Rhizoctonia-Rhizobium Interactions in Relation to Yield Parameters of Soybean

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ABSTRACT

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Rhizoctonia solani significantly reduced top and nodule weights of Lee and Kent soybeans inoculated with *Rhizobium japonicum* and grown in a N-free sand-nutrient substrate as compared with plants grown with *Rhizobium* alone. For Lee soybeans, a 63% decrease in fixed N per plant due to the fungus was demonstrated. In *Rhizobium*-inoculated Kent soybeans grown in the presence of *Rhizoctonia* at either 15, 20, or 25 C, top and nodule weights and the total N content per plant were also reduced. Because resistance in soybeans to Rhizoctonia root rot is not presently available and the direct effects of this fungus on *R. japonicum* are unknown, this fungal pathogen may be a yield-limiting factor under a wide range of ecological conditions.

Additional key words: Thanatephorus cucumeris, symbiotic nitrogen fixation, legumes.

The parasitism of *Rhizoctonia solani* Kühn on soybean, *Glycine max* (L.) Merrill, was first studied by Boosalis (2). The survival of this fungus as affected by inoculum concentration and soil amendment was studied by Papavizas (7). The pathogen causes pre- and post-emergence damping-off, root rot and hypocotyl rot, and foliar blight of soybean (1, 5). Even though resistance in soybeans to Rhizoctonia root rot has not been identified (8) and significant reduction in the yield of some soybean cultivars has been reported from Iowa (10), the economic impact of this disease on soybean production needs further investigation.

Except for a preliminary report (6), the effect of *R. solani* on nodulation and N fixation in soybeans by *Rhizobium japonicum* (Kirchner) Buchanan, to our knowledge, has not been studied. Consequently, we investigated the manner in which the *Rhizoctonia-Rhizobium* interaction and the temperature of the plant's growing substrate may affect the yield parameters of this plant. Hopefully, these results will provide guidelines for elucidating the effect of this fungal pathogen on both the host plant and the N-fixing bacterium.

MATERIALS AND METHODS

Lee and Kent soybeans, maturity groups VI and IV, respectively, were used. The cultivars were moderately susceptible and susceptible (respectively) to Rhizoctonia root rot, as determined in preliminary greenhouse tests. *Rhizoctonia japonicum* isolate 110 (RJ), obtained from the U.S. Department of Agriculture Rhizobium Culture Collection, Beltsville, Maryland, and known to form effective nodules on soybean, was grown for 3 weeks in yeast extract-mannitol broth (pH 6.5) in either stationary or shake cultures at 30 C. The cell density of broth cultures used in sand inoculation was standardized at approximately 13×10^6 cells per gram of sand by means of a hemacytometer. The pathogenic isolate of *R. solani* IV (RS) was grown on potato-dextrose agar enriched with 5.0 g/liter casamino acids until sclerotia developed profusely in about 5 weeks. The density of sclerotial suspensions of RS was standardized to approximately 100 sclerotia per gram of sand.

Growing conditions and inoculation method.—Lee and Kent soybean plants were grown from surface-disinfested (1 minute in 95% ethyl alcohol and rinsed twice in sterile water) seed in medium-fine quartz sand with a modified Crone's N-free complete nutrient solution (4) in a Leonard's modified (3) bottle-jar assembly (Fig. 1) in the greenhouse. Kent soybean plants were grown in cylindrical drainless 25×12 cm porcelain crocks which were kept immersed to five cm from the rim

Fig. 1. Lee soybean plants grown in medium fine quartz sand with Crone's N-free nutrient solution in a Leonard bottle-jar assembly. Left, plants inoculated with *Rhizobium japonicum* strain 110; right, uninoculated plants.

in water tanks maintained at 15, 20, 25, or 30 C. Daily temperature fluctuation in the sand substrate was less than 3 C. Day and night ambient greenhouse temperatures were 23-20 ± 4 C. Plants in the sand-nutrient substrate either in the bottle-jar assembly or in the crocks received the following treatments: (i) inoculated with the standardized cell suspension of RJ; (ii) inoculated with the standardized sclerotial suspension of RS; (iii) inoculated with both the standardized suspension of RJ and of RS; and (iv) noninoculated. Plants in ii and iv were used as controls. Each treatment consisted of four replicated bottles or crocks with two plants per container. The nutrient solution was renewed once a week or as needed until harvest (50-60 days from plant emergence). Tops, roots, and nodules were harvested when the pods began to form, and fresh and oven-dry weights were determined.

