

Anatomical Response of Grain Sorghum Roots to *Meloidogyne incognita acrita*¹

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Abstract: The cotton root-knot nematode, *Meloidogyne incognita acrita*, reproduced on the roots of grain sorghum, causing syncytia in the cortex or stele of lateral roots. Giant cells developed either singly with few nuclei or in groups with many nuclei. Giant cells that developed in groups appeared the same as those which developed singly. The pericycle and endodermis were interrupted at the site of nematode invasion. Large areas of these tissues were absent for one-third of the circumference of the stele and extended 1.5 mm longitudinally along the root. In the area where pericycle and endodermis were absent, the parenchyma of the cortex extended to the vascular elements, and abnormal xylem surrounding giant cells extended into the region of the cortex. Root-knot galls appeared on sorghum roots as elongate swellings, discrete knots, or swellings with root proliferation. Galls were not observed on brace roots. **Key Words:** giant cells, pathogenesis, root-knot nematode.

Grain sorghum (*Sorghum bicolor* (L.) Moench) is a host for the cotton root-knot nematode, *Meloidogyne incognita acrita* (Kofoid & White) Chitwood. Root-knot-infested sorghum plants have been reported to be stunted, to bloom later, and to have suppressed yields. Root systems were small, and galls were observed on lateral roots of infested plants. The growth (dry weight) of sorghum and cotton was retarded by 15 and 40% respectively by root-knot nematodes (12), suggesting that sorghum is the more resistant crop.

Among the histopathological changes that contribute to formation of galls are hypertrophy of cortex, xylem parenchyma, and metaxylem; hyperplasia of the pericycle and xylem parenchyma; and production of giant cells (16). Hodges and Taylor (8) reported that, in roots of creeping bentgrass, the number of cell layers increased in the cortex; hyperplasia of the inner cortex occurred in old infected roots; and giant cells were always formed within the stele.

McClure, Ellis, and Nigh (11) reported three types of histological responses in infected resistant cotton roots. Small galls contained only fragments of nematodes; others contained no detectable traces of developing larvae; and druses were formed in galls of both susceptible and resistant cotton roots, but not in healthy tissue.

Monocotyledonous roots are character-

ized by the absence of secondary growth; therefore, lateral roots of sorghum remain fibrous (6). Upon maturation, the large lateral roots of sorghum contain an epidermis with root hairs. Beneath the epidermis is an exodermis with prominent wall thickening on the outer paraclinal wall. The cortex is composed of 12-15 layers of cells, with 12 the most consistent number. The xylem thickens and lignifies with age, so that in old roots the entire vascular ring is composed of thick-walled lignified tissue, in which only the phloem cells remain thin-walled and not lignified. The cortices of many rootlets eventually break down and become disorganized. However, functional capacity of rootlets as organs of conduction is probably unimpaired because the vascular tissue, protected by the thick-walled endodermis, appears normal (1). The endodermis, which separates the stele and cortex, varies in thickness according to the sorghum cultivar. Prominent silica deposits, which probably strengthen the root to withstand the high pressures developed under drought stress (4), are found in the endodermis (Fig. 9). Also, tertiary thickening of the endodermis and pericycle would supply considerable strength to withstand stress.

The purpose of this work was to study the anatomical response that may contribute to resistance of grain sorghum roots to invasion by root-knot nematodes.

MATERIALS AND METHODS

Meloidogyne incognita acrita were obtained from a population maintained on cotton (*Gossypium hirsutum* L.) and

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tomato (*Lycopersicon esculentum* Mill.) in greenhouse ground beds. Heavily infested tomato roots were washed in tap water and homogenized in water for 2 min in a blender. Aliquots of this solution containing 1,000 larvae were added to sterile sand in 15-cm clay pots in which 'RS626' sorghum had been planted.

Twenty pots were placed in a growth chamber at 30 C (day) and 25 C (night) temperatures and with a 14-h photoperiod. Three weeks after emergence, the plants were harvested, and sections of lateral roots were fixed in FAA (Formalin-Acetic Acid-Alcohol) (14). Galled roots were embedded in paraffin, sectioned transversely and longitudinally 10 to 12 μ m thick, and stained with safranin and fast green (14).

Brace roots were taken from four older sorghum plants (which had grown in the same bed from which the previously described infected tomato plants were harvested), stained in acid fuchsin-lactophenol (10), and examined for root-knot nematode infestation under a binocular microscope.

