Antagonistic Interaction between Heterodera glycines and Rhizobium japonicum on Soybean

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ABSTRACT

Greenhouse experiments and histological investigations were conducted to determine the basis for the antagonistic interaction between the soybean cyst nematode, *Heterodera glycines* (SCN), and *Rhizobium japonicum* (R). Treatments included inoculation of 5- to 7-day-old Lee soybean plants with 200 crushed cysts or 20,000 eggs of SCN and/or 200 mg of commercial inoculum of R, in the following combinations: SCN with R added simultaneously and at 2 to 14 days later; R with SCN added simultaneously and at 2 to 14 days later; R or SCN alone at the above time intervals; and noninoculated controls. Greatest inhibition of nodule development occurred with simultaneous inoculations of R and race 1 of SCN. A 14-day delay in introducing SCN resulted in only slight to moderate inhibition of nodulation. Race 1 of SCN, which inhibits nodulation, penetrated nodular tissues at a much greater rate than race 4, which has little effect on nodulation. Nodular tissue, however, was unfavorable for the development of larvae of either race. Most nematodes that matured in nodules were males. Histological studies also showed nodular tissues to be unsuitable for development of race 1. Infection of nodules by race 1 of SCN produced necrosis in the area around the nematode. Although a few mature cysts developed on nodules, most infections of these tissues by larvae failed to induce syncytia.

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Additional key words: bacteria, histology, *Glycine max,* nodulation.

Several species of *Heterodera* reduce numbers of nodules induced by *Rhizobium* spp. on legumes. Most investigations (9, 12, 15, 19) have indicated that the nematodes play a competitive role in reducing the number of nodules. Taha & Raski (16) found, however, that *H. trifolii* and *Meloidogyne javanica* on white clover (*Trifolium repens* L.) reproduced readily in nodular tissues and only affected numbers of nodules per plant by reducing the size of the root system. They also found that the basic structure of nodular tissues was not disturbed by nematode infection, although giant cells were formed in the vascular bundles.

Other types of parasites also interfere with nodule development on soybean, *Glycine max* (L.) Merr., under certain conditions. Mew & Howard (14) described an antagonistic interaction between *R. japonicum* and *Fusarium oxysporum*. Tu et al. (17) found that soybean mosaic virus inhibits nodule development, especially with early virus infection.

Since certain races of *H. glycines* (SCN) greatly inhibit nodulation on soybean (5, 6, 13), this investigation was initiated to determine (i) the effects of timing and sequence of inoculations with SCN and/or *Rhizobium japonicum* (R) on nodule development; (ii) rates of penetration and development of "inhibitory" and "noninhibitory" races of SCN in nodular tissues; and (iii) the histological responses of nodular tissues of soybean infected with SCN.

MATERIALS AND METHODS.—Two populations of SCN (7) were used: race 1, Wilmington, N.C., which inhibits nodulation; and race 4, Lanoke, Ark., which has little effect on nodulation. Four 5- to 7-day-old Lee soybean seedlings, germinated in vermiculite, were transferred to each 1-liter plastic pot containing 35-mesh silica sand (13) at the time of initial inoculations. Two hundred crushed cysts (yellow to light brown) or about 20,000 eggs of SCN were added to each pot receiving nematodes. Egg suspensions were prepared by the crushing of cysts in a Ten-Broeck homogenizer. Ten ml of a suspension containing 200 mg of a commercial powder of R (Kalo Inoculant®, Kalo Inoculant Co., Quincey, Ill.) were added to each pot receiving the bacterium. All plants received one-half strength Hoagland's nutrient solution (11), minus nitrogen, as needed. Pots were flushed semiweekly with water.

All experiments were repeated twice; three or four replicates/treatment were harvested at different times. Shoot, root, and nodule weights, and numbers of nodules and nematodes were recorded. To determine rates of penetration and development of SCN in nodular tissues, nodules were stained with acid-fuchsin lactophenol (8). We recovered cysts by washing infected roots with a high-pressure spray of water and by the Fenwick can (8) for those remaining in the sand.

