Effects of Meloidogyne incognita on Nitrogen Fixation in Soybean[1]

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Abstract: The effects of a North Carolina population of *Meloidogyne incognita* on N_2 fixation on root-knot-susceptible 'Lee 68' and moderately resistant 'Forrest' soybean were evaluated 50, 75, 100, and 135 days after inoculation with nematodes. Nematodes stimulated N_2 fixation in Lee 68 by 50 days and in Forrest by 75 days. At all other intervals, N_2 fixation was either depressed or unaffected by nematodes. Additional observations indicate that the susceptibility of Lee 68 is associated with greater rates of penetration by larvae and more favorable responses of host tissues to nematodes than occur in Forrest. With time, however, the histological reactions of both hosts became less favorable for nematode development. Resistant or hypersensitive responses became common in Forrest by 75 days but not in Lee 68 until 90 days after inoculation. This population of *M. incognita* may stimulate N_2 fixation at a specific time interval and depress it at others; therefore, disease of susceptible soybeans caused by this nematode is probably not primarily due to a net loss of fixed nitrogen but to pathogenicity similar to that which occurs on nonlegume hosts. *Key Words: Glycine max,* histopathology, nematode, nitrogen fixation.

Meloidogyne incognita (Kofoid and White) Chitwood has been described as the limiting factor to production of soybean [*Glycine max* (L.) Merr.] in some localities, and in extreme cases it may cause yield losses of more than 90% (12). *Heterodera glycines* (Ichinohe, 1952), the soybean cyst nematode, is also destructive to soybean. It inhibits N_2 fixation by *Rhizobium japonicum* (Kirch.) Buchanan, and therefore may result in N-deficient plants (1, 13). In contrast, *Meloidogyne* spp. are not known to severely inhibit N_2 fixation, regardless of their tendency to invade the *Rhizobium* nodule (16). Crittenden (6) observed that *M. incognita* resulted in more galls on nodulating soybeans than nonnodulating lines, although the nematodes matured more rapidly on nonnodulating forms. Bird (3) noted that small numbers of *M. javanica* (Treub, 1885) Chitwood accelerated growth in N-deficient plants, and Ross (15) found that seeds from soybeans infected with *M. incognita* had a higher percent protein content than seeds from uninfected plants. Those observations suggest that *Meloidogyne* spp. could be favored by *Rhizobium* development and that, under certain conditions, N_2 fixation may be enhanced by the presence of this nematode. However, reactions of soybeans to *M. incognita* are highly variable among nematode populations and host cultivars. These differences are reflected in the histopathology and histochemistry of soybeans infected with *Meloidogyne* spp. (7, 8, 17).

For the most part, previous studies of *M. incognita* on soybeans have been during relatively early development of the host through pod formation. Our preliminary investigations suggested that *R. japonicum*- and *M. incognita*-soybean interactions may vary as the soybean continues to mature. Therefore, the present investigation was designed to examine this interaction at intervals between 50 and 135 days after inoculation of seedlings with nematodes on root-knot-susceptible 'Lee 68' and moderately resistant 'Forrest' soybeans.

MATERIALS AND METHODS

Nematode penetration. Sixteen nine-day-old soybean seedlings of cultivar Lee 68 and 16 of Forrest were transplanted to 150-ml beakers filled with a mixture of equal parts 35- and 65-mesh sand. Concurrent with transplanting, roots of all 32 seedlings were inoculated with 100 mg Nitragin "S" *R. japonicum* suspended in 10 ml distilled water. The nine-day-old seedlings were also inoculated with a 10-ml aqueous suspension of 500 freshly hatched larvae of *M. incognita* from a North Carolina population. At 3 and 7 days after inoculation, roots of eight seedlings of each

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cultivar were washed, stained with acid-fuchsin-lactophenol (14) and destained in lactophenol at room temperature for 10 days. The destained roots were cut in small pieces and placed in glycerin between glass plates, and numbers of larvae in each root system were counted under a dissecting microscope.

Nematode development and N_2 fixation. Sixty-four nine-day-old soybean seedlings of Lee 68 and 64 of Forrest were transplanted to 15-cm-diam clay pots filled with a sterile 1:1 mixture of a sandy-loam soil and 35-mesh sand. Concurrent with transplanting, all 128 seedlings were inoculated with *R. japonicum,* and 32 seedlings of each cultivar were inoculated with *M. incognita* by adding a 50-ml aqueous suspension of 15,000 eggs (10). Modified Hoagland's nutrient solution minus N was added biweekly to each pot. Greenhouse light was supplemented with multivapor ballast lights to provide a 12-h day. Fifty, 75, 100, and 135 days after inoculation, eight replicates of each treatment were harvested. At each harvest, shoot and root weights, nodule numbers, and nodule dry weights were determined. Nematode eggs were extracted from roots with 1.20% sodium hypochlorite in water (4), and larvae were obtained from soil with a semiautomatic elutriator (5) followed by centrifugation flotation and counting. Nitrogen-fixing capacity was measured for each root system with acetylene-ethylene assay utilizing an F and M model 700 gas chromatograph (Hewlett Packard Co., Avondale, Pennsylvania) with an H_2-flame ionization detector (9, 13). The degree of deviation of data from means for given treatments changed with age of plants and, thus, harvests. Natural log transformation was carried out before factorial analysis of variance in order to equalize these deviations from means in the combined analysis.

