. The enlargement of giant cells occurred in all planes. In serial sections through giant cells the cell walls between the

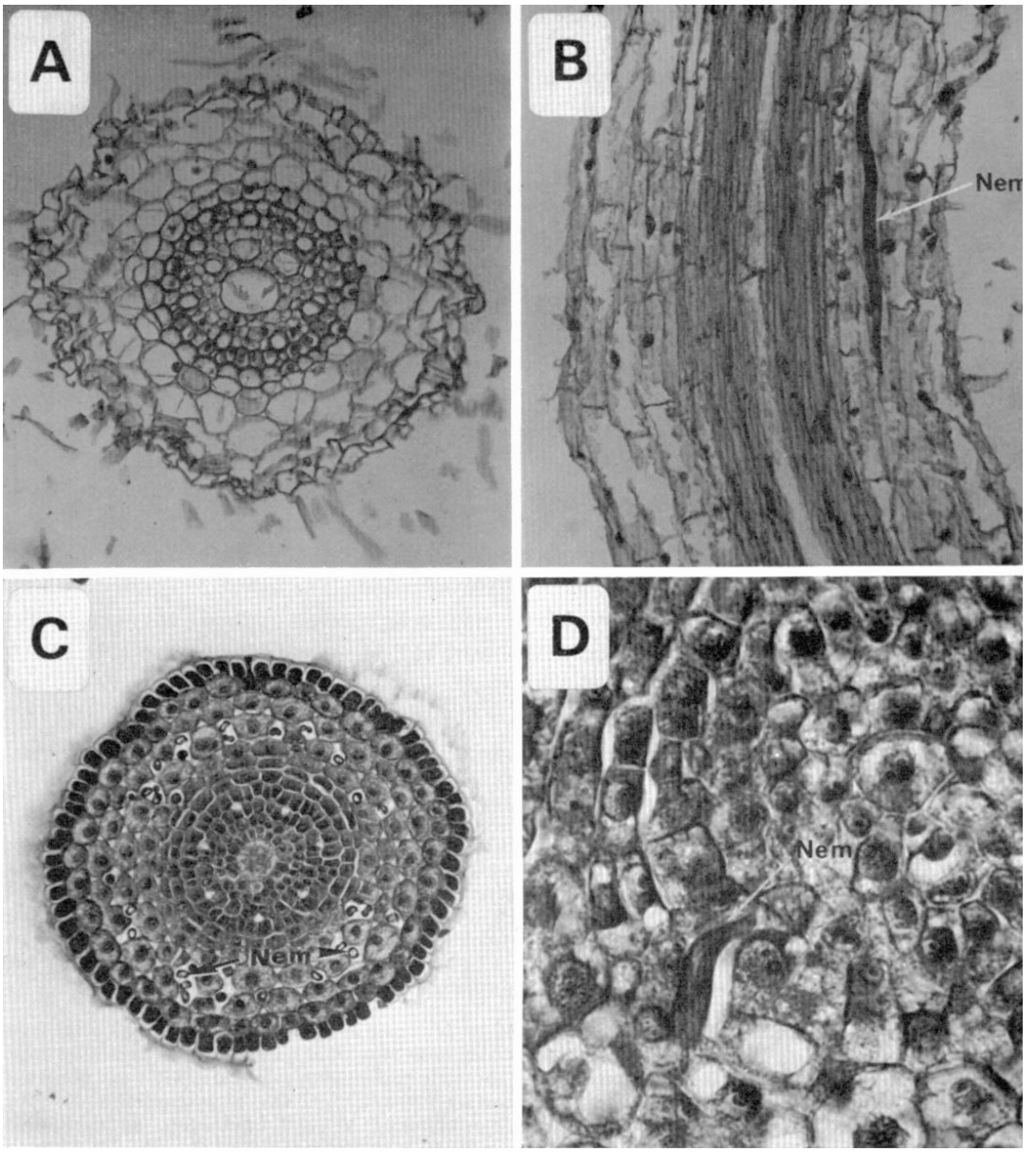

FIG. 1 A–D. Non-infected and infected roots of wheat, showing the early stages of infection by second-stage larvae of *M. naasi*. **A.** Transverse section of a non-infected root, 20 days after germination (× 105); **B.** Intracellular larvel migration in the cortex, 1 day after inoculation (× 105); **C.** Transverse section showing 31 larvae (Nem) embedded in the cortex 2 days after inoculation. Note the disruption of adjacent cortical cells (× 105). **D.** Nematode with its lips in the pericycle region, 3 days after inoculation (× 375).