RESULTS AND DISCUSSION

Many workers have noted that the size and general appearance of galls depend not only on the number and species of the nematode, but also on the host plant species (5). Galls resulting from infection with *Meloidogyne hapla* Chitwood can often be distinguished from those of other root-knot species by the matted appearance of roots formed by a combination of relatively small galls and numerous lateral roots growing out of each gall (15). The root-knot nematode galls we found on the lateral roots of grain sorghum were elongate swellings, discrete knots, or swellings with root proliferation (Figs. 1, 2).

Lateral roots of sorghum were more heavily infested than were brace roots. On lateral roots, 13.6 sites of root-knot infection were observed/gm of root, whereas on brace roots, only 1.7 sites were found. Galls were not found on brace roots, but some slight swellings were observed. Sites of nematode infection on brace roots appeared as brown necrotic areas and were often associated with fungi.

The anatomy of lateral roots was

changed at the site of nematode infection. In longitudinal view, the development of the endodermis was interrupted for a distance of 1,570 μ m. In cross-sectional view, depending on the diameter of the root, one-third or more of the circumference of the root contained no endodermis and pericycle. These tissues were normal in the remainder of the root. Where endodermis and pericycle were absent, the parenchyma of the cortex extended to the vascular cylinder (Fig. 3).

Five metaxylem points are normal for sorghum roots. In Fig. 3, one metaxylem point was replaced by giant cells. Some of the giant cells were small with few nuclei, whereas others were large and contained up to 15 nuclei (Figs. 5, 7). Root primordia were often initiated from the site of nematode injury. Giant cells formed in the cortex (Fig. 7) and in the stele (Fig. 6) of sorghum roots.

Grain sorghum roots were not as readily invaded by root-knot nematodes as were roots of cotton and tomato. More galls were found on fibrous lateral roots than on brace roots of grain sorghum. Nematode infection sites were often associated with necrotic fungal lesions where *Pythium* sp. was observed (Fig. 9). Batten and Powell (2, 13) reported that, when root-knot invasion preceded fungus invasion, six genera of fungi caused necrotic symptoms on roots. None of the fungi induced disease unless root-knot nematodes were present. Golden (7) found that okra and tomato roots were readily invaded by *Pythium* when nematode-free roots contained no appreciable invasion by the fungus. These data support our observation of more extensive root necrosis and injury to sorghum plants by the nematode and disease complex.

Different reactions were found in root tissue associated with root-knot nematodes. Sections were observed that contained larvae, but no sign of giant cell initiation. Giant cells were formed without nematodes present; perhaps the root-knot larvae had initiated the giant cell, then migrated away, or were males. Other giant cells had become highly vacuolated, and fragments of nematode were found in the root tissue, indicating that the nematodes were unsuccessful in completing life cycles. Some syncytia were highly developed and could

