Histopathology of Corn Hybrids Infected with Root Knot Nematode, Meloidogyne incognita

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ABSTRACT

Histopathological responses of corn (*Zea mays*) hybrid Coker 911 to *Meloidogyne incognita* were similar to those of other good hosts with respect to the development of granular, multinucleate giant cells and egg-laying females within 25 days after inoculation. In hybrid Pioneer 309B, a poor host, giant cells were often collapsed and associated with apparently dead larvae. No reproduction was evident in this host at 25 days after inoculation, but a few females with eggs were detected after 58 days. Higher numbers of larvae of *M. incognita* had penetrated Coker 911 at 4 and 8 days after inoculation than had penetrated the poor host, Pioneer 309B. Phytopathology 60:1195-1198.

Inbred lines of corn (*Zea mays* L.) have been shown to vary considerably in their susceptibility to *Meloidogyne incognita* (Kofoid & White) Chitwood (4, 8). Certain corn hybrids such as Coker 911, a good host (GH), support greater reproduction of this nematode than other hybrids such as Pioneer 309B, a poor host (PH) (2). Although the host-parasite relationship of root knot nematodes has been described on many hosts, no reports on the histopathology of corn infected with *Meloidogyne* spp. were found. Investigations of the histological response of several soybean varieties with various degrees of susceptibility to *Meloidogyne* spp., however, indicated a relationship between host susceptibility and the characteristics of its giant cells (5). These investigations on corn hybrids were initiated to compare the histopathology of hybrids that were relatively good and poor hosts for *M. incognita*.

MATERIALS AND METHODS.—In a survey of numerous corn hybrids, *M. incognita* reproduced at different rates on Coker 911 (GH) and Pioneer 309B (PH). Therefore, these hybrids were selected for histopathology investigations. Eight days after seeds were planted in vermiculite, seedlings were placed in 3.8-cm plastic pots with the bottoms sealed with Zut® (Bennett's, Salt Lake City, Utah) to prevent drainage. The roots of each plant were covered with Quartzite® sand with a mean diam of 235 μ and a range of 158 to 593 μ (Pennsylvania Glass Sand Corp., Pittsburgh) and inoculated. Inoculum consisted of an aqueous suspension of 10,000 larvae prepared by placing galled tomato roots in a modified Seinhorst mist chamber and drawing off a few ml of water from each Baermann funnel after 8 hr. Three days after inoculation, 12 seedlings of each hybrid were transplanted to 15-cm pots containing 35-mesh sand with a mean diam of 578 μ and a range of 255 to 1418 μ. In addition to providing water as needed, plants were given a modified Vhph® nutrient solution [700 g Vhph (Miller Chemical and Fertilizer Corp., Baltimore, Maryland), 123 g KNO_3, 227 g $Mg(SO_4)$/133 liters of H_2O] on alternate days.

To determine host-parasite relations as affected by time, one seedling of each hybrid from each of the three replicated blocks was harvested 5, 10, 20, and 25 days after inoculation. Several root pieces approximately 1-cm long were cut from each root system and fixed in formalin-alcohol-acetic acid (FAA). The tissue was dehydrated with a tertiary-butyl-alcohol series, embedded in Tissuemat® (Scientific Products, Minneapolis, Minn.) and cut into 12-μ sections with a rotary microtome. The sections were mounted with Haupt's adhesive and 4% formalin and stained with Johansen's safranin and fast green.

Preliminary tests had indicated differences in larval penetration between the two hybrids. Twelve plants of each hybrid were inoculated as described above. At 4 and 8 days after inoculation, six seedlings (replicates) of each hybrid were washed from the pots. After examining the roots for necrosis or galls, they were stained with acid fuchsin lactophenol, destained in lactophenol by autoclaving for 6 min at 120 C and about 1 atm pressure (1), and examined for larvae under a stereoscopic microscope.

RESULTS.—Stained root sections of both corn hybrids fixed 5 days after inoculation were very similar. At this time, anaplasia was observed and had apparently originated near the head of the larvae either within the stele or endodermis. Cytoplasm of proliferating cells was granular and dense, and cell walls were thinner than normal and partially dissolved. One or two enlarged nuclei with safranin-stained nucleoli were often observed near the head region of the larvae.

Differences between host responses of the two hybrids were first apparent in roots fixed 10 days after inoculation (Fig. 1-A, B). In Coker 911 (GH), granular masses of cytoplasm were delimited into several giant cells by walls of normal thickness (Fig. 1-A). Cells adjacent to giant cells appeared turgid. Hyperplasia was minimal. Cytoplasm of some giant cells appeared to be continuous at some point in the cell wall with that of neighboring giant cells. From serial sections it was estimated that about 20 enlarged nuclei with safranin-stained, irregularly shaped nucleoli were present in a single giant cell. Although the foregoing responses were most characteristic of GH at 10 days after inoculation, in several instances giant cell cytoplasm was vacuolated and there was some necrosis.

