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Influence of Environmental Factors on Interstrain Competition in *Rhizobium japonicum*†

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The effect of several biotic and abiotic factors on the pattern of competition between two strains of *Rhizobium japonicum* was examined. In two Minnesota soils, Waseca and Waukegan, strain USDA 123 occupied 69% (Waseca) and 24% (Waukegan) of the root nodules on *Glycine max* L. Merrill cv. Chippewa. USDA 110 occupied 2% of the root nodules in the Waseca soil and 12% of the nodules in the Waukegan soil. Under a variety of other growth conditions-vermiculite, vermiculite amended with Waseca soil, and two Hawaiian soils devoid of naturalized *Rhizobium japonicum* strains-USDA 110 was more competitive than USDA 123. The addition of nitrate to or the presence of antibiotic-producing actinomycetes in the rhizosphere of soybeans did not affect the pattern of competition between the two strains. However, preexposure of young seedlings to USDA 110 or USDA 123 before transplantation into soil altered the pattern of competition between the two strains significantly. In the Waseca soil, preexposure of cv. Chippewa to USDA 110 for 72 h increased the percentage of nodules occupied by USDA 110 from 2 to 55%. Similarly, in the Hawaiian soil Waimea, nodule occupancy by USDA 123 increased from 7 to 33% after a 72-h preexposure.

In many soils of the North Central United States, inoculations of soybeans with highly effective strains of *Rhizobium japonicum* have failed to increase yields (for review, see reference 3). This is thought to be at least partly due to the failure of the inoculant strains to displace highly competitive, yet often less efficient, indigenous strains from the nodules. In particular, *R. japonicum* serogroup USDA 123 is among the most successful of the native strains in these soils (13).

Several studies have attempted to correlate the nodulating success of serogroup USDA 123 with various soil parameters (for review, see reference 13). The presence of *R. japonicum* serogroup USDA 123 in nodules recovered from field-grown soybeans has been found to be affected by planting dates (7) and by soil pH (8, 14). Attempts to correlate the distribution of USDA 123 with organic nitrogen levels in soils have been inconclusive. In one study, a negative correlation with organic nitrogen was observed (3), although in another study, the addition of combined nitrogen had no effect on nodule occupancy by USDA 123 (24). The competitive success of USDA 123 was not found to be related to its ability to colonize the root surfaces of fieldgrown soybeans (22). The susceptibility of USDA 123 to antimicrobial activity in soil was also negligible (9).

In this study, the competitive abilities of *R. japonicum* strains USDA 110 and USDA 123 were examined under a variety of environmental conditions. USDA 110 was chosen because of its frequent use as a commercial inoculant strain (6). However, inoculation with USDA 110 does not always result in yield increases because of its failure to form a significant proportion of nodules on soybeans when serogroup USDA 123 is present in the soil (13).

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MATERIALS AND METHODS

Bacterial cultures. Cultures of *R. japonicum* strains USDA 110, USDA 123, USDA 138, and USDA 31 were obtained from E. L. Schmidt, University of Minnesota, St. Paul, Minn. *R. japonicum* strain CB1809 was obtained from the NifTAL (Nitrogen Fixation in Tropical Agricultural Legumes) Project, Paia, Hawaii. The ineffective strain SM-5 was obtained from W. Brill, University of Wisconsin, Madison. Nodule isolates representative of serogroups USDA 110 and USDA 123 were obtained as described by Vincent (27). *Rhizobium* cultures were maintained on yeast extract-mannitol (YEM [5]) slants at 4°C. For inoculation, 1.0 ml of 4-day-old broth cultures was used. Soil bacteria and actinomycetes associated with soybean roots, grown in Waseea soil, were isolated by the methods of Waksman (28). Stock cultures were maintained on tryptone-yeast extract (TY [16]) slants, containing 1.0 g of arginine per liter.

Soils. Two Minnesota soils, Waseca and Waukegan, were obtained from E. L. Schmidt. Both soils are mollisols and contain a background population of indigenous rhizobial strains, including *R. japonicum* serogroups USDA 110 and USDA 123. Two Hawaiian soils, Waimea and Kawaihae, were collected from the island of Hawaii. A third Hawaiian soil was obtained from the Mauka Field Station, University of Hawaii, Oahu, Hawaii. Waimea soil is an inceptisol and is devoid of indigenous *R. japonicum* strains. The Kawaihae soil is classified as an aridisol and also lacks indigenous *R. japonicum* strains. The soil from the Mauka Field Station is also an inceptisol and contains a background population of *R. japonicum* strains.

