

Histological Study of the Compatible and Incompatible Interaction of Soybeans and *Meloidogyne incognita*¹

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INTRODUCTION

The host-parasite interaction of several sedentary endoparasitic nematode genera and soybean plants has been studied histologically (1, 2, 3), ultra-structurally (9, 10, 24, 25), biochemically (7, 11, 22), and histochemically (4, 5, 6, 29), as well as in greenhouse and field studies (14, 15, 19, 20, 21, 23, 26, 28). This histological study differs from the above in that it attempts to provide basic information regarding the incompatible response of the soybean cultivar (cv.) Centennial to the root-knot nematode which is associated with the accumulation of a phytoalexin (12). This study discusses: 1) the relative susceptibility of the closely related cvs. Centennial and Pickett 71 to California single-egg-mass populations of *Meloidogyne incognita*, *M. javanica*, and *M. hapla*; and 2) macroscopic and microscopic changes in root morphology of the two cultivars infected by *M. incognita* in relation to phytoalexin accumulation (12).

MATERIALS AND METHODS

Susceptibility study. Three seeds of the

soybean [*Glycines max* (L.) Merr.] cvs. Centennial and Pickett 71 were soaked for 24 h in water and planted in separate 10.0-cm-diam pots containing a loamy sand. Twenty-four hours later, eggs were extracted from greenhouse cultures of single-egg-mass populations of *Meloidogyne javanica* grown on lima bean (*Phaseolus lunatus* L.) cv L-136, *M. incognita* grown on chili pepper (*Capsicum frutescens*) cv. New Mexico, and *M. hapla* grown on tomato (*Lycopersicon esculentum* Mill.) cv. Rutgers (17). Five thousand eggs were added to each pot. The study was repeated twice at 25 C in a growth chamber with five replications per host-parasite combination. Control plants were grown under the same conditions but were not inoculated.

Forty-five days after inoculation, root systems were washed free of soil, weighed, and their relative galling was determined. Larvae were collected from the root systems incubated in a mist chamber for seven days. The number of larvae/g of root fresh weight was determined.

Histological study. A procedure similar to that of McClure and Robertson (18) was used to ensure uniform infection of soybean roots with *M. incognita*. Aluminum trays (49 × 29 × 2 cm) used as inoculation chambers were filled with 600 g of silica sand-60, covered with aluminum foil, and autoclaved. Sterile tap water was added with a hand-pumped sprayer to increase the moisture of the sand in each tray to ca. 10%.

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Five sets of two sterilized 2-cm-wide Miracloth strips (Chicopee Mills, New York, New York 10018) were placed equidistant in each of the trays.

Seeds of the soybean cultivars Centennial, resistant to *M. incognita*, and Pickett 71, susceptible to *M. incognita* (12, 16), were dusted with Thiram (tetramethylthiuramdisulfide) and germinated in rag dolls at 30 C. Two days later, seedlings with roots of similar size were selected and placed along each set of Miracloth strips with their root tips positioned between the two layers.

Inoculum consisted of second-stage larvae from a single-egg-mass population of *Meloidogyne incognita* grown in greenhouse cultures on chili pepper. Root systems of culture plants were rinsed and aerated in tap water. Larvae collected during the first 24-h hatch period were discarded, whereas larvae hatching over the next 24-h hatch period were collected on a 500-mesh sieve. The larvae were concentrated to 50 larvae/0.5 ml of water. Fifty larvae were applied to the Miracloth strips around each of the seedling roots. The inoculation chambers (trays) were incubated at 25 C for 24 h. At the end of this period, cotton thread was tied onto the root at the upper edge of the inoculation zone. Roots of inoculated plants were rinsed with tap water to remove nematodes from the root surface which had not yet infected the root. Root systems were placed in Lucite chambers (10 × 15 × 2.5 cm) filled with moist vermiculite. The chambers had a removable side panel which enabled observation of macroscopic changes in inoculated material of 60× as well as excision of this material at appropriate intervals.

Infected tissue of each variety was harvested 1, 2, 3, 4, 6, 8, and 12 days after inoculation. The material was fixed in FAA and dehydrated through an alcohol series (8). Tissue was infiltrated with, and embedded in, Paraplast at 58 C. Longitudinal sections, 12 μm thick, were stained with safranin and fast green (8). Slides were examined and photographed with a Zeiss photomicroscope at 60×, 400×, and 1000×.