Histology. Lee 68 and Forrest soybeans were prepared as for the N_2-fixing capacity study with five replications and were harvested 50, 75, and 90 days after inoculation. Roots and nodules were fixed, dehydrated, embedded, sectioned, and mounted as in previous studies (1). Staining was with safranin and fast green (11).

RESULTS

Nematode penetration. Penetration of larvae was significantly greater in Lee 68 than in Forrest. Three and seven days after inoculation, respective means of 22 and 49 larvae were counted per root system from Lee 68, whereas means of 10 and 17 larvae were counted from Forrest. No difference was noted between the two varieties with respect to maturity of larvae at a given harvest.

Nematode development and N_2 fixation. No differences were noted between rates of maturation of Lee 68 and Forrest soybeans under the conditions of this experiment. Flowering began before the first harvest, and by 50 days, pod formation and filling had begun. Although soybeans were most vigorous, having many pods at 75 days, by 100 days they had begun to senesce, and plants inoculated with nematodes appeared to senesce more rapidly than uninoculated controls. *Meloidogyne incognita* increased in number on both Lee 68 and Forrest throughout the experiment (Fig. 1). At 135 days, Lee 68 had about 550,000 eggs and larvae per plant, whereas Forrest had about 150,000.

Nitrogen fixation did not differ significantly between nematode-free Forrest and Lee 68 soybeans except at 50 days, when fixation was less ($P = 0.01$) in Lee 68 than Forrest (Table 1). Although the numbers of nematodes on Forrest remained relatively constant between 50 and 75 days (Fig. 1), the change in their effect on N_2 fixation was striking (Table 1). At 50 days, N_2 fixation was suppressed by nematodes on Forrest, whereas at 75 days N_2 fixation was much greater than in nematode-free controls. After 75 days, N_2 fixation in both Forrest and Lee declined rapidly in treatments with nematodes, but by 135 days there was no difference between the two Forrest treatments (Table 1). The nematodes increased rapidly on Lee 68 (Fig. 1) by 50 days and slightly stimulated N_2 fixation (Table 1). From 75 days to the termination of the experiment, large numbers of nematodes on Lee 68 tended to depress fixation (Table 1). By 100 days, when plants had begun to senesce, N_2 fixation declined for all treatments (Table 1), but generally declined to an only slightly greater extent in soybeans with *M. incognita* than in uninoculated checks. In Forrest, however, N_2 fixation changed from greater than uninoculated

TABLE 1. Influence of *Meloidogyne incognita* (Mi) on nodule development and nitrogen-fixation capacity (measured in μmoles ethylene) by *Rhizobium japonicum* on Forrest and Lee 68 soybeans at different time intervals.[a]

Treatment	Time after Inoculation			
	50 days	75 days	100 days	135 days
Number of nodules[b]				
Forrest + Mi	130.0	217.4	163.1	131.7
Forrest	107.0	177.4	171.6	98.4
Lee 68 + Mi	96.9	155.7	125.9	30.4**
Lee 68	99.3	124.7	135.1	97.4
Dry weight of nodules (mg)[b]				
Forrest + Mi	409.9	423.0	477.7*	386.9
Forrest	420.4	541.7	692.9	347.7
Lee 68 + Mi	525.9	548.4	639.2	105.2**
Lee 68	401.0	697.3	639.3	534.4
μmoles ethylene[b]				
Forrest + Mi	7.1**	36.6**	3.7*	2.4
Forrest	14.3	12.0	5.2	2.1
Lee 68 + Mi	10.0**	9.0*	2.2	.6*
Lee 68	5.3	14.1	3.9	1.2

[a]Asterisks (*, **) indicate a significant difference from respective nematode-free controls at $P = 0.05$ and 0.01. Significance was determined from LSD's for a combined analysis including all time intervals. (All data were transformed to natural logs for statistical analyses.)
[b]Per root system; each figure is the arithmetic mean of 8 replicates.

checks at 75 days to less than checks at 100 days (Table 1).