Soybean cultivars. Seeds of *Glycine max* L. Merrill cv. Lee were obtained from H. H. Keyser, U.S. Department of Agriculture, Beltsville, Md. Soybean seeds of cv. Chippewa 64 were obtained from E. L. Schmidt. Soybean seeds of cv. Davis were obtained from the NifTAL Project. For all of the experiments, seeds were surface sterilized for 20 min in 4% calcium hypochlorite and washed five times in sterile water.

TABLE 1. Effect of abiotic or biotic components (Waseca soil) on the pattern of competition between *R. japonicum* strains USDA 110 and USDA 123[a]

Substratum	Treatment		Mean % total nodules (±SE) composed of[b]:			
	Amendment	Inoculum	USDA 110	USDA 123	USDA 110 ± USDA 123	Other
Waseca soil	None	None	2 (±1)	70 (±1)	2 (±1)	26 (±1)
Waseca soil	None	USDA 110	15 (±1)	60 (±1)	9 (±1)	16 (±1)
Vermiculite	None	USDA 110 + USDA 123	53 (±16)	31 (±6)	16 (±16)	None
Vermiculite	1 g of Waseca soil	None	34 (±12)	24 (±12)	5 (±4)	37 (±4)
Vermiculite	1 g of Waseca soil	USDA 110 + USDA 123	69 (±9)	10 (±3)	0	21 (±6)

[a] Strains USDA 110 and USDA 123 were culture strains obtained from E. L. Schmidt.
[b] The pattern of competition between USDA 110 and USDA 123 was not affected by the addition of gamma-irradiated or heat-treated Waseca soil. Results are for three replications.

The seeds were pregerminated either on water agar plates or in sterile vermiculite.

Strain identification. The percentage of nodules occupied by each strain was determined by immunofiuorescence. At least 25% of the nodules from each replicate were identified. Nodule smears were stained with strain-specific fluorescent antibodies by the method of Schmidt et al. (23). Gelatinrhodamine isothiocyanate was used to suppress nonspecific adsorption (4). Microscopy was done as described previously (20).

Effect of rhizosphere microorganisms on competition. The sensitivity of USDA 110 and USDA 123 to antimicrobial products formed by soybean rhizosphere bacteria and actinomycetes was examined on TY-arginine plates by using the cross-streaking method (28). The root isolates were inoculated either 1 or 2 days before inoculation of USDA 110 and USDA 123. Plates were incubated at 28°C. Controls consisted of plates inoculated with sterile distilled water in place of the root isolates.

The effect of four antibiotic-producing actinomycete isolates on the pattern of competition between USDA 110 and USDA 123 was examined in vermiculite. Uniform, 2-day-old seedlings of cv. Chippewa were planted in sterile vermiculite moistened with 1:4 Hoagland nitrogen-free solution (17) in 250-ml Erlenmeyer flasks. The seedlings were inoculated with a 1.0-ml portion of either a spore suspension of one of the four isolates or a mixture of the four isolates. Of a mixture of YEM broth cultures of USDA 110 and USDA 123, 1.0 ml (10^9 cells per ml) was added to the flasks 7 days later. After inoculation, the top of the vermiculite was covered with a 2-cm layer of sterile perlite and a 2-cm layer of paraffin-coated sand. Plants were grown in a Sherer model CEL4-7 controlled-environment growth chamber at 27°C with a flux density of 170 microeinsteins/m^2 per s and a photoperiod of 16 h. The plants were harvested 4 weeks after the inoculation with USDA 110 and USDA 123, and nodule occupancy was determined. In a similar experiment, the pattern of competition between USDA 110 and USDA 123 was examined in vermiculite, either left unamended or amended with 1.0 g of Waseca soil.

