Influence of nematodes and light sources on growth and nodulation of soybean[1]

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Abstract: The influence of nematodes on nodulation of soybean varied according to their modes of parasitism. In the greenhouse, nodule formation was stimulated by the endoparasites, *Meloidogyne hapla* and *Pratylenchus penetrans,* but was inhibited slightly by the ectoparasite, *Belonolaimus longicaudatus.* In an experiment under controlled conditions in a phytotron, *Heterodera glycines* severely inhibited nodule formation, whereas plants inoculated with *B. longicaudatus* and *P. penetrans* had more nodules per g root than nematode-free plants. Nitrogen-fixing capacity, however, was inhibited by all three nematode species. Different light sources used in the phytotron experiment also influenced growth and nodulation of soybean. A fluorescent plus incandescent light regime resulted in plants with the greatest shoot weight, pod number, and nodules per g root. Plants grown under Lucalox lamps had excessive stem elongation. *Key Words: Meloidogyne* species, *Belonolaimus longicaudatus, Pratylenchus penetrans, Glycine max,* nitrogen fixation, *Rhizobium.*

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The inhibitory effects of plant-parasitic nematodes on nodulation of roots of leguminous plants has been the subject of several reports (1, 10, 13, 14). Plant-parasitic nematodes used in previous studies, however, were limited to sedentary endoparasites with most of the work concerned with the role of *Heterodera* species in these interactions. Although poor nodulation has been previously associated with severe infection by *H. glycines* (4, 9), Lehman et al. (10) recently showed that only certain races of this parasite greatly suppress nodula-

tion and N_2-fixation. Nematode-*Rhizobium* interactions, on the other hand, are not always antagonistic. Taha and Raski (13) reported that *H. trifolii* and *Meloidogyne javanica* reproduce readily in nodular tissues on white clover and have little effect on nodulation. Leguminous plants are good hosts for nematodes with other types of feeding habits. Interactions of ectoparasitic and migratory endoparasitic nematodes with *Rhizobium* species on legumes have not been investigated.

Growing certain leguminous plants under controlled conditions or during winter in greenhouses often requires supplemental light. The influence of different lighting systems on nodulation and growth of legumes is little known. Lie (11, 12) recently found that far-red light greatly inhibits nodulation on leguminous plants.

The objectives of this study were: to determine the interaction of ectoparasitic, migratory endoparasitic, or sedentary endoparasitic nematodes with *Rhizobium* species on soybean and to determine the influence of different light sources and intensities on growth and nodulation of soybean. A preliminary report of this work has been published (9).

MATERIALS AND METHODS

For the greenhouse experiment, 5-day-old 'Lee' soybeans [*Glycine max* (L.) Merr.] germinated in vermiculite, were transplanted individually to 1-liter plastic pots containing 35- and 65-mesh silica sand (1:1). Simultaneous inoculations included 200 mg of a commercial preparation of *Rhizobium japonicum* (Nitragin Company, Milwaukee, Wisconsin) combined with each of the following nematode population densities: *Belonolaimus longicaudatus* Rau (BL), 2,000; *Pratylenchus penetrans* (Cobb) Filip. and Schuur.-Stekh. (PP), 6,000; *Meloidogyne incognita* (Kofoid and White) Chitwood (MI), 7,000 eggs; and *M. hapla* Chitwood (MH), 10,000 eggs. Control plants were inoculated only with *Rhizobium*. Root-knot nematode eggs were collected as previously described (7). Supplemental light (800 ft-c from GE mercury and Lucalox lamps) were used to provide a 16-h day. All plants received one-half strength Hoagland's nutrient solution (6), minus nitrogen, as needed. Pots were flushed semiweekly with water. Treatments were replicated four times in a randomized block arrangement.

After 60 days, fresh shoot and root weights, nodule dry weight, and number of nodules per plant were recorded. Nitrogen-fixing capacity of nodules was determined by using the acetylene-ethylene assay (5). Excised root systems with attached nodules were used in the assays. Ethylene concentration was determined with an F and M Model 700 gas chromatograph equipped with an H_2-flame ionization detector. Chromatographic conditions were: column (180 cm x 3.1 mm) packed with 80/100 mesh Porapack N (Waters Associate, Inc.). Temperatures were: detector—250 C, injector—140 C and column—50 C. Helium was used as the carrier gas at 80 ml per min. Assays were conducted for 30 min and 100 μliters of the gas were analyzed.