giant cells and the cells surrounding them appeared to be diffused (Fig. 2 B). The cytoplasm was dense and highly granular. Only one to four nuclei were present in each giant cell. These nuclei were either highly hypertrophied, multinucleolate and dispersed in the cytoplasm, or small, with one or two nucleoli, and clustered in the center of a giant cell.

Abnormal xylem cells with characteristic wall reticulations were observed for the first time around the giant cells. The larvae in association with the giant cells were still in the second stage and those in the cortex did not show any sign of development.

NINE DAYS AFTER INOCULATION: Cell differentiation ceased to occur in the heavily infected roots. Proliferation of hyperplastic parenchyma was intense in the galled areas. Two types of alteration were displayed by the cortex; an enlargement of the cortical cells without any increase in the size or number of nuclei, or a compaction of the cortical cells over a small area. The second reaction was the result of the pressure exerted by the formation of giant cells and rapid proliferation of hyperplastic parenchyma in the area.

The giant cells measured 60–90μ long × 32–50μ wide in cross section. A slight thickening of giant cell walls was apparent. Nuclear mitoses in the giant cells were first observed on the ninth day after inoculation (Fig. 2 C–D). None of the mitotic divisions observed in giant cells were accompanied by cytokinesis. All the divisions within a giant cell were observed to be synchronous. However, mitotic divisions between giant cells of the same complex did not display this synchronization. The larvae associated with the giant cells showed midbody enlargement but were still in second larval stage (Fig. 3 A).

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FIG. 2 A–D. Histopathological alterations caused by *M. naasi* in wheat roots, 5–9 days after inoculation. **A.** Incipient giant cell with hypertrophied nucleus (N), nucleolus (Nu) and nematode (Nem), 5 days after inoculation (× 485); **B.** Longitudinal section of an expanding giant cell (GC) with diffused cell boundaries (F-arrow), 6 days after inoculation (× 370); **C.** Nuclear mitoses in a giant cell, 9 days after inoculation. Note the rearrangement of chromatin (CHR) at prophase (× 930); **D.** Longitudinal section showing two mitotic apparatuses (Chr) in the lower giant cell. Nuclei in the upper two giant cells display no signs of mitosis, 9 days after inoculation (× 370).

FIG. 3 A–D. Histopathological alterations caused by *M. naasi* in wheat roots, 9–18 days after inoculation. **A.** Late second-stage larva intimately associated with two giant cells (GC), 9 days after inoculation (× 265); **B.** Longitudinal section showing the stele packed with giant cells (GC) and abnormal xylem (AX). Note the formation of two additional growing points (GP), 12 days after inoculation (× 100); **C.** Enlarged view of a giant cell with dense, granular cytoplasm (Cyt) and a pentagonal inclusion (X). The nematode lips in contact with giant cell, 18 days after inoculation (× 1,415); **D.** Photomicrograph showing hypertrophied nuclei (N) with dense granular nucleoplasm and highly vacuolate nucleoli (Nu), 12 days after inoculation (× 1,125).

FIG. 4 A–D. Histopathological alterations caused by *M. naasi* in wheat roots, 27–40 days after inoculation. **A.** Transverse section through a lateral root gall showing a complete deformation of the stele and cortex, 27 days after inoculation (× 100); **B.** Partially degenerated giant cell (GC) with vacuolate cytoplasm and thick cell walls, 27 days after inoculation (× 450); **C.** Photomicrograph showing the anterior end of an adult female nematode (Nem) in contact with a giant cell, proliferation of abnormal xylem (AX) around the giant cell and ruptured cortex (RC) around the parasite, 40 days after inoculation (× 125); **D.** Partially degenerated giant cell surrounded by reticulated abnormal xylem cells, 40 days after inoculation (× 350).