Effect of combined nitrogen on nodule occupancy. Three soils with different nitrogen contents were used to test the effect of available nitrogen on the pattern of competition between the two strains. The soil was distributed in portions of 1.5 kg per pot. Available nitrogen in the soils was immobilized by adding finely ground bagasse (sugar cane waste) to give a final concentration of 1.1% (wt/wt). Calcium nitrate was added at either 70 ppm of $N0_3$ N [0.41 g of $Ca(N0_3)_2$ per kg] or 300 ppm of $N0_3$ N (1.75 g/kg), to replenish available nitrogen in the three soils. The percent moisture for the Waimea and Kawaihae soils was maintained at 10 kPa of water potential. The percent moisture for the Waukegan soil was maintained at 60% of its water-holding capacity (corresponding to 8 kPa of tension).

Seeds (cv. Chippewa 64) in the Waimea and Kawaihae soils were inoculated by placing 1.0 g of peat, containing an equal mixture of USDA 110 and USDA 123, under each seed (4 x 10^6 cells per seed). In the Waukegan soil, seeds were planted without inoculation. Soil surfaces were covered with a 2-cm layer of perlite. The 10 to 12 seeds that were planted initially were thinned to 8 uniform seedlings 2 to 3 days after germination. Plants were grown in a greenhouse and harvested 4 to 5 weeks from planting. Nodule number, dry weight, shoot dry weight, and nodule occupancy were determined.

Effect of harvest time on nodule occupancy. For the field experiment, the inoculum strains were grown separately in modified YEM broth (containing 1:4 mannitol and yeast-extract). A peat inoculum, containing an equal number of the desired strains (10^8 cells per g), was used to inoculate the seeds. A randomized complete block design with three replicates was used. Plants were harvested after 1, 2, and 3

TABLE 2. Effect of antibiotic-producing actinomycetes on the pattern of competition between *R. japonicum* strains USDA 110 and USDA 123 for nodulation of soybeans grown in vermiculite

Treatment[a]	Sensitivity of strain[b]:		% Nodule occupancy[c] by:		
	110	123	110	123	Both
110 + 123[d]			58 (±12)	22 (±14)	20 (±3)
110 + 123 + Actinomycete no. 1	+	+	50 (±0)	20 (±7)	30 (±7)
110 + 123 + Actinomycete no. 2	+	−	53 (±18)	25 (±14)	22 (±4)
110 + 123 + Actinomycete no. 3	−	−	50 (±4)	20 (±4)	30 (±7)
110 + 123 + Actinomycete no. 4	+	+	53 (±4)	35 (±14)	12 (±11)
110 + 123 + Actinomycete no. 1–4			77 (±15)	8 (±10)	15 (±9)

[a] Two to three replicates per treatment. 110, USDA 110; 123, USDA 123.
[b] +, Growth in the presence of the isolate; −, no growth in the presence of the isolate.
[c] Mean ± SE No significant differences (P = 0.05) between treatments were found.
[d] Strains USDA 110 and USDA 123 isolated from nodules of soybeans grown in Waseca soil.

TABLE 3. Effect of combined nitrogen on competition among *R. japonicum* strains in three soils

Soil[a] and amendment (ppm NO_3)	Growth results per plant[b, c]			% Nodule occupancy by:			
	Shoot dry wt (g)	Nodule dry wt (mg)	No. of nodules	USDA 110	USDA 123	USDA 110 + USDA 123	Negative
Waimea							
None	4.0 (±0.2)^d	502 (±104)^d	115 (±16)^d	66	24	10	None
Bagasse	3.0 (±0.4)^d	370 (±29)^d	56 (±6)^e	52	35	13	None
$Ca(NO_3)_2$ (70)	2.2 (±0.2)^e	282 (±16)^e	60 (±15)^e	64	23	13	None
$Ca(NO_3)_2$ (300)	5.1 (±1.0)^f	321 (±84)^e	120 (±11)^d	53	38	9	None
				d	e	f	
Kawaihae							
None	1.3 (±0.2)^g	78 (±8)^f	60 (±8)^f	86	8	6	None
$Ca(NO_3)_2$ (70)	1.3 (±0.2)^g	27 (±6)^g	20 (±6)^g	80	10	10	None
				g	h	h	
Waukegan							
None	3.1 (±0.7)^h	195 (±23)^h	46 (±6)^h	12	24	13	51
Bagasse	3.6 (±0.5)^h	248 (±83)^h	65 (±8)^h	13	23	12	52
$Ca(NO_3)_2$ (300)	3.3 (±1.2)^h	246 (±83)^h	55 (±19)^h	22	18	7	53
				i	i	j	k