For the histological investigations, roots with nodules were fixed in Formalin-alcohol-acetic acid (FAA), dehydrated with a tertiary-butyl-alcohol series, and embedded in Tissuemat. Twelve-μ sections were cut with a rotary microtome, mounted with
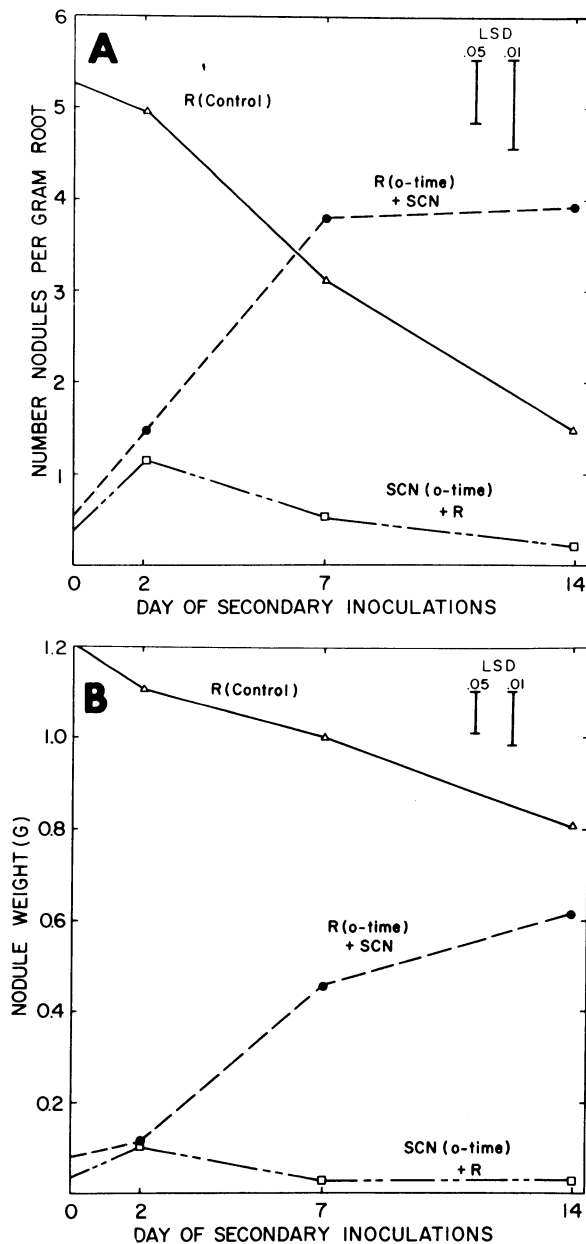
Fig. 1. Influence of timing of inoculation of soybean with *Rhizobium japonicum* (R) and *Heterodera glycines* (SCN) on nodule development. A) Number of nodules per gram of root. B) Nodule weights per four plants (per pot). Data are means of the 42- and 60-day harvests.

TABLE 1.   Effect of *Rhizobium japonicum* on reproduction of race 1 of *Heterodera glycines* on soybean

| Treatment[a] | Number cysts recovered/pot/harvest | | |
	42-day	60-day	Means of both harvests
SCN (only)	5001	6104	5553
SCN + R	4520	4761*[b]	4641**
R + SCN	3468**[c]	6394	4931**

[a]SCN = 200 crushed cysts of *H. glycines*; R = 200 mg of commercial inoculum of *R. japonicum* per pot. Data are means of four inoculation periods: simultaneous (0-time), 2, 7, and 14 days.

[b]* = significant difference at 5% level when compared to respective SCN (only) treatment.

[c]** = significant difference at 1% level when compared to respective SCN (only) treatment.

Haupt's adhesive and 4% Formalin, and stained with Johansen's quadruple stain (10).

RESULTS.—*Nodule and nematode development as affected by timing and sequence of inoculations.*—Various combinations of inoculation treatments with race 1 of SCN and R were used: SCN at 0-time with R added simultaneously, and at 2, 7, and 14 days later; R at 0-time with SCN added simultaneously, and at 2, 7, and 14 days later; R or SCN alone at these same times; and noninoculated controls. Of the eight replicates of each treatment, four were harvested at 42 days and four at 60 days after initial inoculations. Greatest inhibition of nodule development, up to 100% as compared to R alone at 0-time, occurred with simultaneous inoculations (Fig. 1). A 7- to 14-day delay in introduction of SCN resulted in only slight to moderate inhibition of nodulation. These relationships were similar for both harvests whether data were expressed as numbers of nodules per g of root (Fig. 1-A) or as nodule weights per treatment (Fig. 1-B).

