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Symbiotic Effectiveness and Host-Strain Interactions of *Rhizobium fredii* USDA 191 on Different Soybean Cultivars†

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Nodulation, acetylene reduction activity, dry matter accumulation, and total nitrogen accumulation by nodulated plants growing in a nitrogen-free culture system were used to compare the symbiotic effectiveness of the fast-growing *Rhizobium fredii* USDA 191 with that of the slow-growing *Bradyrhizobium japonicum* USDA 110 in symbiosis with five soybean (*Glycine max* (L.) Merr.) cultivars. Measurement of the amount of nitrogen accumulated during a 20-day period of vegetative growth (28 to 48 days after transplanting) showed that USDA 110 fixed 3.7, 39.1, 4.6, and 57.3 times more N_2 than did USDA 191 with cultivars Pickett 71, Harosoy 63, Lee, and Ransom as host plants, respectively. With the unimproved Peking cultivar as the host plant, USDA 191 fixed 3.3 times more N_2 than did the USDA 110 during the 20-day period. The superior N_2 fixation capability of USDA 110 with the four North American cultivars as hosts resulted primarily from higher nitrogenase activity per unit nodule mass (specific acetylene reduction activity) and higher nodule mass per plant. The higher N_2-fixation capability of USDA 191 with the Peking cultivar as host resulted primarily from higher nodule mass per plant, which was associated with higher nodule numbers. There was significant variation in the N_2-fixation capabilities of the four North American cultivar-USDA 191 symbioses. Pickett 71 and Lee cultivars fixed significantly more N_2 in symbiosis with USDA 191 than did the Harosoy 63 and Ransom cultivars. This quantitative variation in N_2-fixation capability suggests that the total incompatibility (effectiveness of nodulation and efficiency of N_2 fixation) of host soybean plants and *R. fredii* strains is regulated by more than one host plant gene. These results indicate that it would not be prudent to introduce *R. fredii* strains into North American agricultural systems until more efficient N_2-fixing symbioses between North American cultivars and these fast-growing strains can be developed. When inoculum containing equal numbers of USDA 191 and of strain USDA 110 was applied to the unimproved Peking cultivar in Perlite pot culture, 85% of the 160 nodules tested were occupied by USDA 191. With Lee and Ransom cultivars, 99 and 85% of 140 and 96 nodules tested, respectively, were occupied by USDA 110.

Bradyrhizobium japonicum nodulates and fixes nitrogen on soybean roots. It grows slowly, with a generation time of 8 to 20 h. In 1982, Keyser et al. (9) reported a number of strains isolated from a Chinese soybean cultivar Peking (*Glycine max* (L.) Merr.) and from *Glycine soja* (L.) Sieb. and Zucc., the wild soybean. These strains grow more rapidly, with a generation time of 2 to 4 h, and have been classified as a new species, *Rhizobium fredii* (18). Generally, *R. fredii* strains fail to form effective symbioses on American soybean cultivars (9). An exception is strain USDA 191 (24), which has been reported to be as effective as *B. japonicum* 61A76 on three soybean cultivars (Maple Arrow, Harosoy 63 and PI840-7-30) and *G. soja* (PI342-619B) by Hattori and Johnson (6). However, extremely low nodule numbers, masses, and acetylene reduction values were reported in this study relative to other values reported for *R. fredii* (11, 20, 24). To clarify the question of the symbiotic effectiveness of *R. fredii* USDA 191 compared with that of the *B. japonicum* strains, the symbiotic effectiveness of USDA 191 was compared with that of *B. japonicum* USDA 110 on five soybean cultivars (Lee, Ransom, Harosoy 63, Pickett 71, and Peking). Nodulation, nitrogenase activity, plant growth, nitrogen accumulation and concentration, and xylem sap nitrogen composition were considered in the comparison of symbiotic effectiveness. Additional data were also collected on the competition between USDA 191 and USDA 110 on three soybean cultivars (Ransom, Lee, and Peking), and these were compared with published data on the competition of fast-growing strains with slow-growing *B. japonicum* Ag 39 and Ag 89 (15).