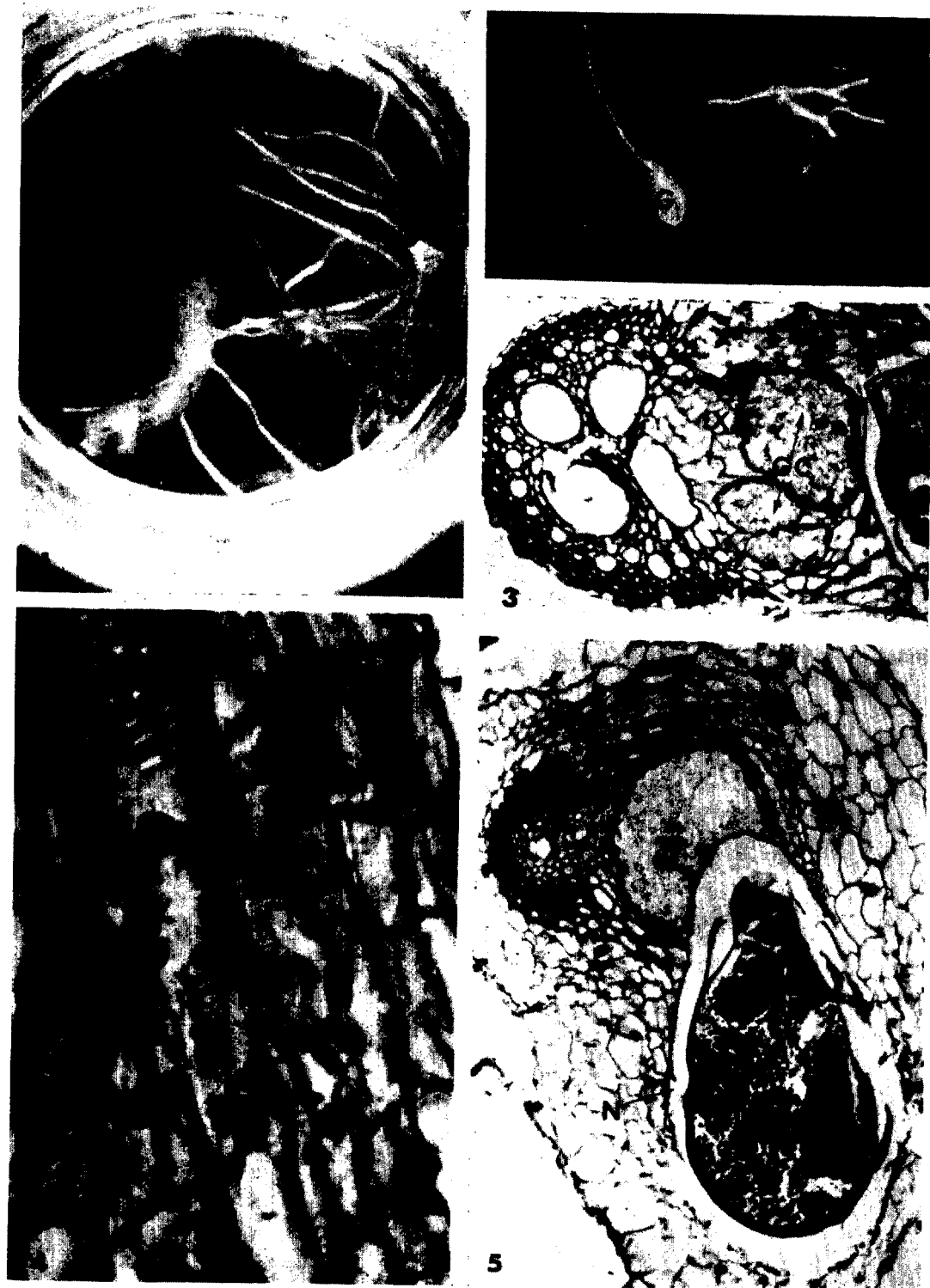


FIG. 1-5. Photomicrographs of reactions of grain sorghum roots to infection by *Meloidogyne incognita* *acrita*. 1 & 2) Gross form of root-knot galls (G) on sorghum roots. 3) Endodermis and pericycle are absent in the area of giant cells (GC) (X 160). Abnormal xylem (IX). 4) Formation of giant cell in a vessel with dissolution of vessel and phloem fiber (PF) cell walls (X 1,000). 5) Partially degenerated single giant cell with nuclei clumped in the center. Root primordia initiated at the site of infection (X 160).