Combination of data for the four inoculation periods made apparent a significant effect of the sequence of inoculation on numbers of cysts that developed. Addition of R after SCN resulted in lower numbers of cysts than developed with SCN alone (Table 1). The R + SCN treatments harvested after 60 days yielded the highest numbers of cysts, which apparently was due to the development of a more extensive root system.

When data for all treatments were considered, the number of nematodes found in nodules from the 42- and 60-day harvests were very different. The mean number of nematodes per nodule harvested after 42 days was 72, and only 5% of these were males; other nematodes, excluding a total of 16 females, were second-stage larvae. The mean number of nematodes per nodule harvested after 60 days was only four, and 50% of these were males.

Comparison of races of SCN.—To determine the rates of penetration and development of races 1 (inhibits nodulation) and 4 (has little effect on nodulation) in soybean nodules, given seedlings were inoculated with: R alone; R with 20,000 eggs of race 1 at 0-time or 5 days later; and R with 20,000 eggs of race 4 of SCN at 0-time or 5 days later. Of the nine replicates/treatment, three were harvested each time at 3, 6, and 9 weeks after initial inoculations. Race 1 penetrated nodular tissues at a much greater rate than did race 4 (Table 2). However, usually less than 1% of the larvae of either race that penetrated nodules

TABLE 2. Differential rates of penetration of nodules on soybean by races 1 and 4 of *Heterodera glycines*[a]

Treatment[b]	Total no. nodules/pot	Weight of nodules (g)	Total no. nematodes in nodules[c]	Mean no. nematodes/nodule
R (control)	33	1.2		
R + race 1 (0-time)	9**	0.2**	477	53
R + race 4 (0-time)	42*	1.0	60**	1.4**
R + race 1 (5 days)	15**	0.5**	590	39
R + race 4 (5 days)	72**	0.9	36**	0.5**

[a]All data are means of three harvests (3 four-plant replicates/treatment per harvest) made at 3, 6, and 9 weeks after initial inoculations.

[b]R = 200 mg of commercial powder of *Rhizobium japonicum*/pot; 20,000 eggs of a given race of *H. glycines* (SCN) were used/pot.

[c]With a few exceptions, all nematodes in nodules were second-stage larvae.

[d]** = Significant difference at 1% level when compared to respective R (control) treatment for numbers or wt of nodules; for numbers of nematodes per nodule, compare with R + race 1 of SCN at 0-time.

[e]* = Significant difference at 5% level when compared to respective R (control) treatment.

matured into females. A *Rhabditis* species invaded and apparently reproduced readily in many older nodules infected with race 1 of SCN.

Race 1 caused much inhibition of nodulation; race 4, however, resulted in increased nodulation as compared to the R control (Table 2). The total fresh weight of nodules from the R + race 4 treatment, however, was slightly less than the R control.

Histological investigations.—Inoculation treatments in this experiment were the same as those outlined for the experiment on the effects of timing and sequence of inoculations on nodule and nematode development. Three of the six replicates were harvested at 30 days and three at 45 days. Results given are limited to race 1, since insufficient nematode-infected nodules were obtained from plants inoculated with race 4. Soybean nodular tissue generally failed to support the development of mature nematodes. Larvae of SCN usually induced much necrosis in nodular tissues (Fig. 2-A). Larvae often induced the development of syncytia, but the nematodes usually failed to mature, and the syncytia degenerated (Fig. 2-B,C,D); considerable necrosis was associated with such infections (Fig. 2-A,B,C,D). Although many of the 3rd- and 4th-stage larvae in the "abortive" infections apparently were males (Fig. 2-B), a few were females (Fig. 2-C). The typical response of nodular tissues to SCN is shown in Fig. 2-D; i.e., both the surrounding tissue and the nematode die.