MATERIALS AND METHODS

Bacterial strains, media, and host plants. *R. fredii* USDA 191 was obtained from H. Keyser (U.S. Department of Agriculture, Beltsville, Md.), and *B. japonicum* USDA 110 was obtained from L. D. Kuykendall (U.S. Department of Agriculture, Beltsville, Md.). Strain USDA 191 was grown in yeast extract-mannitol broth (22) and strain USDA 110 was grown in yeast extract-gluconate broth (10). Seeds of soybean cultivars Lee, Ransom, Pickett 71, and Peking were obtained from J. Burton (U.S. Department of Agriculture and Department of Crop Science, North Carolina State University, Raleigh) and seeds of Harosoy 63 were obtained from R. Bernard (U.S. Department of Agriculture and Department of Agronomy, University of Illinois, Urbana, Ill.).

Symbiotic tests. Plant tests were conducted on five soybean cultivars: Lee, Pickett 71, Harosoy 63, Ransom, and

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Peking. A randomized complete block design was used with sampling dates of 28 and 48 days after inoculation and transplanting. Each strain by cultivar by sampling date combination was randomized within each of four blocks. Inocula were grown to stationary phase (3×10^9 to 4×10^9 CFU/ml). Plants were pregerminated for 72 h at 30°C and 95% relative humidity, and their roots were dipped in inoculum just before transplantation. Immediately after transplantation, 2.5 ml of inoculum was applied to the base of each seedling. Two seedlings were transplanted into each 6-liter pot. At 14 days after transplantation, the seedlings were thinned to one per pot.

The plants were grown hydroponically in sterile horticultural Perlite which was amended by the addition of 300 g of $CaCO_3$ in the form of oyster shells to control the acidification of the rhizosphere (7). The pots were flushed with tap water and supplied nitrogen-free nutrient solution daily. The composition of the nitrogen-free nutrient solution was as described by McClure and Israel (13), except that 1.0 mM KH_2PO_4 was the sole source of phosphorus and the initial solution pH was 6.2. Experiments were conducted during the winter and spring of 1985 in a greenhouse. Natural light intensity was supplemented for 18 h each day with metal halide lamps that provided 150 microeinsteins $\cdot m^{-2} \cdot s^{-1}$ of photosynthetically active radiation at pot level. The 18-h photoperiod was sufficient to prevent the flowering of all cultivars.

Acetylene reduction assays were performed on the excised root systems of 28-day-old plants by the methods of Sloger (19). After incubation, the nodules were removed from the root systems, counted, and weighed. Nitrogenase activities were estimated as micromoles of ethylene per hour per plant and per gram (fresh weight) of nodule tissue.

Plants that were 28 days old were separated into shoot, root, and nodule fractions, and plants that were 48 days old were separated into leaflet, stem plus petiole, and root plus nodule fractions. All plant material was dried at 65°C for 72 h, weighed, and ground to pass a 1-mm screen. The nitrogen content of dried tissue was determined by using a semi-micro Kjeldahl procedure (4, 16).

Xylem sap was collected from 48-day-old plants between 0900 and 1230 h for 20 min after the sap began to exude from the base of the cut stem (13). Total nitrogen and ureide nitrogen concentrations in the xylem sap were determined by using a modified Kjeldahl procedure (13) and by the Rimini-Schryver reaction (25), respectively.

At both the 28- and 48-day harvests, two nodules from each plant were surface sterilized, crushed in sterile distilled water, and streaked onto yeast extract-mannitol broth plates containing bromthymol blue (pH 6.7) to characterize inocula as either acidic or basic in reaction (21, 22). The nodules from plants inoculated with USDA 191 exhibited an acidic reaction that changed the medium from green to yellow, and nodules from plants inoculated with USDA 110 exhibited a basic reaction that changed the medium from green to blue. These tests indicated that all plants were nodulated by the appropriate bacterial strain. In addition to these tests, three putative USDA 191 cultures from these plants were shown to have the appropriate plasmid profile (24), and three putative USDA 110 cultures were shown to have restriction patterns similar to those of USDA 110 derivatives I and L1-110 (12).