Nodule numbers were highly variable and were little affected by nematodes in Forrest, including at 75 days, when N_2 fixation was greatly stimulated by *M. incognita* (Table 1). In Lee 68, however, nodulation was highly inhibited by nematodes after 100 days and many nodules were deteriorated. Nodule number on plants without nematodes was quite consistent throughout the experiment and seemed to have little relation to N_2-fixation capacity (Table 1). Total nodule dry weight per plant was also highly variable, but respectively at 100 and 135 days, nematode-infected Forrest and Lee 68 had less nodule weight than uninoculated controls (Table 1). Shoot growth was not retarded by nematodes in either soybean cultivar, and root weight was generally greater in nematode-inoculated treatments of both cultivars.

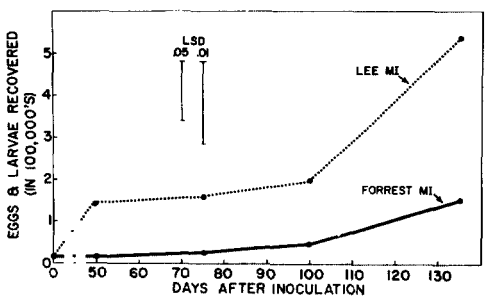
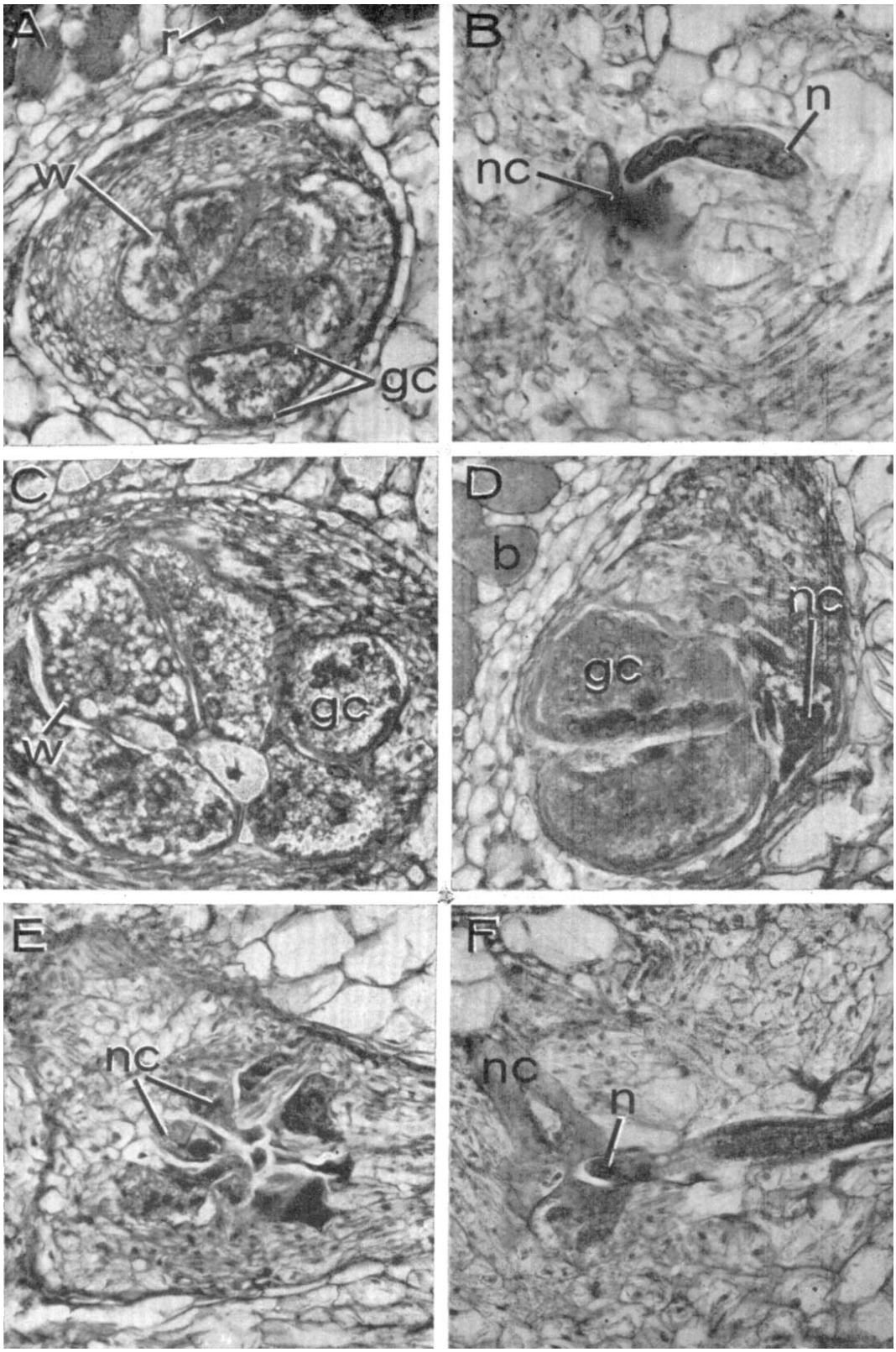


FIG. 1. Number of eggs and juveniles of *Meloidogyne incognita* on Lee 68 and Forrest soybeans, recovered at different time intervals.