Fresh roots infected with *M. incognita* were observed under Normarski optics. Steles of infected roots which had been mechanically excised with forceps and a dissecting needle were examined for the

presence of larvae four days after inoculation. The condition of cells closely associated with larvae was noted.

RESULTS

The galling index correlated well with the reproduction of the three *Meloidogyne* species tested on soybean roots (Table 1). The cultivar Centennial was clearly resistant to our *M. incognita* population, but was susceptible to *M. javanica* and *M. hapla*. Cultivar Pickett 71 was susceptible to all three root-knot nematode species.

Macroscopic differences in roots of the two varieties inoculated with *M. incognita* first appeared during the third day after inoculation, when Centennial, but not Pickett 71, exhibited faint brown streaks. These streaks were parallel to the root axis and became more intense with time. Mechanical separation of the cortex and stele in these roots indicated that the brown cells were located primarily within the stele. No such streaks were observed in roots of Pickett 71 infected with *M. incognita*.

Light-microscope observations of sectioned Centennial and Pickett 71 roots infected with *M. incognita* are summarized in Table 2. Cellular changes occurred within two or three days following inoculation in the incompatible roots that were clearly different from those in compatible roots. The response of incompatible Centennial roots were considered a classical hypersensitive response (Fig. 2). Most larvae observed in stelar tissues were sur-

TABLE 1. Comparative galling and reproduction of three root-knot nematode species in roots of soybean cultivars Centennial and Pickett 71.

Nematode species	Centennial		Pickett 71	
	Galling index	Larvae/g fresh weight	Galling index	Larvae/g fresh weight
<i>M. incognita</i>	0 ^b	10.3 ^c	2.6	446.8 ^{ab}
<i>M. javanica</i>	2.6	705.1 ^a	2.2	193.9 ^{bc}
<i>M. hapla</i>	1.0	239.5 ^{bc}	1.2	463.3 ^{ab}
Control	0	0	0	0

*r = 0.76.

^bGalling index: 1 = 1-10%, 2 = 11-20%, 3 = 21-30%, 4 = 31-50%, 5 = 51-100%.

^cColumn means followed by the same letter are not significantly different (P = 0.05) according to Duncan's multiple-range test.