Nitrogen determinations.—Oven-dried plant top tissues were pooled into a composite sample of all replicates for each treatment and cultivar, ground in a laboratory mill equipped with a 0.5-mm screen, and analyzed for total N content on the basis of mean dry tissue weight by means of a Coleman II Nitrogen Analyzer. The relative net amount of N fixed per plant in Lee soybean was determined by subtracting the total N content in the controls, as described in ii and iv, from the total N content in plants grown with RJ alone or in those grown with the RJ plus RS. Composite samples of Kent soybean grown at four temperatures were analyzed only for total N.

RESULTS

Rhizobium-Rhizoctonia interaction in relation to fixed nitrogen for Lee soybean.—Rhizoctonia root and hypocotyl infections of Kent and Lee soybeans, caused by artificial inoculation, are shown in Fig. 2. The symptoms of the disease were characterized by root hair decay, severe necrosis of lateral roots and the hypocotyl, and moderate to severe discoloration of the main root. Mean top and nodule weights per plant (oven-dry basis) of Lee soybean (Table 1) inoculated with RJ and grown in the N-free substrate in the presence of RS were significantly lower than those of plants grown with RJ alone. However, differences in root weights were not significant. The low top, root, and nodule weights for the control plants, namely those grown with *Rhizoctonia* alone and those noninoculated, resulted from root infection and/or N deprivation after the N supply of the cotyledons and in the fungal inoculum itself was exhausted. The N analysis of plant top tissue (Table 2) showed that plants grown with *Rhizobium* alone contained a greater amount of total N than plants grown with RJ plus RS. The calculated net amount of N fixed per plant was also higher for the RJ treatment. The relative net loss in N fixed due

TABLE 1. Yield parameters (tops, roots, and root nodules) of Lee soybean plants inoculated and noninoculated with *Rhizobium japonicum* and grown in sand with a N-free nutrient solution in the presence or absence of *Rhizoctonia solani* in the greenhouse

Treatment	Mean weight per plant (g) [y]		
	Tops	Roots	Root nodules
Rhizobium	2.00 a[z]	1.18 a	0.24 a
Rhizobium + *Rhizoctonia*	1.54 b	1.00 a	0.11 b
Rhizoctonia (Control)	1.00 c	0.82 ab	0 c
Noninoculated (Control)	0.71 d	0.57 b	0 c

[y]On a dry weight basis.
[z]Duncan's multiple range test: values followed by the same letter are not significant, $P = 0.05$.

TABLE 2. Nitrogen content of tops of Lee soybean plants inoculated and noninoculated with *Rhizobium japonicum* and grown in sand with a N-free nutrient solution in the presence or absence of *Rhizoctonia solani* in the greenhouse

Treatment	Grams of N per plant[a]		Relative fixed-N loss due to *Rhizoctonia* (g per plant)
	Total[b]	Fixed[b]	
Rhizobium	0.072	0.057	
Rhizobium + *Rhizoctonia*	0.043	0.021	0.036
Rhizoctonia	0.022	0	
Control	0.015	0	

[a]On a dry weight basis.
[b]Determined as described in "Materials and Methods".

Fig. 2. Root and hypocotyl infections incited by artificial inoculation of Kent (first plant on left) and Lee soybeans with *Rhizoctonia solani.*

to RS was 63% per plant. As expected, no fixed N was associated with plants grown in absence of RJ.

Rhizobium-Rhizoctonia interaction as affected by substrate temperature in relation to yield parameters of Kent soybean.—Data in Fig. 3 show that mean top weights (oven-dry basis) of Kent soybean grown with RJ alone at 15, 20, and 25 C were significantly greater than those of plants grown with the RJ-RS combination in the N-free sand substrate. Mean top weights for plants grown with RJ plus RS at these three temperatures differed significantly also and were lower than top weights for plants grown with RJ alone. These data also show that the highest top mean weight loss (0.71 g per plant), which occurred at 25 C, apparently was associated with the harmful effect of the fungus. Except for a small nodule weight increase at 30 C for plants grown with RJ plus RS, the fungus consistently reduced nodule weight at 15, 30, and 25 C.

Total N (grams of N per plant) of tops of plants grown with RJ alone was higher than for plants grown with RJ plus RS at 15, 20, and 25 C (Table 3). The loss in total N (0.020 g N per plant) due to RS at 25 C corresponded to losses in top and nodule weights as shown in Fig. 3 when treatments with RJ alone and with RJ plus RS at this temperature are compared. The increase in total N (0.003 g N per plant) in the latter treatment at 30 C may have resulted from late-forming, very small nodules that developed as the plant matured. All plants inoculated with RS in treatments grown in the absence of RJ as the only source of N, besides being stunted, were severely chlorotic. The RJ cell density used in these experiments was adequate as it allowed satisfactory nodulation. The RS inoculum at the sclerotial density used did not kill the plants even though the disease was severe enough to impair nodulation and plant growth. *Rhizoctonia solani* was reisolated from root lesions and the nodule surface.