Competition between USDA 191 and USDA 110. All competition experiments were performed with cultures of approximately equal CFU/ml (3×10^9 to 4×10^9 cells per ml). USDA 191 (50 ml) was mixed with USDA 110 (50 ml). The seedlings were then dipped, and 2.5 ml of additional inocula per plant was added as described in symbiotic tests. Plant culture was carried out as described for symbiotic tests. Then, 24 to 32 days after transplantation, at least 100 randomly chosen nodules from at least five plants were crushed and plated onto yeast extract-mannitol broth plates containing bromthymol blue (21, 22) and on plates containing 2 μg of rifampin per ml, which is inhibitory to USDA 191. Plates were then scored as USDA 191 on the basis of an acidic reaction, as USDA 110 on the basis of a basic reaction, or as double infections on the basis of an acidic reaction and the ability to grow on rifampin.

Additional data were also collected on the ability of USDA 191 or USDA 110 to inhibit the growth of one another. Sterile antibiotic disks (diameter, 0.7 cm) with 5, 10, 20, and 25 μl of stationary-phase culture of USDA 110 and USDA 191 were tested in duplicate for inhibition of one another when spread as a bacterial lawn on yeast extract-mannitol broth plates. Nodule extracts were also made by crushing nodules in 0.2 ml of sterile H_2O and then applying 10, 20, and 25 μl of the resulting solution to a sterile 0.7-cm disk. Each cultivar-strain combination of USDA 191 and USDA 110 discussed in symbiotic tests was tested against spread plates of the other strain, either USDA 191 or USDA 110.

Analysis of data. Data were subjected to analysis of variance by using the Statistical Analysis System (5). Strains, sampling dates, and cultivars were designated as fixed effects for statistical tests.

RESULTS

Nodulation and nitrogenase activity. Significant host plant, bacterial strain, and host plant-bacterial strain interaction effects were observed for all symbiotic parameters measured (Table 1). When cultivar Peking was the host plant, USDA 191 was superior to USDA 110 for nodule number, nodule mass, and total acetylene reduction activity per plant. Nodules formed by these strains had equal specific acetylene reduction activities, but mass per nodule was higher for USDA 110 than it was for USDA 191. The higher mass per nodule for USDA 110 was associated with an almost fourfold reduction in nodule number per plant relative to USDA 191.

Strain USDA 110 was superior to strain USDA 191 for nodule mass and total acetylene reduction activity per plant with all four North American cultivars as host plants and for specific acetylene reduction activity with Lee, Harosoy 63, and Ransom as host plants. With cultivars Pickett 71, Harosoy 63, and Lee, USDA 110 was superior to USDA 191 for nodule number per plant (Table 1). The two strains produced similar numbers of nodules on the Ransom cultivar and similar mass per nodule on Pickett 71, Harosoy 63, and Lee cultivars. USDA 110 produced significantly larger mass per nodule on the Ransom cultivar than did USDA 191. When USDA 191 was the bacterial symbiont, differences in total and specific acetylene reduction activity among the four North American cultivars were not statistically significant, although Pickett 71 and Ransom tended to have the highest and lowest activities, respectively.

Dry weight and nitrogen accumulation. Highly significant host plant, bacterial strain, and host plant-bacterial strain interactions were observed for whole-plant dry weight accumulated by 28 and 48 days after transplanting (DAT) and between 28 and 48 DAT (Table 2). With Peking as the host plant, USDA 191 was superior to USDA 110 for whole-plant dry matter accumulated by 28 and 48 DAT. On the other hand, USDA 110 was superior to USDA 191 for these parameters when the four North American cultivars were

TABLE 1. Nodulation and acetylene reduction activity of *R. fredii* USDA 191 and *B. japonicum* USDA 110 in association with various soybean cultivars[a,b]