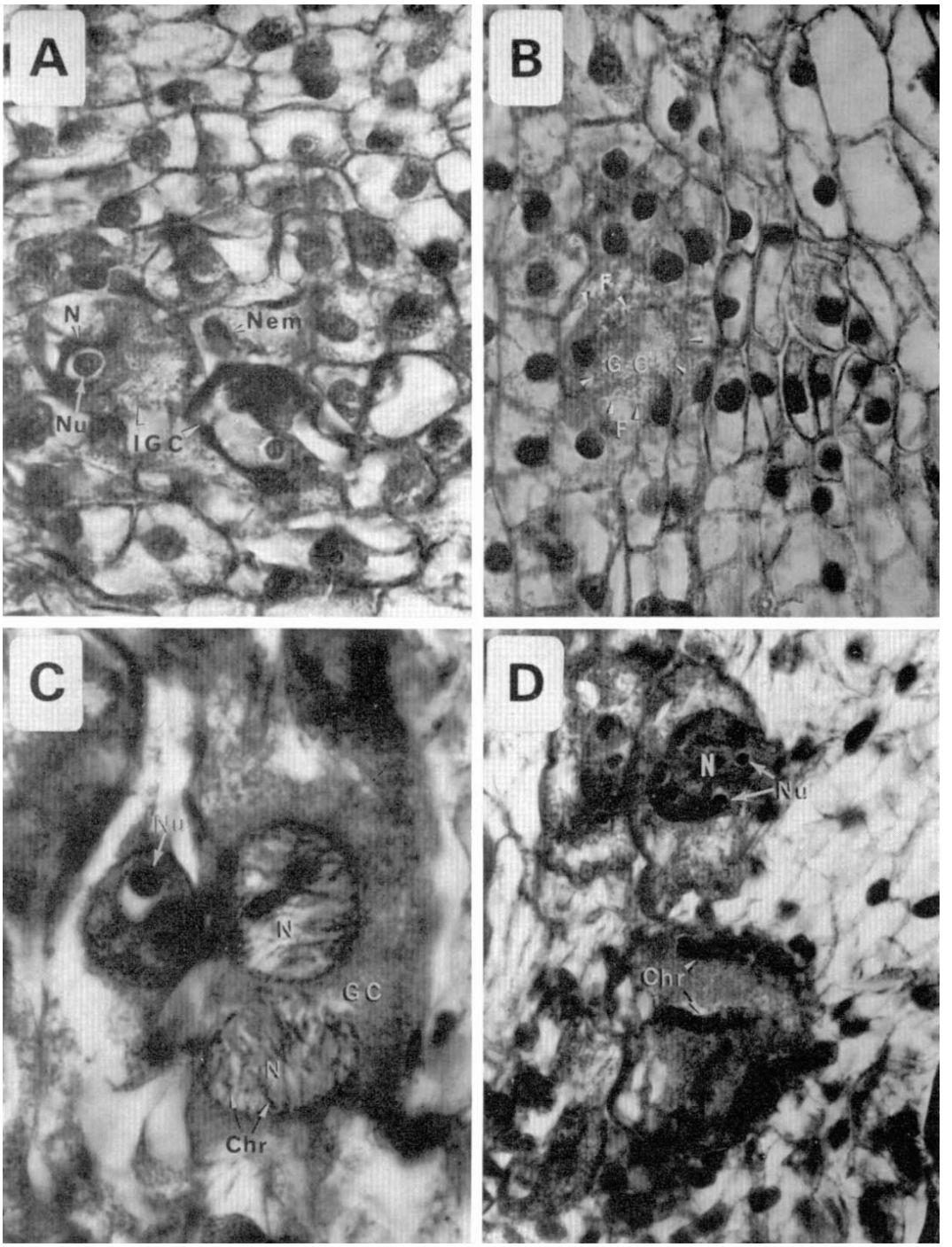

FIG. 2

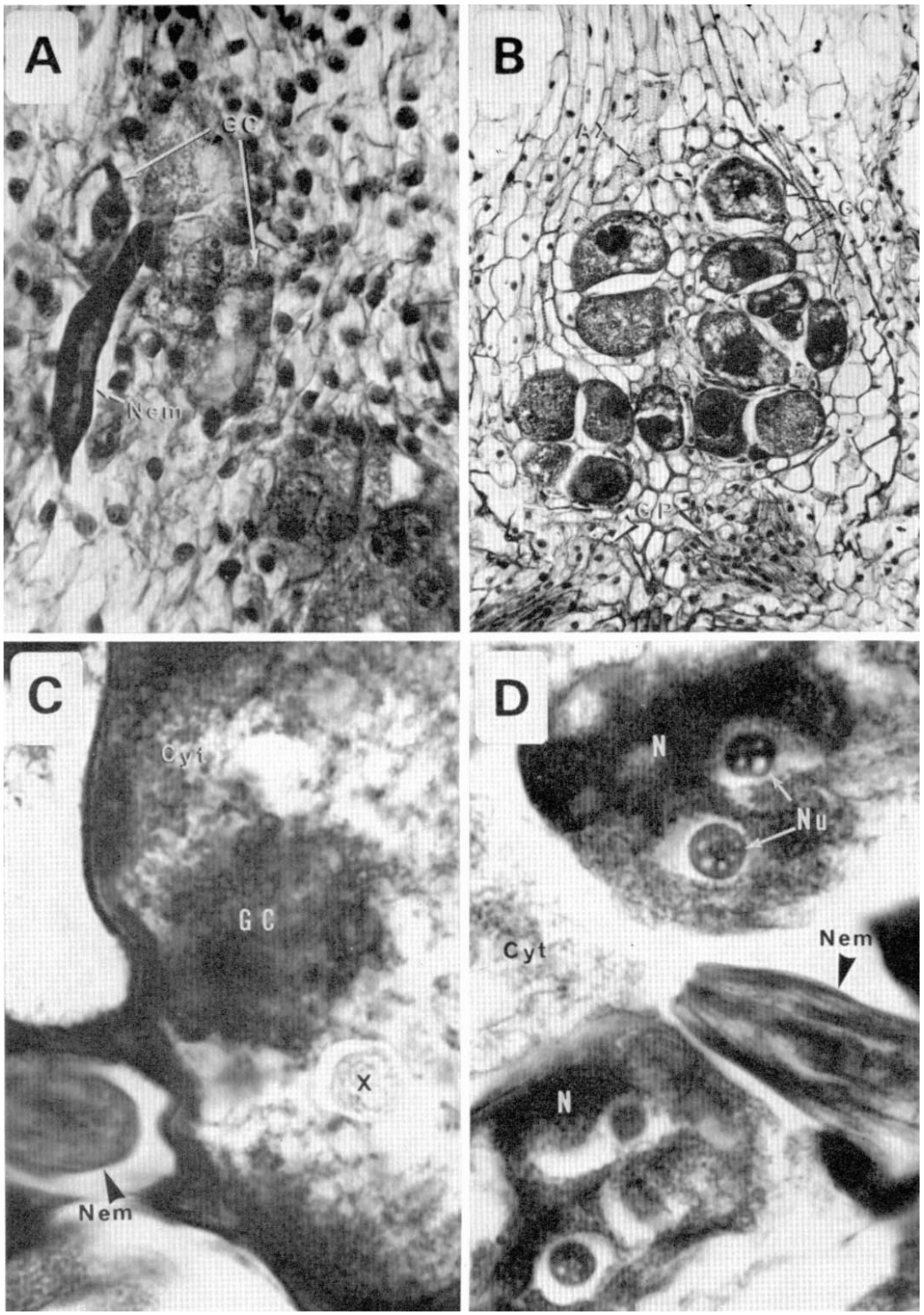

FIG. 3

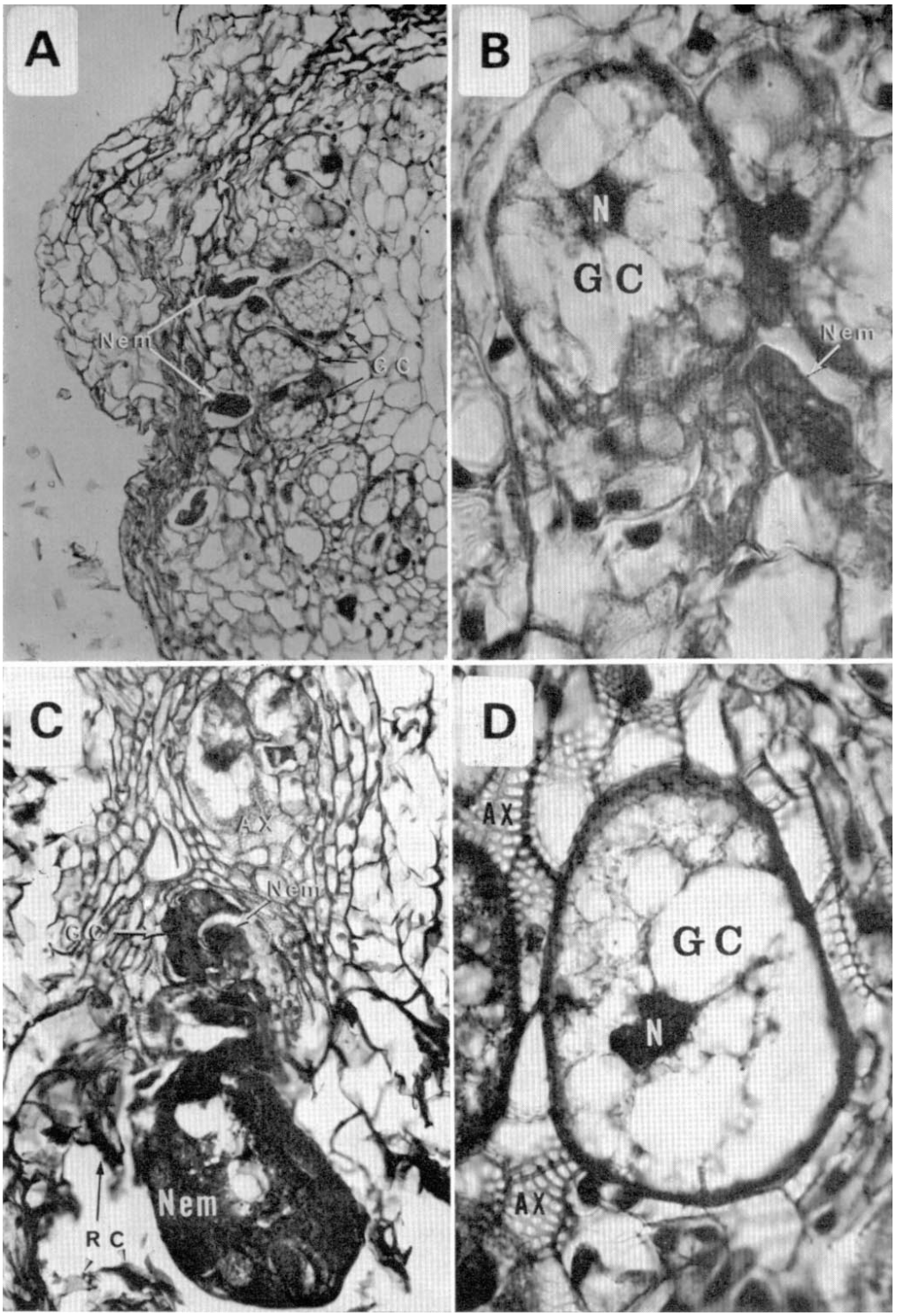

FIG. 4

TWELVE DAYS AFTER INOCULATION: The giant cells displayed an irregular pattern of secondary wall thickening (Fig. 3 C). The cytoplasm became highly granular and dense. Nuclear mitoses were frequently observed. The nuclei were agglomerated and were located in the center of giant cells (Fig. 3 B). From two to eight nuclei were observed in each giant cell. The nucleoli were severely hypertrophied. Occasionally, the nucleolar material exhibited an unusual arrangement of four to eight radially distributed vacuoles (Fig. 3 D). Abnormal xylem surrounded the giant cells.

By the 12th day many second-stage larvae in the tissue were sexually differentiated. Those that were still in the cortex and had failed to establish a feeding relationship disintegrated *in situ*. Figure 3B shows a longitudinal section of a root with its stele completely transformed into giant cells at the level of the gall; terminal growth ceased completely and in its place, the pericycle cells produced two new growth points.