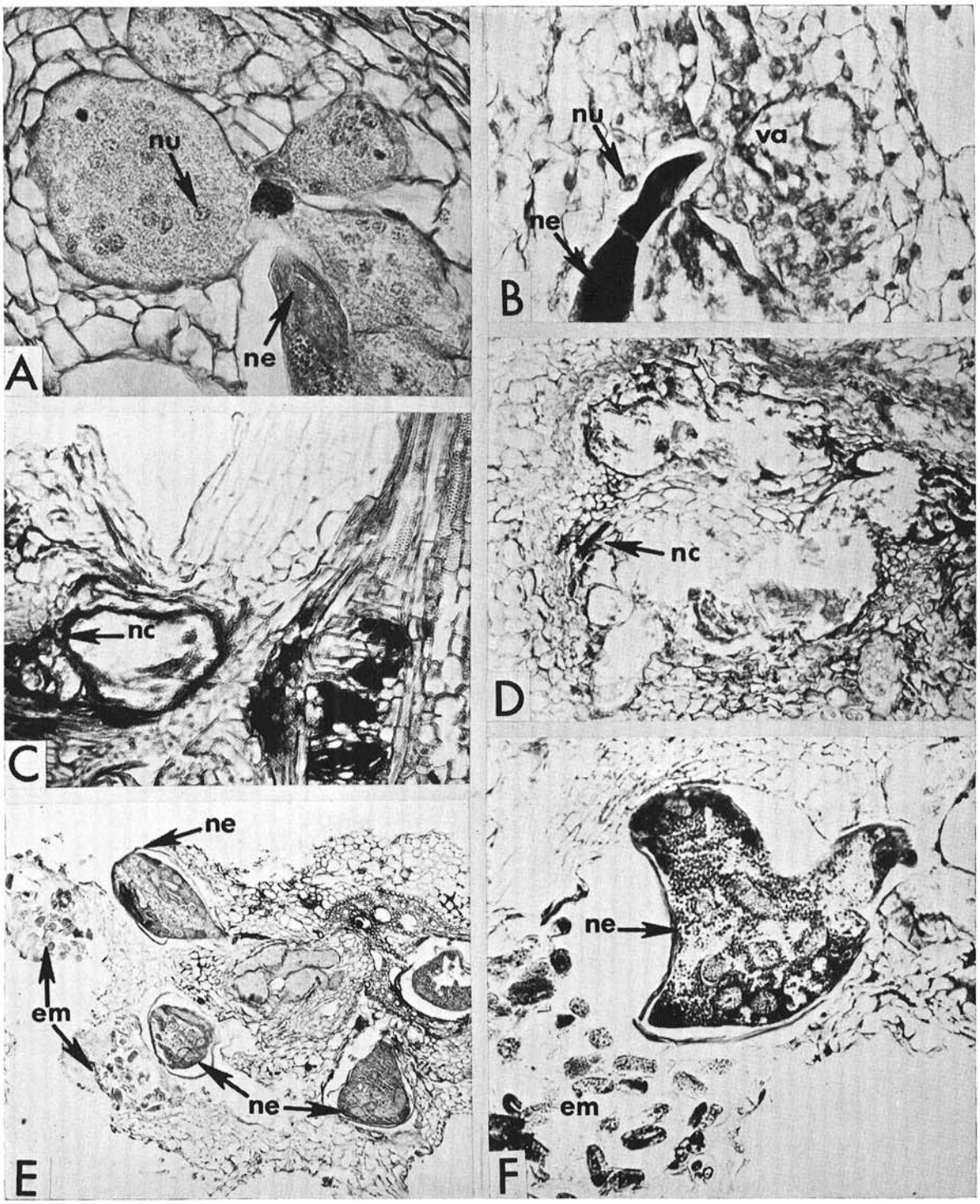

Fig. 1. Cellular reactions of Coker 911 (good host) and Pioneer 309B (poor host) hybrids of corn to *Meloidogyne incognita*. **A)** Coker 911 10 days after inoculation (×300). **B)** Pioneer 309B 10 days after inoculation (×280). **C)** Pioneer 309B 20 days after inoculation (×100). **D)** Pioneer 309B 25 days after inoculation (×85). **E)** Coker 911 25 days after inoculation (×40). **F)** Pioneer 309B 58 days after inoculation (×120). em = egg mass; nc = necrotic plant tissue; ne = nematode; nu = nucleus of giant cell; va = vacuole.

The latter cases were usually associated with small roots which had been invaded by numerous larvae.

In contrast, vacuolated and empty giant cells were typical of PH, including tissues where invasion was not especially dense (Fig. 1-B). Cell walls were not always clearly defined, and fewer nuclei were present than in giant cells of GH (Fig. 1-A, B). Vacuoles and apparently empty regions were often associated with the cytoplasm of giant cells in PH. Cells surrounding the nematode and poorly defined giant cells often seemed to lack turgidity, in comparison to adjacent, apparently healthy tissue. There was some evidence of hyperplasia.