Strain	Cultivar	Nodule no. per plant	Nodule mass (g fresh wt · plant^{-1})	Mass per nodule (mg fresh wt)	Acetylene reduction	
					Total activity (μmol of C_2H_4 · plant^{-1} · h^{-1})	Sp. act. (μmol C_2H_4 · gfw^{-1} · h^{-1})
USDA 191	Peking	87	1.34	15.4	20.8	15.5
USDA 110	Peking	23	0.73	31.7	11.7	16.0
USDA 191	Pickett 71	74	1.22	16.5	12.8	10.5
USDA 110	Pickett 71	112	1.79	16.0	27.0	15.1
USDA 191	Harosoy 63	40	0.56	14.0	4.6	8.2
USDA 110	Harosoy 63	88	1.38	15.7	24.4	17.7
USDA 191	Lee	72	1.30	18.0	11.0	8.5
USDA 110	Lee	150	2.60	17.3	49.1	18.9
USDA 191	Ransom	89	0.82	9.2	3.9	4.7
USDA 110	Ransom	107	1.76	16.4	34.3	19.5

[a] Plants were sampled 28 days after transplanting.
[b] Since significant cultivar-strain interactions were obtained, least significant differences can be used to compare any two treatment means. Least significant difference at P = 0.05: nodule number, 32; nodule mass, 0.36 g; mass per nodule, 4.4 mg; total activity, 13.0 μmol; specific activity, 6.4 μmol.

host plants (Table 2). There was significant variation for these parameters among the four North American cultivars when USDA 191 was the bacterial symbiont. Pickett 71 and Lee exhibited significantly more growth than did Harosoy 63 and Ransom with USDA 191 as the bacterial symbiont.

Host plant and bacterial strain effects on whole-plant nitrogen accumulated by 28 and 48 DAT and between these dates are presented in Table 3. Since plants were grown in a nitrogen-free system, nitrogen accumulation was entirely due to N_2 fixation. With Peking as the host plant, USDA 191 was superior to USDA 110 for N_2 fixed by 28 and 48 DAT. With the four North American cultivars as host plants, USDA 110 was superior to USDA 191 for these parameters. There was also significant variation among the North American cultivars for both parameters when USDA 191 was the nodule symbiont. Pickett 71 and Lee exhibited significantly more N_2-fixation capability with strain USDA 191 than did Harosoy 63 and Ransom (Table 3).

Host plant, bacterial strain, and host plant-bacterial strain interaction effects were highly significant for concentration of nitrogen in different plant tissues (data not shown). With Peking as the host plant, USDA 191 was superior to USDA 110 for all of these parameters except root plus nodule nitrogen concentration at 48 DAT. The converse was true for the four North American cultivars. There was also significant variation among the four North American cultivars for nitrogen concentration parameters when USDA 191 was the nodule symbiont, with Pickett 71 and Lee being superior to Harosoy 63 and Ransom.

Nitrogen transport. Bacterial strain exhibited significant effects on the total nitrogen and ureide nitrogen concentrations and on the exudation rate of total nitrogen in the xylem sap of 48-day-old plants (Table 4). The bacterial strain did not significantly affect the relative ureide nitrogen content of the xylem sap. Host-plant cultivar had no significant effects on any of the nitrogen transport parameters (Table 4).

Competition experiments. When approximately equal numbers (3×10^9 to 4×10^9 CFU/ml) of USDA 191 and USDA

TABLE 2. Comparison of dry weight accumulated between 28 and 48 DAT for various soybean cultivars nodulated by *R. fredii* USDA 191 and *B. japonicum* USDA 110[a]

Strain	Cultivar	Dry wt (g · plant^{-1}) after:			Δ wt USDA 110 / Δ wt USDA 191
		28 days	48 days	Δ wt	
USDA 191	Peking	2.9	34.5	31.6	0.4
USDA 110	Peking	1.5	14.9	13.4	
USDA 191	Pickett 71	2.1	17.4	15.3	2.8
USDA 110	Pickett 71	3.6	47.2	43.6	
USDA 191	Harosoy 63	1.6	3.3	1.7	18.9
USDA 110	Harosoy 63	3.4	35.1	31.7	
USDA 191	Lee	2.0	13.3	11.3	3.5
USDA 110	Lee	4.8	44.0	39.2	
USDA 191	Ransom	1.4	2.7	1.3	26.2
USDA 110	Ransom	3.2	36.8	33.6	