FIFTEEN, EIGHTEEN, AND TWENTY-ONE DAYS AFTER INOCULATION: After the 15th day no further increase in giant cell size was observed. Vacuoles were observed in the giant cell cytoplasm; however, nuclei and nucleoli were still intact. This change in the giant cell condition coincided with the onset of the second molt of the parasite. The giant cell walls showed further irregular thickening (Fig. 3 C). Rod-like projections, as a part of the secondary thickening of the cell walls, extending into the cell body as reported by Krusberg & Nielson (10) and Huang & Maggenti (8) were not observed. A pentagonal body of unknown nature and origin was occasionally observed in the cytoplasm of giant cells (Fig. 3 C).

TWENTY-FOUR, TWENTY-SEVEN, AND THIRTY DAYS AFTER INOCULATION: The contents of the giant cells became further deteriorated (Fig. 4 A). The cytoplasm became highly vacuolate, with two areas of concentration of cytoplasm around the nuclei and along the walls connected by fine cytoplasmic strands between them (Fig. 4 B). After 30 days, many giant cell nuclei degenerated and their contents were diffused into the cytoplasm (Fig. 4 C). The prevalence of abnormal xylem around the giant cells increased as the latter showed signs of degeneration (Fig. 4 A–D).

By the 24th day, many larvae had reached the third or fourth stage. Cortical cells around the nematode body were either compressed due to the pressure applied by the enlargement of the nematode, or were collapsed (Fig. 4 A). The galls on secondary roots displayed a severe necrosis in the hyperplastic parenchyma and endodermis. A similar reaction was observed by Krusberg & Nielson in *M. incognita acrita*-infected sweet potatoes, which they designated as a lytic reaction (10).

FORTY DAYS AFTER INOCULATION: Many giant cells were partially or completely degenerated. Meanwhile, the abnormal xylem completely surrounded and walled off the giant cells (Fig. 4 B–D). The giant cell cytoplasm, including the nuclei and nucleoli disappeared. Necrosis of the cortical cells around the gelatinous matrix, or egg mass of the female nematode, was frequently encountered. The enlarged nematode body and the formation of an egg mass caused disruption of many cortical cells (Fig. 4 C).

DISCUSSION

The preceding observations on gall formation in wheat roots indicate the sequence of histopathological changes that occur in a susceptible host following infection by *M. naasi*.

The development of abnormal xylem was not noticed in the early stages of giant cell formation. Abnormal xylem and hyper-

plastic parenchyma cells divided actively in the central and peripheral regions, respectively, in the galled roots. It appears that these two types of symptoms developed in response to the injured xylem parenchyma and other vascular elements involved in the giant cell formation, and they serve to contain the giant cell development in a susceptible host. Furthermore, the failure of giant cells to increase in size 15 days after inoculation and an increased incidence of abnormal xylem and hyperplastic parenchyma at the same time support the notion of the host's effort to contain the giant cell development.

Giant cells induced by *M. naasi* in wheat roots were smaller than those in tomato (1, 3), sweet potato (10) and soybean (4) by other species of *Meloidogyne*.

Since no mitotic division was observed in the giant cells until the 9th day and the cell walls at several points were diffused and the cytoplasm appeared to be continuous, it is possible that the initial multinucleate condition in the giant cells resulted from the incorporation of nuclei of adjoining cells. After 9 days, the increase in the number of nuclei in giant cells was due to synchronous mitotic divisions, as previously reported by Bird (1) and Owens & Specht (11). However, recent ultrastructural studies by Huang & Maggenti (7), working on the host-parasite relationships between *Vicia faba* and *M. javanica*, indicated that the multinucleate condition was solely derived from repeated mitoses without cytokinesis. As also reported by the previous workers, all nuclei in a giant cell displayed a synchronization of mitotic divisions. Owens & Specht (11) postulated a cytoplasmic regulation of nuclear behavior in giant cells. This phenomenon and the origin of the multinucleate condition in giant cells induced by other species of *Meloidogyne* in various hosts should be investigated at the ultrastructural level.

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