DISCUSSION

Significant reduction in the yield parameters of the soybean plant; i.e., plant top, root, and nodule weights (oven-dry basis) per plant of Lee soybean, is demonstrated in plants inoculated with *Rhizobium japonicum* and grown in a defined N-free sand-nutrient substrate in the presence of *Rhizoctonia solani.* These yield reductions were associated with impaired plant growth brought about by root hair decay and secondary root infection, which apparently reduced main sites of N fixation on the root rhizoplane during the plant's vegetative growth.

Our results showed also that root hairs and lateral roots were more susceptible to fungal infection than the main root. Sloger et al. (9) demonstrated that lateral root nodules support nearly 60 percent of the $N_2(C_2H_2)$-fixing activity per plant per hour at the full-bloom stage and 80 percent at mid-bean development during the growth of the soybean plant. In our experiments, plants grown with RJ alone were larger and contained more N than smaller, stunted plants grown with RJ in the presence of RS. Although the net amount of N fixed by Kent soybean plants was not calculated, it is inferred that the net loss in fixed N due to RS was comparable to that for Lee soybeans.

The results of this investigation indicated also that

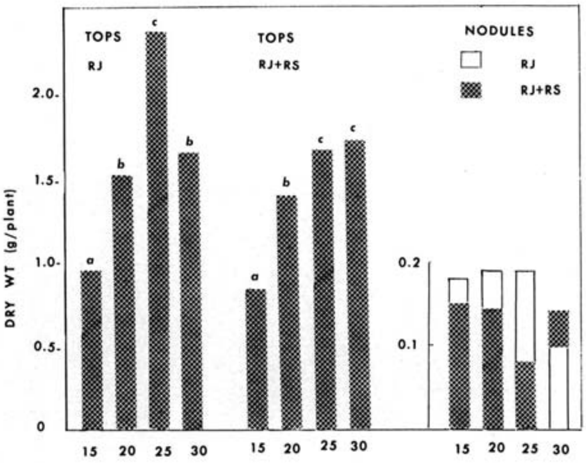

Fig. 3. Tops and nodule weights (oven-dry basis) of Kent soybean plants inoculated with *Rhizobium japonicum* isolate 110 (RJ) and grown in the absence or presence of *Rhizoctonia solani* isolate IV (RS) in sand with a N-free nutrient solution at 15, 20, 25, or 30 C. In each four-column group, columns with the same letters are not significantly different, $P = 0.05$ according to Duncan's multiple range test.

TABLE. 3. Total nitrogen content of plant tops of Kent soybean plants inoculated with *Rhizobium japonicum* and grown in a sand substrate with a N-free nutrient solution in the presence or absence of *Rhizoctonia solani* at four sand substrate temperatures

	N per plant (g)[a]			
Treatment	15 C	20 C	25 C	30 C
Rhizobium	0.033	0.049	0.057	0.054
Rhizobium + *Rhizoctonia*	0.025	0.045	0.037	0.057
Difference	0.008	0.004	0.020	(0.003)[b]

[a]On a dry weight basis.
[b]Weight increase.

plant growth, nodulation, and N fixation in soybeans are greatly influenced by the temperature of the plant's growing substrate. The importance of the effect of soil temperature on *Rhizobium* root nodules of soybeans was demonstrated by Weber and Miller (11). In our experiments top, root, and nodule weights (oven-dry basis) per plant were lower at 15 C than at 20 and 25 C for plants grown with RJ plus RS than were weights for plants grown in the N-free sand-nutrient substrate with RJ alone. The greatest reduction in top and nodule weight at 25 C, which is near the optimum for the growth of RS, is of biological significance in the epidemiology of the disease. Increased nodulation at 30 C indicated that the RJ remained viable in the sand substrate and was capable of causing late nodulation in plants approaching maturity. The present results demonstrate, therefore, that RS interferes with plant growth, N fixation, and yield in soybeans. These effects can be alleviated by nodulation by means of efficient strains of the symbiotic N-fixing bacterium. Under field conditions, the yield potential in soybeans would be affected by the tolerance or susceptibility of either or both the host plant and the *Rhizobium* strain to *Rhizoctonia* under a wide range of ecological conditions.

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