For the phytotron experiment, 5-day-old 'Lee' soybeans, germinated in vermiculite, were transplanted singly at the time of inoculation to 15-cm diam clay pots containing the previously described mixture of silica sand. Nematodes and densities used were: BL, 3,000, PP, 5,000; and *H. glycines* Ichinohe (Race 1) (HG), 18,000 eggs. Plants were simultaneously inoculated with nematodes and 200 mg of *R. japonicum*. Treatments were replicated eight times in a randomized block arrangement. Plants were grown under five different light sources with the following output wattage: (i) two General Electric Lucalox lamps (Lu-2), 3,350 ft-c (at plant height), 175 w/m^2; (ii) four Lucalox lamps (Lu-4), 6,350 ft-c 340 w/m^2; (iii) five General Electric metal halide lamps (Mh), 4,400 ft-c, 185 w/m^2; (iv) five metal halide lamps plus four incandescent flood lamps (Mh + I), 4,650 ft-c, 250 w/m^2; and (v) 84 General Electric fluorescent plus 48 100-w incandescent bulbs (Fl + I), 4,800 ft-c, 260 w/m^2. The spectral emission of these light sources has been described (3). For the Mh + I, the metal halide lamps provided 77% of the input wattage and for the Fl + I, 49% of the input wattage was provided by the cool white fluorescent lamps. Light duration was 12 h during the vegetative stage and was reduced to 9 h at flowering. The air temperature was 19 C ± 1 during the dark period

and 25 C ± 1 during the light period. The chamber with Lu-2, however, was 2 to 3 C lower than the other chambers during the light period. Plants were irrigated daily with distilled water and fertilized with one-half strength Hoagland's nutrient solution, minus nitrogen, as needed. The experiment was terminated after 50 days and data were recorded as previously described.

RESULTS

Influence of nematodes on nodulation and plant growth: Influence of the nematode species on greenhouse-grown soybean varied according to their modes of parasitism (Table 1). Tops were stunted by MI, MH and BL but not by PP. Nodulation was stimulated by the endoparasites, MH, MI, and PP, but was inhibited slightly by the ectoparasite, BL. Nodules on plants with increased nodulation were smaller than nodules formed on plants free of nematodes, whereas, nodules on plants inoculated with BL were significantly larger and had increased nodular efficiency. Nitrogen-fixing capacity (μ moles C_2H_4/plant/h), however, was not greatly affected by any of the nematode species.

In the controlled environment in the phytotron, a more striking interaction occurred between the nematodes and *Rhizobium* on soybean than when plants were grown in the greenhouse (Table 1). In this experiment HG was used as the sedentary endoparasite in lieu of the *Meloidogyne* species. Shoot and root weights were significantly lower with all three nematode species with different feeding habits than in noninoculated controls. Nodule formation was suppressed on plants inoculated with HG and BL, with HG inhibiting nodulation by 95%. Nitrogen-fixing capacity, on the other hand, was significantly lowered by all three nematode species, although the nodular efficiency was only affected by HG. The few nodules that developed on the plants inoculated with HG were more efficient than nodules on the other plants. The loss in root weights on plants inoculated with BL and PP resulted in significantly more nodules per g root when compared with the plants free of nematodes.

Influence of light source and intensity on plant growth: The different light sources and intensities used in this study had variable effects on soybean growth. The Fl + I light source resulted in soybeans with the greatest shoot weight and number of pods, whereas the low intensity Lucalox lamps (Lu-2) resulted in plants with the lowest

TABLE 1. Effects of nematodes with different feeding habits on nodulation and growth of soybean.

Nematode[a]	Plant weights (g) Shoot	Root	Nodules no./plant	Nodules/ g root	Av. nodule dry wt. (mg)	Nitrogen-fixing capacity[b]	Nodule efficiency[c]
Experiment 1 (in Greenhouse)							
MI	28*	27	139*	5.0	4.4**	28.1	232
MH	28*	23	187**	8.6**	3.4**	33.0	180
PP	33	24	137*	6.0*	4.8*	22.6	176
BL	28*	22	61	3.0	8.6**	23.9	395*
Check	33	22	94	4.3	6.0	19.9	216
Experiment 2 (in Phytotron)							
BL	40**	34**	204**	6.1**	3.8	23.0**	133
HG	28**	31**	15**	0.4**	0.8**	2.8**	524**
PP	49**	46**	260	5.9*	4.1*	28.2**	133
Check	56	52	275	5.3	3.7	34.8	149

[a]MI = *Meloidogyne incognita;* MH = *M. hapla;* PP = *Pratylenchus penetrans;* BL = *Belonolaimus longicaudatus;* HG = *Heterodera glycines.*
[b]μ moles C_2H_4/plant/h.
[c]n moles C_2H_4/nodule/h.
*Significantly different from check at the 5% level.
**Significantly different from check at the 1% level.

shoot weight and number of pods (Table 2). Both intensities of the Lucalox lamps resulted in plants with excessive stem elongation when compared to plants grown under the other light sources. Root development was greatest on plants given the metal halide light source.

Nodulation was affected by light source but not intensity. The Fl + I supported greatest nodulation. The low nodule number on plants grown under the low-intensity Lucalox lamps was due to an overall smaller root system. A slight suppression of nodulation occurred on plants grown under the Mh + I light source. The higher nodulation with the Fl + I light source resulted in smaller nodules with lower N_2-fixing capacity. Nodules on plants grown under the high-intensity Lucalox lamps had the greatest N_2-fixing capacity.