Histology. Nematode-free Lee 68 and Forrest were histologically similar. *Rhizobium* nodules were surrounded by a network of vascular cylinders as previously described (2), and this developing vascular tissue provided numerous sites for nematode infection.

At 50 days after inoculation, clusters of about five giant cells were associated with mature females in both cultivars (Fig. 2-A,C). Giant cells of Lee 68 and Forrest

FIG. 2-(A-F). Sections of Lee 68 and Forrest soybean 50, 75, and 90 days after inoculation with *M. incognita* (130X). A) Lee 68 at 50 days with giant cells (gc) that have wall ingrowths (w). Note adjacent bacterial (*Rhizobium*-filled) cells (r). B) Lee 68 at 90 days, showing necrotic tissue (nc) and nematode (n). C) Forrest at 50 days, with giant cells (gc) that have wall ingrowth (w). D) Forrest at 75 days, with two well-formed giant cells (gc) and adjacent nectrotic tissue (nc); b, bacteroid-filled cells. E) Forrest at 75 days, with necrotic tissue (nc). F) Forrest at 90 days, showing necrotic tissue (nc) and nematode (n).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were generally typical of those previously described for susceptible reactions (7) and had clearly defined boundaries with wall ingrowths and optically dense cytoplasm (Fig. 2-A,C). Giant cells were often larger in Forrest than in Lee 68 (Fig. 2-A,C). At 75 days, the predominant response in Lee 68 was similar to that at 50 days (Fig. 2-A), but in Forrest, tissues densely stained with safranin and typically interpreted as resistant necrotic responses (1, 7, 17) were common (Fig. 2-E). Although many well-formed giant cells were also present, only two or three such cells were associated with a given nematode, and these cells were often partially surrounded by necrotic tissue (Fig. 2-D). At 90 days, reactions in Forrest were unchanged (Fig. 2-F); however, the predominant response in Lee 68 was necrosis typical of resistant reactions (Fig. 2-B). In all treatments, care was taken that large giant cells or cavities resulting from females which had reached maturity and subsequently died were differentiated from giant cells associated with larvae and young females.

DISCUSSION

The effects of *M. incognita* on N_2 fixation varied with soybean cultivar and harvest; harvest includes the combined influence of age of host and development of nematode population. Development of the nematode population was determined by assay of eggs and larvae at each harvest. However, there is a lag between these young stages and the maturing nematodes which exerted a pathogenic effect on the host. As small numbers of nematodes became established, stimulation of N_2 fixation occurred in Forrest at 75 days and, to a lesser degree, in Lee at 50 days. Several investigators have suggested that hosts sometimes respond to small numbers of *Meloidogyne* species by an increased metabolism (3, 18). Similarly, race 1 of *H. glycines* initially increased but subsequently retarded N_2 fixation in soybean (13). We are unable to explain the depression of N_2 fixation in Forrest by small numbers of nematodes at 50 days; however, histology indicates that Forrest responses were less likely to be hypersensitive and resistant at 50 days than at 75 days. This response was in contrast to the early hypersensitive response noted in highly resistant soybean cultivars (7, 8).

By 100 days, N_2-fixation generally declined for all treatments, probably primarily from senescence of the hosts. Hardy *et al.* (9) also observed that N_2 fixation increases rapidly during pod-formation and filling, followed by decreased fixation and senescence. The senescence and a decline in N_2-fixation capacity in healthy soybeans was only slightly greater in treatments with nematodes than in uninoculated controls.

Resistance of Forrest to *M. incognita* is partly due to lower rates of penetration and partly to a physiological response reflected in the histopathology; resistant responses of Lee 68 are delayed and occur in association with denser populations of nematodes and increased host senescense.

Pathogenicity is highly variable among populations of *M. incognita* on many hosts. Further investigations are needed to determine the extent to which additional populations of *M. incognita* differ from the present population in host-parasite relations and influence on N_2 fixation. The effects of this North Carolina population on N_2 fixation varied with the combined influence of the age of host and time since inoculation with the nematode; there was apparently little if any net loss of fixed nitrogen. Thus, damage of *M. incognita*-susceptible soybeans is not necessarily due primarily to inhibition of N_2 fixation, as is true for certain races of *H. glycines*, but may be due to pathogenicity similar to that of *Meloidogyne* spp. on nonlegume hosts.

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