Some giant cells of both hybrids were nearly empty 20 days after inoculation, and some hyperplasia occurred adjacent to the giant cells and nematodes. Most giant cells in the GH were multinucleate and granular, whereas in the PH, giant cells were often surrounded by necrotic tissue and thick, safranin-stained walls (Fig. 1-C). These cells were usually associated with poorly developed or dead larvae. Occasionally in the PH, well-developed nematodes were observed associated with giant cells similar to those developed in the GH.

Twenty-five days after inoculation, differences in responses of the two corn hybrids were greater than previously observed (Fig. 1-D, E). Roots of the GH typically included large numbers of mature egg-laying females. Where several females were closely associated in roots of this hybrid, there was little evidence of root necrosis (Fig. 1-E). Giant cells had uniformly dense cytoplasm, numerous large nuclei, and thickened walls. Serial sections indicated that more giant cells were associated with a given female in GH than in PH. Unlike those nematodes in GH, no egg masses were associated with any of the females in PH at 25 days after inoculation. In some cases necrotic cells partially surrounded the nematode and giant cell in PH, and giant cells often appeared to be collapsed or empty; in such cases nearby nematodes were apparently dead. No nematodes were present in the vicinity of some empty giant cells (Fig. 1-D). Giant cells and nematodes in both hybrids were often surrounded by hyperplastic cells 25 days after inoculation. Roots inoculated with 20 egg masses in preliminary experiments and fixed 58 days after inoculation showed that at least some females eventually mature and produce eggs on Pioneer 309B (PH) (Fig. 1-F).

The mean number of larvae found in destained roots of Coker 911 was considerably higher than in Pioneer 309B at both 4 and 8 days after inoculation (Table 1). In both hybrids, larvae tended to penetrate primarily, just behind the root cap, but they migrated extensively. The larvae were most frequently aligned parallel to the stele with the head directed away from the root cap at both 4 and 8 days after inoculation. Occasionally, some individuals were found almost perpendicular to the stele. No differences in penetration sites or in positions of larvae in roots were observed between hybrids. Although neither hybrid was conspicuously galled, PH roots had more necrosis than GH roots.

TABLE 1. Number of larvae of *Meloidogyne incognita* penetrating good and poor corn hosts

Time after inoculation	No. larvae penetrating/plant[a]		
	Coker 911	Pioneer 309B	LSD
days	*good host*	*poor host*	*.01*
4	276	143	110
8	522	325	141

[a] Mean number for six replicates.

DISCUSSION.—The higher rate of reproduction of *M. incognita* on Coker 911 (GH) as compared to Pioneer 309B (PH) can be attributed, in part, to more larvae penetrating the former. In addition, experiments indicated that more favorable cellular responses to root knot nematodes in Coker 911, as compared to Pioneer 309B, partially explains the higher rate of nematode reproduction on the Coker hybrid. This is consistent with observations on certain vetch varieties in which resistance was due to low penetration rates as well as cellular responses unfavorable for nematode development (7). On both hybrids, larvae seemed to be particularly attracted to the area a short distance behind the root caps, as reported for root knot nematodes on other hosts (5, 6, 11).

The general sequence of giant cell formation in GH, under conditions most favorable for root knot nematode development, was similar to the sequence outlined for other suitable hosts (3, 5, 9, 10). Giant cells in corn apparently develop only from cells in the stele, as is found to be the case in most plants. When enlarging giant cells expand into the cortex, the endodermis persists and at least partially encloses the giant cells. Disruption and hyperplasia of cells adjacent to nematodes and giant cells have been reported in root knot on most hosts (5, 6).

Giant cell walls in corn may not thicken as early in development as those observed in most other hosts. In tomato the cell walls were extensively thickened only 10 days after inoculation (10), and in soybean, thickening of giant cell walls occurred during the early stages of their development (5). Giant cells most commonly associated with the GH were basically similar to those observed in highly susceptible soybeans, whereas cellular responses in PH such as necrosis and vacuolated cytoplasm were described in resistant soybeans (5). Groups of empty giant cells not in contact with nematodes were prevalent in PH after 25 days. Similar cells have been reported in sweetpotato infected with root knot nematodes (6), and were attributed to previous feeding by migratory root knot nematode males. Since fewer empty giant cells having no nematodes were found on GH, fewer larvae may have developed into males on this host than in PH. Although vacuolate giant cells might have resulted from frequent feeding on given cells, the findings of Rubinstein & Owens (12) (i.e., that continued feeding is required to sustain DNA synthesis) indicate that the opposite, lack of feeding, could be the case.

The lack of eggs in PH at 25 days after inoculation and the subsequent presence of eggs at 58 days suggest that those nematodes that did not die in Pioneer 309B developed very slowly. Peacock (11) reported

that root knot nematode females in necrotic tomato roots may develop to maturity after 8 weeks or more. Therefore, fewer generations probably complete their life cycles during the growing season under field conditions on PH than on GH.

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