[a] Since significant strain-cultivar interactions were obtained, least significant differences can be used to compare any two treatment means. Least significant difference at P = 0.05: dry weight (28 days), 0.6 g; and dry weight (48 days), 6.9 g. No statistical parameters are given for Δ weight (dry weight) and Δ weight (USDA 110/USDA 191) because values were calculated by subtracting treatment means.

TABLE 3. Comparison of the amount of nitrogen accumulated between 28 and 48 DAT for various soybean cultivars nodulated by *R. fredii* USDA 191 and *B. japonicum* USDA 110[a]

Strain	Cultivar	N accumulation (g · plant^{-1}) after:			ΔN USDA 110 / ΔN USDA 191
		28 days	48 days	ΔN	
USDA 191	Peking	0.099	1.264	1.165	0.3
USDA 110	Peking	0.047	0.400	0.353	
USDA 191	Pickett 71	0.050	0.487	0.437	3.7
USDA 110	Pickett 71	0.133	1.757	1.624	
USDA 191	Harosoy 63	0.029	0.060	0.031	39.1
USDA 110	Harosoy 63	0.114	1.326	1.212	
USDA 191	Lee	0.047	0.378	0.331	4.6
USDA 110	Lee	0.176	1.698	1.522	
USDA 191	Ransom	0.026	0.049	0.023	57.3
USDA 110	Ransom	0.112	1.430	1.318	

[a] Since significant cultivar by strain interactions were obtained, least significant differences can be used to compare any two treatments means. Least significant difference at P = 0.05: N accumulation (28 days), 0.017g; (48 days), 0.242 g. No statistical parameters are given for ΔN (N accumulation) and ΔN (USDA 110/USDA 191) because values were calculated by subtracting treatment means.

110 were inoculated onto the North American cultivars Lee and Ransom grown in Perlite, a significantly higher proportion of nodules was formed by USDA 110 (Table 5). Conversely, USDA 191 formed a significantly higher proportion of nodules than did USDA 110 with Peking as the host plant. Disks of USDA 110 and USDA 191 containing up to 25 μl of stationary-phase culture did not inhibit the growth of the opposite strain when applied to a lawn of bacteria. Also, disks containing up to 25 μl of extracts of nodules formed by all possible combinations of host plant and bacterial strain did not inhibit the growth of the opposite strain.

DISCUSSION

Nodulation, acetylene reduction activity, and total nitrogen and dry matter accumulation by nodulated plants growing in a nitrogen-free culture system were used to evaluate the symbiotic effectiveness of different host plant-bacterial strain symbioses. With four North American cultivars as host plants, the symbiotic effectiveness of *B. japonicum* USDA 110 was superior to that of *R. fredii* USDA 191 (Tables 1 through 3). This result is in agreement with the report that the symbiotic effectiveness, as measured by total and specific acetylene reduction activities, of USDA 110 was superior to that of USDA 191 (24). On the other hand, Hattori and Johnson (6) have reported that on the basis of total and specific acetylene reduction activities, USDA 191 and another slow-growing strain, *B. japonicum* 61A76, had similar symbiotic effectiveness with three North American cultivars (Maple Arrow, Harosoy 63 and PI840-7-30) as host plants. This apparent discrepancy may be related to the nutritional status of the host plants, but unfortunately, dry-weight data were not given in the latter study (6). Total and specific acetylene reduction activity in this study and in the study of Yelton et al. (24) was 10-fold or higher than that in the study of Hattori and Johnson (6). This suggests poor plant growth in the latter study. It seems likely that some factor or factors other than N_2-fixation capability may have limited plant growth in the study of Hattori and Johnson (6) and hence prevented the expression of differences in the symbiotic effectiveness of the two strains.