A number of females developed on roots adjacent to nodules (Fig. 2-E), especially when SCN was introduced after R. In treatments where inoculations were initially with R and subsequently with SCN, most cysts developed on secondary roots rather than on nodules. An almost mature female (2 weeks old) on a plant initially inoculated with R and 2 weeks later with SCN is shown in Fig. 2-F.

DISCUSSION.—Our results indicate that developing nodules are most sensitive to race 1 of SCN in the early stages, since greatest inhibition occurred when SCN was added with R simultaneously or short-ly after adding R. In addition to sequence of inoculation, timing of harvests greatly influenced the numbers of cysts that developed on plants which received R initially, followed by SCN. These plants had a much larger root system, especially those of the later harvest interval. Different races of SCN vary greatly in their capacity to inhibit nodule development (6) and N_2 fixation on soybean by R (13). The greater rate of penetration of nodular tissues by race 1 of SCN, as compared to that of race 4, may be partially responsible for the greater capacity of race 1 to inhibit nodulation and N_2 fixation. The failure of either race 1 or 4 to reproduce in significant numbers in nodular tissues was somewhat unexpected, since Taha & Raski (16) found that *M. javanica* and *H. trifolii* actually preferred such tissues on white clover.

The histological responses of soybean nodules are also very different from those of clover to *M. javanica* and *H. trifolii* (16); clover nodular tissues were very compatible "hosts" for these nematodes, whereas our results indicate soybean nodular tissues to be hypersensitive to SCN. Although a few cysts developed on nodules, most nematode infections caused considerable tissue necrosis, and most syncytia did not develop fully.

In studying the responses of soybean, Endo (3) indicated that SCN, especially males, often resulted in necrosis and degeneration of syncytia as the nematodes matured. He suggested that the position of the syncytia in relation to the vascular tissues may determine the sex of larvae. If this is true, the type of vascular tissues in nodules may not be suitable for female development. Although such host or environmental factors do not have a direct role in sex determination in a bisexual species such as SCN, the recent work of Viglierchio & Croll (18) with *Ditylenchus dipsaci* and *Heterodera schachtii* shows that less favorable host tissues may support the development of greater numbers of males than females. Such differential development may explain why most of the SCN that reached maturity in nodular tissues were males. In later work, Endo (4) found that SCN in-

duced much necrosis in resistant soybean plants. Some of the reactions that he observed were very similar to those of nodular tissues (Fig. 2-B, D), where the surrounding tissue as well as the nematode died. This type of reaction may partially explain the reduced numbers of SCN obtained when nematodes and R were added simultaneously (1). However, the effect of this interaction on the population dynamics of SCN probably is minor under field conditions.