DISCUSSION

Stimulation of nodule formation on leguminous plants by plant-parasitic nematodes has not been previously reported. Most of the earlier reports concerned the suppression of nodulation of legumes by pathogenic nematodes (1, 10, 13, 14). Taha and Raski (13), on the other hand, reported that *M. javanica* and *H. trifolii* had no influence on nodule number or size on white clover. The increase in nodule number on the legumes used in our study, however, was apparently not beneficial to the plant since the nodules were usually smaller and less efficient in N_2 fixation than nodules on comparable plants free of nematodes. When *Meloidogyne* species stimulated nodulation, nodules often developed on the surface of the root galls. This interaction was also observed in the study with white clover (13). The histopathology of these nematode-*Rhizobium* interactions is reported elsewhere (8).

The severe antagonistic interaction of *H. glycines* and *R. japonicum* observed in our study supports the earlier work with these two organisms (1, 10), although an even greater suppression of nodulation was observed in the present study in the phytotron.

Some differences were observed between the greenhouse and phytotron experiments. Although *P. penetrans* increased the number of nodules per g of root under both growing conditions, N_2-fixing capacity was inhibited only on plants grown in the phytotron. Also, about three times as many nodules developed on nematode-free plants grown in the phytotron as on plants grown in the greenhouse. This greater number resulted in the nodules being smaller and less efficient on plants grown in the phytotron.

Light source and intensity also had an influence on nodulation on soybean. Although soybeans grew best and had the greatest number of nodules under the Fl + I light source, these plants had the lowest

TABLE 2. Influence of light source and intensity on growth and nodulation of soybean.

Light Source[a]	Fresh plant weights (g) Shoot	Root	Plant height (cm)	Pods no./plant	Nodules no./plant	Nodules/ g root	Av. nodule dry wt. (mg)	Nitrogen-fixing capacity[b]	Nodule efficiency[c]
Lu-2	37	29	82	22	115	4.0	3.5	23.4	330
Lu-4	42	42	61	25	189	4.3	3.2	28.6	296
MH	44	45	29	29	174	3.8	3.4	20.4	135
MH + I	41	47	29	27	177	3.7	3.0	22.4	211
Fl + I	52	41	28	34	287	6.4	2.5	16.4	199
LSD									
.05	4	5	4	3	28	0.6	0.4	5.0	NS
.01	5	7	5	4	36	0.8	0.6	6.6	NS

[a]Total output for lights were: Lu-2 (2 Lucalox lamps) = 3,350 ft-c, 175 w/m^2; Lu-4 (4 Lucalox lamps) = 6,350 ft-c, 340 w/m^2; Mh (5 metal halide lamps) = 4,400 ft-c, 185 w/m^2; Mh + I (5 metal halide + 4 incandescent flood lamps) = 4,650 ft-c, 250 w/m^2; and Fl + I (84 fluorescent lamps + 48 100-w incandescent bulbs) = 4,800 ft-c, 260 w/m^2.
[b]μ moles C_2H_4/plant/h.
[c]n moles C_2H_4/nodule/h.

N_2-fixing capacity. Plants grown under this light source appeared physiologically older than those under the other light sources. Lie (11, 12), has found that far-red light suppressed nodulation on pea plants. This effect could be counteracted by exposure to red light which suggests that nodule formation might be controlled by the phytochrome system. In our system, the Fl + I light regime provided the least amount of far-red light, whereas the Lucalox lamps emitted the greatest amount of far-red light (3). Plants grown under the Lucalox lamps showed excessive stem elongation which is characteristic of plants whose shoots have been exposed to far-red light. Lie suggested that some of the inhibition of nodulation on plants with elongated stems may be due to altered distribution of carbohydrates, which are necessary for nodulation, to the shoot and the root (11).

In our study plants grown under the Lucalox lamps had more nodules per g root than those grown under light regimes involving the metal halide lamps. Similarly, the N_2-fixing capacity and nodule efficiency were greatest on plants grown under the Lucalox lamps. Neither the N_2-fixing capacity nor the nodule efficiency was studied by Lie (11).

The various effects that light sources used in our study had on nodulation and growth of soybean indicate the relative value of different types of supplemental light when used in greenhouse or growth-chamber studies with leguminous plants. Nematode reproduction was also reported to be influenced by these light sources (2). Effects of nematodes on nodulation can vary according to the feeding habits of the nematode, leguminous host plant, and environmental conditions. Race 1 of *H. glycines* apparently has a more drastic effect on nodulation of soybean than does any other nematode species and may be the only one to affect nodulation directly under field conditions (4, 9). Nodules infected with *Meloidogyne* species, however, are susceptible to earlier deterioration (13).

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