While it was possible to elucidate on the basis of acetylene reduction activity a gross difference in the N_2-fixation capabilities of USDA 191 and USDA 110 in symbiosis with four North American cultivars, the assay was not sensitive enough to establish significant differences in the N_2-fixation capabilities of the four North American cultivar-USDA 191 symbioses (Table 1). Whole-plant nitrogen accumulation by 28 and 48 DAT and between these dates indicated large and highly significant differences in the N_2-fixation capabilities of the USDA 191-North American cultivar symbioses (Table 3). These results indicate that nitrogen accumulation by nodulated plants growing in a nitrogen-free culture system is a much more reliable and sensitive measure of the N_2-fixation capabilities of symbiotic systems than are acetylene reduction assays at a single time point. Therefore, such assays should not be used as the sole criterion for assessing the N_2-fixation capability of symbiotic systems.

The symbiotic superiority of USDA 191 with Peking as

TABLE 4. Nitrogen composition of xylem sap from various 48-day-old soybean cultivars nodulated by *R. fredii* USDA 191 and *B. japonicum* USDA 110[a]

Strain	Cultivar	Concn		% Total N as ureide	Exudation rate of total N (μg · min^{-1})
		Total N (μg · ml^{-1})	Ureide N (μg · ml^{-1})		
USDA 191	Peking	384	257	66.9	25.0
USDA 110	Peking	281	216	76.9	8.3
USDA 191	Pickett 71	157	110	70.1	5.8
USDA 110	Pickett 71	309	198	64.1	24.1
USDA 191	Harosoy 63	ND[b]	ND	ND	ND
USDA 110	Harosoy 63	314	215	68.5	19.8
USDA 191	Lee	100	78	78.0	3.1
USDA 110	Lee	328	218	66.5	26.8
USDA 191	Ransom	ND	ND	ND	ND
USDA 110	Ransom	326	210	64.4	29.4

[a] Since significant cultivar-strain interactions were obtained, least significant differences can be used to compare any two treatment means. Data for cultivars Peking, Pickett 71, and Lee were used in the analysis of variance. Least significant differences at $P = 0.05$: total N, 88 μg; ureide N, 39 μg; percent total N as ureide, not significant; and exudation rate of total N, 11.2 μg.

[b] ND, not determined because insufficient sap was obtained for chemical analysis.

host plant (Tables 1 through 3) was associated with a 1.8-fold higher nodule mass per plant relative to USDA 110. This greater nodule mass was a consequence of a 3.8-fold higher nodule number per plant and a 2-fold lower average mass per nodule (Table 1). The specific acetylene reduction activities of nodules formed by the two strains in symbiosis with Peking were the same (Table 1). Thus, the nodule number was the symbiotic property that limited the N_2-fixation capability of the USDA 110-Peking symbiosis relative to the USDA 191-Peking symbiosis.

With all four North American cultivars, a higher N_2-fixation capability of USDA 110 relative to USDA 191 (Table 3) resulted from significantly higher specific acetylene reduction activities and nodule mass per plant (Table 1). With Pickett 71, Harosoy 63, and Lee cultivars as host plants, higher nodule mass per plant for USDA 110 resulted almost entirely from higher nodule numbers per plant (Table 1). With the Ransom cultivar as the host plant, higher nodule mass per plant for USDA 110 resulted almost entirely from a higher average mass per nodule (Table 1).

Differences in acetylene reduction activity per unit nodule mass imply differences in nitrogenase specific activities. Such differences in nitrogenase activity could result from differences in the ratio of bacteroidal to plant tissue in the nodules or from differences in nitrogenase activity per unit of bacteroid. *Lotus pedunculatus* has been shown to have a higher specific acetylene reduction activity in symbiosis with the slow-growing *Bradyrhizobium lotus* CC8145 than in symbiosis with the fast-growing *Rhizobium loti* NZP2037 (17). A morphometric study of nodules formed by these two strains showed that the degree of infection and bacterial proliferation in the host tissue partially accounts for measured differences in the specific acetylene reduction activity (23). Microscopic studies are required to determine whether this phenomenon can account for differences in the specific acetylene reduction activity of the *R. fredii* USDA 191- and *B. japonicum* USDA 110-North American soybean cultivar symbioses.