The invasion of nodules infected with SCN by
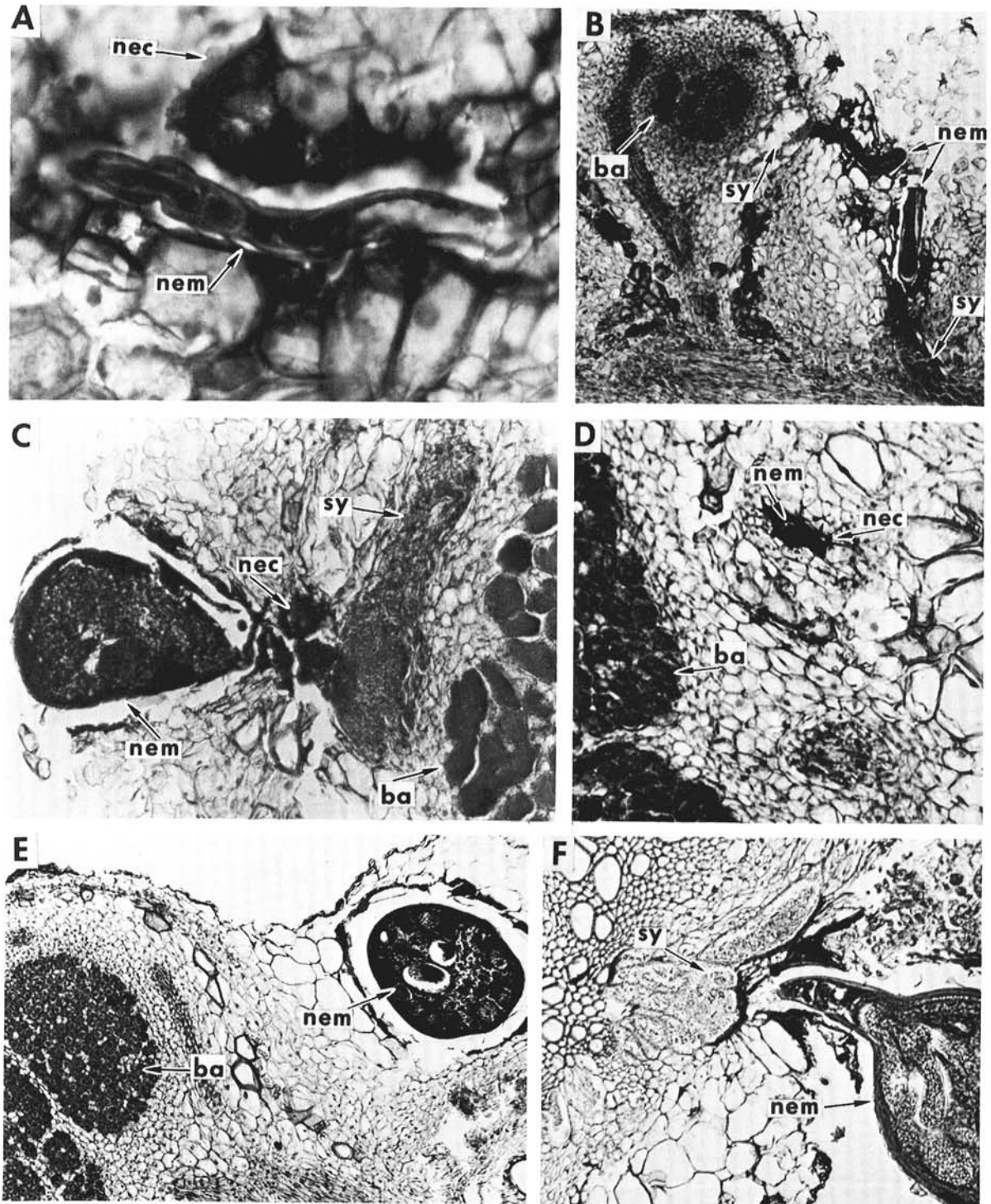
secondary organisms may accentuate the deterioration of these structures, especially under field conditions. The occurrence of high numbers of a *Rhabditis* sp. in many older SCN-infected nodules from greenhouse plants indicates that saprophagous nematodes may be involved in such an interaction.

Although all basic events leading to the formation of bacteriods and the complete nodule are not fully known (2), the sequence of events is sufficiently complex so that the nematode could interfere with their development at a number of stages. These nematodes may secrete hydrolytic and oxidative enzymes or growth regulators which could play determinative roles in this inhibition of nodule development.

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Fig. 2. Responses of soybean nodular tissues to race 1 of *Heterodera glycines* (SCN). A) Necrotic tissue surrounding an apparently dead SCN larva in nodule on plant inoculated 30 days previously with SCN and 16 days after addition of *R. japonicum* (R) (× 450). B) Necrotic reaction of tissue adjacent to nodule showing degeneration of syncytium and poorly developed nematodes at 45 days after inoculation with R and 31 days after addition of SCN (× 72). C) Section of nodule showing large syncytium, bacterioids, and a poorly developed female 45 days after inoculation of plants with R and 31 days after the addition of SCN (× 130). D) Typical response of nodular tissues adjacent to bacteroids with tissue surrounding the larva as well as the latter dying (30 days after inoculation with R and 16 days after adding SCN) (× 140). E) An unusual section of a female and adjacent nodule developing normally on a plant 30 days after receiving R and 16 days after the addition of SCN (× 76). F) Almost mature female on plant 30 days after introduction or R and 16 days after the addition of SCN (× 76). F) Almost mature female on plant 30 days after introduction of R and 16 days after adding SCN (× 130); nec = necrotic plant tissue; nem = nematode; ba = bacteroids; sy = syncytium.