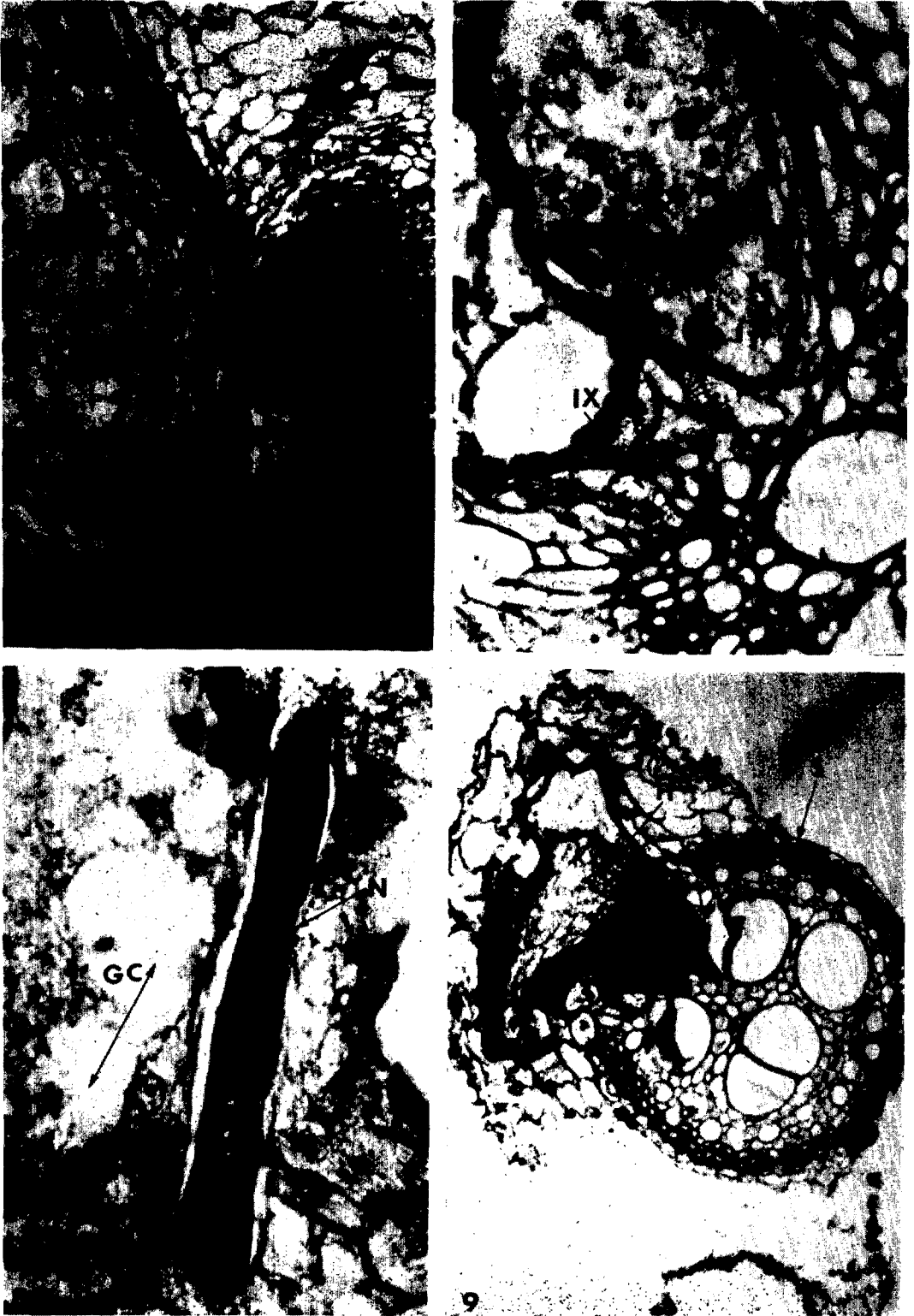


FIG. 6-9. Longitudinal section of giant cell in the stele. Developing root primordia at right (X 160). 7) Giant cells in the cortex with abnormal xylem (X 400). 8) A root-knot nematode among previously formed giant cells (X 400). 9) *Pythium* spp. (H) in association with root-knot injury. Note silica deposits (S) in the endodermis.

not be distinguished from giant cells in other susceptible plant species. Krusberg (9) also noted that nematode feeding stimulated the formation of several atypical tissues: giant cells, "abnormal xylem," hyperplastic parenchyma, and cork.

Giant cells were initiated in both the cortex and stele of grain sorghum roots, and giant cells developed in either of those tissues (Fig. 6, 7). In the developed giant cells, the cytoplasm was dense with numerous nuclei. Giant cells initiated in the cortex developed abnormal xylem which was independent of surrounding cells. Parenchyma type cells bordering the giant cell were small, densely protoplasmic, and irregular in shape. Crystals were not found in the area of nematode development (Fig. 3).

Cells some distance away from the nematode's head were often affected (3). Root-knot nematode invasion suppressed the development of the endodermis and pericycle. At the site of nematode invasion (Fig. 3), parenchyma cells of the cortex were found to border the vascular elements and extend through the endodermis and pericycle. The endodermis and pericycle were absent for one-fourth or more of the circumference of the stele and for 1.5 mm or the length of the root surrounding the nematode.

Christie (3) reported instances in which certain walls of xylem elements near giant cells became greatly thickened or swollen. Those swollen walls stained deep red with Fleming's triple stain in contrast to the purplish-to-blue color usually assumed by such elements. Sometimes these modified cell walls blended into the giant cells as if the substance of the wall were being absorbed.

Walls of the vessel trace (Fig. 4) showed stages of dissolution. Cell walls close to the nematode were diffuse, whereas progressively up the vessel trace the walls became more distinct and more prominently stained. Dense granular cytoplasm of the developing syncytia was observed in cells on either side of the vessel trace. Walls of those cells had become diffuse, and their nuclei were enlarged and clumped. Some of the nuclei were undergoing mitotic division. The wall of the forming giant cell was observed to extend half the distance into a

vessel cell. The phloem fiber (Fig. 4) next to the vessel containing the portion of the giant cell was distorted. The fiber was swollen at the proximal end and appeared to be dissolving.

These several reactions clearly show that although grain sorghum is a poor host for *M. incognita acrita*, significant nematode reproduction does occur.

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