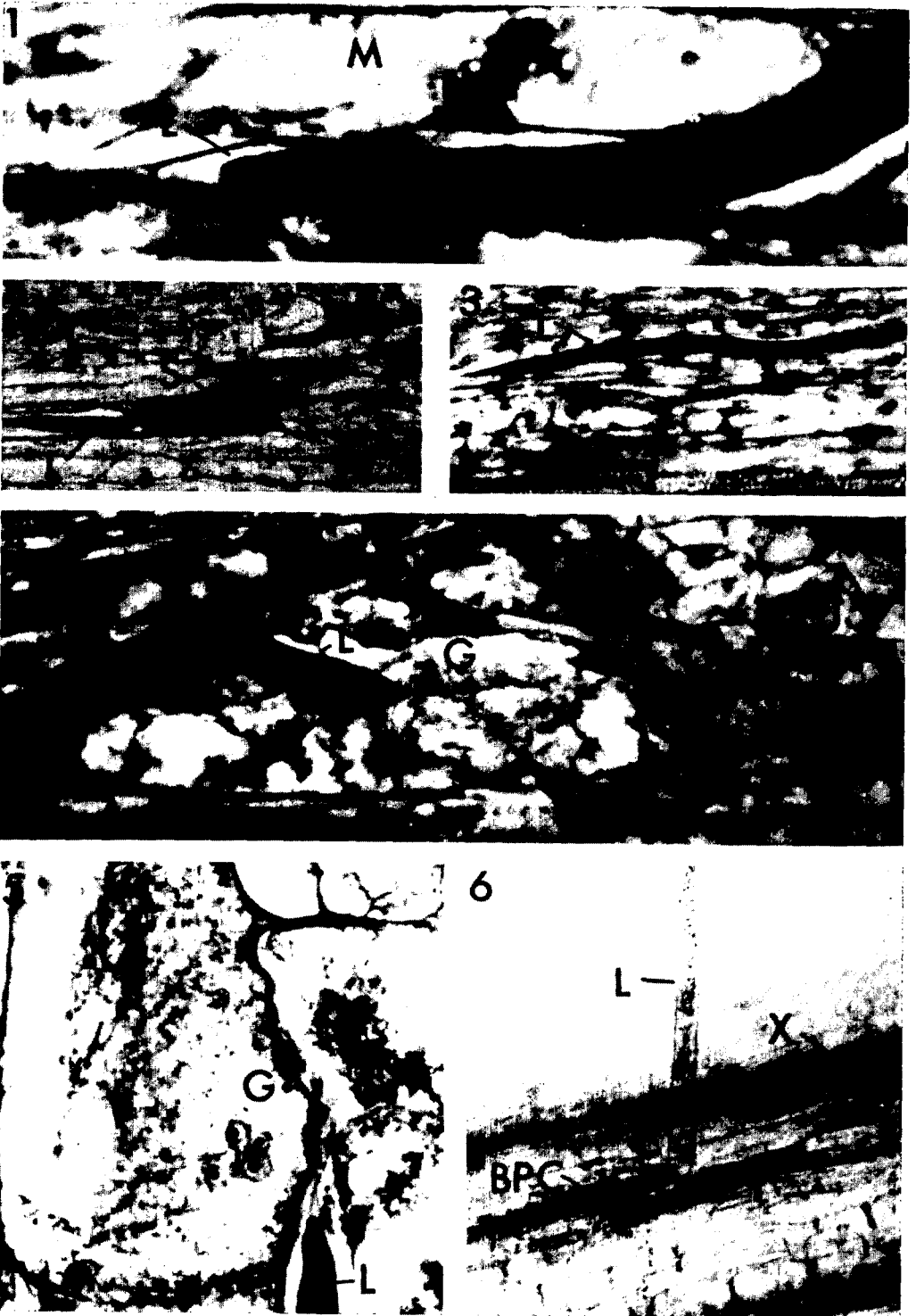


FIG. 1. Longisection of a root of Pickett 71 demonstrating mitosis in phloem cells adjacent to *M. Incognita* larva two days after inoculation. (M = mitotic cell, L = larva). 1,000 \times .
FIG. 2. Phloem cells associated with head regions of *M. incognita* second-stage larvae were differentially stained by safranin in root of the soybean cv. Centennial. (400 \times , 4 days after inoculation) (S = safranin staining, L = larva).

TABLE 2. Comparison of light-microscope observations of *M. incognita*-infected roots of the soybean cvs. Centennial (incompatible) and Pickett 71 (compatible).

Day after inoculation	Incompatible (Centennial)	Compatible (Pickett 71)
1	Infection of roots and migration of most second-stage larvae to protosteles. Some larvae present in the cortex.	Infection of roots and migration of most second-stage larvae to protosteles. Some larvae present in the cortex.
2	Cells in the vicinity of the heads of larvae within the phloem are generally uninucleate, optically dense cytoplasm surrounds the nuclei in many of these cells. Some cells are stained with safranin. Some larvae remain in the cortex. These larvae and some larvae within the stele have no optically distinguishable effect on adjacent cells.	Nuclei of phloem cells in the vicinity of larvae's heads appear normal, binucleate, or in the process of mitosis. Occasionally cells in the vicinity of larvae stained with safranin. Some larvae remained in the cortex (Fig. 1).
3	An increased number of larvae were surrounded by cells which stained with safranin. No multinucleate cells were observed. Some larvae do not appear to affect adjacent cells. Some larvae were still present between cortical cells.	Multinucleate cells were associated with the head regions of many of the larvae present in the stele. Other larvae were associated with uninucleate and binucleate cells. The number of cells which stained with safranin was small and limited.
4	Safranin staining of cells adjacent to larvae has increased both in intensity and in frequency within the stele (Fig. 2). Necrotic-appearing cavities filled with granular material have developed within the stele where many larvae have associated together. Some larvae in the stele did not affect adjacent cells, but remained inter-cellular as if they were still migrating.	More larvae are associated with multinucleate cells in the stele.
6	No giant cells were observed in the steles; however, an occasional larva was associated with binucleate unstained cells in the stele. Most larvae in the stele were associated with cells intensely stained with safranin.	Second-stage larvae were associated with giant cells.
8	Second-stage larvae in the phloem were associated with: <ol style="list-style-type: none"> 1) Cells stained with safranin 2) Cells which appeared normal (Fig. 3) 3) Occasional giant cells with vacuolated cytoplasm and small nuclei (Fig. 4) 	Extensive giant cell formation associated with third- and fourth-stage larvae (Fig. 5).
12	Vermiform larvae remain in the phloem associated with: <ol style="list-style-type: none"> 1) Cells stained with safranin 2) Cells which appeared normal 3) Occasional small giant cells 	Large giant cells present in the stele with granular cytoplasm were associated with fourth-stage larvae with enlarged ovaries.