All *B. japonicum*-North American soybean cultivar symbioses tested have been shown to transport high relative concentrations of ureide (70 to 90% of total nitrogen) in the xylem sap when grown under nitrogen-free conditions (8, 13, 14). The relative ureide concentration of xylem sap is a good indication of the expression of the ureide formation pathway in soybean nodules. While genetic information for enzymes of the ureide formation pathway is presumably encoded by the host plant genome (1, 3), the infecting bacterial strain could influence the expression of these genes. The availability of two different genera of bacteria that nodulate the same host plant provides an opportunity for examination of the effects of bacterial strains with markedly different genetic backgrounds on the expression of the ureide formation pathway in soybean nodules. In this study, the bacterial strain had no significant effect on the relative ureide concentration in xylem sap from three host plant cultivars, Peking, Pickett 71, and Lee (Table 4). This result suggests that the host plant has relatively tight control over the expression of the ureide formation pathway in soybean nodules.

Sanogho and Keyser showed that two fast-growing *R. fredii* strains (USDA 205 and USDA 208) were more competitive for nodule occupancy on cultivar Peking than were two slow-growing *B. japonicum* strains (USDA 110 and USDA 122) in a field soil free of indigenous rhizobia (Agron. Abstr., p. 196, 1982). Also, the slow-growing strains were more competitive than were the fast-growing strains for nodule occupancy on Lee. Similar results were obtained in this study. USDA 110 was more competitive for nodule occupancy on Lee and Ransom than was USDA 191, while the opposite result was obtained when Peking was the host (Table 5). McLoughlin et al. (15) observed that *B. japonicum* strains (Ag 89 and Ag 39) were more competitive for nodule occupancy on both the North American cultivar Jacques 130 and the unimproved cultivar Peking than were eight *R. fredii* strains. These experiments were performed in growth pouches at inoculum ratios up to 10-fold in favor of the *R. fredii* strains. Thus, the slow-growing *B. japonicum* strains are consistently more competitive for nodule occupancy on the North American soybean cultivars than are the fast-growing *R. fredii* strains. The apparent lack of production of bacterial growth inhibitors by free-living cultures of USDA 110 and USDA 191 or by the interaction of these strains and host plants during nodule development is consistent with the conclusion that differences in competitiveness were due to competition for sites on the root surface.

TABLE 5. Competition between *R. fredii* USDA 191 and *B. japonicum* USDA 110 on three soybean cultivars[a]

Cultivar	No. of plants[b]	No. of nodules screened	% Nodule occupancy of:		
			USDA 191	USDA 110	Mixed
Ransom	5	96	15	85	0
Lee	10	140	1	99	0
Peking	10	160	85	14	1

[a] Nodules from Lee and Ransom were screened 24 days after transplanting and nodules from Peking were screened 32 days after transplanting.

[b] All individual plants were not significantly different from the pooled values.

Devine (2) reported that the effective nodulation of the unimproved Peking cultivar by *R. fredii* USDA 205 was conditioned by a single recessive allele. He cautioned, however, that the discovery of this gene does not necessarily imply that the total incompatibility (including both the effective nodulation and efficiency of N_2-fixation) of North American cultivars with *R. fredii* strains is controlled by a single gene. Other genes may, therefore, condition incompatibility at other stages of nodule development and function. The wide range in symbiotic N_2 fixation capabilities of the North American cultivar-USDA 191 symbioses (19-fold) (Table 3) observed in this study is consistent with the notion that the total incompatibility of host and *R. fredii* strains is regulated by more than one plant gene. Since *R. fredii* USDA 191 is not as efficient in fixing N_2 in symbiosis with North American soybean cultivars as is *B. japonicum* USDA 110, it would not be prudent to introduce fast-growing *R. fredii* strains into North American agricultural systems until more efficient N_2-fixing symbioses can be developed.

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