FIG. 3. An *M. incognita* larva which may have been migrating in the stele of a root of the soybean cv. Centennial. Appearance of adjacent cells suggests that they were not adversely affected by the presence of the nematode. (60 \times , 8 days after inoculation) (L = larva).

FIG. 4. Giant cells stimulated by an *M. incognita* larva in the soybean cv. Centennial. The cells and nuclei were smaller than those which developed in the roots of the compatible host. The cytoplasm was highly vacuolated (400 \times , 8 days after inoculation) (L = larva, G = giant cell).

FIG. 5. Giant cells associated with *M. incognita* in the roots of the soybean cv. Pickett 71. (400 \times , 8 days after inoculation) (L = larva, G = giant cell).

FIG. 6. Photomicrograph of a stele dissected out of a root of the soybean cv. Centennial showing the association of brown phloem cells with the larval head region. The posterior portion of the nematode's body was liberated from the cortical tissue which was removed. (Nomarski optics, 400 \times , 4 days after inoculation). (BPC = brown phloem cells, L = larva, X = xylem).

rounded by phloem cells that stained intensely with safranin. When fresh inoculated root tissue was studied by Nomarski optics, the phloem cells surrounding the anterior end of the nematodes appeared brown (Fig. 6). However, some nematodes in Centennial roots survived and formed at least rudimentary giant cells which were visible 8-12 days after inoculation (Fig. 4). Thus, although apparent hypersensitive reactions occurred at most nematode infection sites in the stele of resistant cultivar roots, exceptions were noted (Figs. 3, 4). Roots of Pickett 71 infected by *M. incognita* and *M. javanica* as well as roots of Centennial infected by *M. javanica* did not exhibit this extensive cellular browning. Instead, the stele had swollen as a result of cell hypertrophy.

DISCUSSION

Our observations indicated that the incompatible response of the soybean cv. Centennial to *M. incognita* may manifest itself in several ways. Although predominantly associated with cellular browning, other host responses capable of limiting pathogen development were noted. The giant cells stimulated by relatively few larvae were small, the cytoplasm was highly vacuolated, and their nuclei were smaller than those which developed in roots of the compatible host. Many of the giant cells which developed in roots of the incompatible host degenerated prior to nematode maturation. The limited number of mature females which developed in roots of Centennial produced significantly fewer eggs than females which developed in roots of the susceptible cultivar (12). In addition, some larvae did not appear to induce beneficial or deleterious changes in adjacent cells. This array of responses observed within a single root of the incompatible host to nematode infection may result from the action of a phytoalexin (12) or they may reflect resistance of a multimechanismal nature. Relatively large concentrations of isoflavonoid phytoalexin glyceollin accumulated in the stele concomitantly with the appearance of cellular browning in response to infection by second-stage *M. incognita* larvae (12). This compound was nematostatic to *M. incognita in vitro* (12, 13) and

may have prevented larvae from migrating away from brown cells within the root.

In contrast, Veech and Endo (29) reported that *M. incognita acrita* larvae were seldom associated with brown cells in roots of the resistant soybean cv. Delmar but rather were thought to move away from cells as they became brown. In addition, giant cell development in the roots of the cv. Delmar was limited only in total number (5, 6, 29). That is, there were no distinguishable differences noted in giant cells developing in the resistant and susceptible cultivars infected by *M. incognita acrita*. However, giant cells stimulated by *M. incognita* in roots of the resistant cv. Centennial were easily distinguished from those which developed in the susceptible cv. Pickett 71. This suggests that the nature of soybean incompatibility to *M. incognita acrita* may differ from incompatibility to *M. incognita*. The incompatible response of Centennial to *M. incognita* is dependent upon glyceollin accumulation (12). Information is not presently available on the chemical basis of the incompatible response of cv. Delmar to *M. incognita acrita*.

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