[a] Waimea characteristics: Soil type, inceptisol; C/N ratio, 9; percent N, 1.2. Kawaihae characteristics: Soil type, aridisol; C/N ratio, 9; percent N, 0.09.
[b] Numbers are means (plus or minus standard errors) of three replications.
[c] Differences among treatments within a soil were not significant ($P = 0.05$). Columns followed by the same letters (d to k) do not differ significantly ($P = 0.05$) for the indicated soil and amendment. Negative, Percentages of nodules which did not react with two fluorescent antibodies.
[d–h] Numbers followed by the same superscript letters within a column do not differ significantly ($P = 0.05$).

months. For each time period, three to five plants per replicate per treatment were harvested, and nodule occupancy was determined.

Time-delay experiments. A time course experiment was used to determine if pre-exposure of soybean seedlings to USDA 110 would enhance the competitiveness of this strain in the Waseca soil. Seeds of cv. Chippewa, were germinated in vermiculite moistened with Hoagland solution containing approximately 108 cells per ml from a 5-day liquid culture of USDA 110. Seedlings were removed after 2, 48, and 72 h and transplanted into pots containing Waseca soil. Controls consisted of uninoculated seedlings and seeds which received a peat inoculum (2 x 10^8 cells per g) of USDA 110 at the time of planting. The soil was maintained at 60% of its water-holding capacity (corresponding to 8 kPa of tension). Plants were grown in a greenhouse and harvested 4 to 5 weeks from planting.

In a similar experiment, soybean seedlings preexposed to USDA 110, USDA 123, or SM-5 were planted in the Waimea soil seeded with a mixture of the same three strains. The inoculum strains were grown separately for 10 days in peat and then introduced into the soil. Mixtures of different peat cultures were made to contain equal numbers of the desired strains. The peat preparations were mixed in with the soil and allowed to equilibrate for 24 h before the seedlings were planted.

Seedlings of cv. Chippewa that were 2 days old were preexposed to USDA 110, USDA 123, or SM-5 for 24, 48, and 72 h and transplanted into the soil. The percentage of moisture in the soil was maintained at 10 kPa of water holding potential. Plants were harvested at 4 weeks (Harvest 1) and at 9 weeks (Harvest II).

Statistical analyses. Analysis of variance and Duncan multiple range tests were done by using the GLM (General Linear Models) program from the SAS statistical package at the University of Hawaii, Honolulu. For data given in percents, the values were coverted to ranks before being analyzed.

RESULTS

The pattern of competition between USDA 110 and USDA 123 in Waseca soil and in vermiculite is given in Table 1. In the soil, USDA 123 occupied 70% of the nodules, although in

TABLE 4. Competition pattern of *R. japonicum* strains in a Hawaiian inceptisol: Mauka field trial

Strain(s)	% Strain in cultivar at Harvest[a]:								
	Lee			Chippewa			Davis		
	I	II	III	I	II	III	I	II	III
USDA 110	3^e	3^j	9^{mn}	1^g	2^j	3^l	0^f	10^{hi}	11^l
USDA 123	36^c	29^{gf}	19^{kl}	44^c	41^h	31^k	54^c	28^g	25^k
USDA 138	7^d	11^{ghi}	18^{lm}	8^{ef}	11^i	3^l	6^{ef}	7^i	14^{kl}
USDA 31	6^{de}	10^{hi}	1^o	1^g	0^j	1^m	1^f	1^j	4^m
CB 1809	10^d	14^{gh}	11^{lm}	12^e	12^i	35^k	9^e	20^{gh}	20^{kl}
Double infection	31^c	15^g	22^k	24^d	16^i	21^k	20^d	20^{gh}	13^{kl}
Triple infection	4^e	4^{ij}	6^n	4^f	1^j	7^l	0^f	1^j	2^m
Negative[b]	1^e	14^{gh}	14^{lm}	6^{ef}	17^i	0^m	10^e	13^{hi}	11^l

[a] For each cultivar, numbers within the same harvest followed by the same superscript letter (*c–m*) do not differ significantly ($P = 0.05$).
[b] Percentage of nodules which did not react with the five fluorescent antibodies.

TABLE 5. Effect of preexposure to USDA 110 on competition patterns of *R. japonicum* strains in a soil containing indigenous *R. japonicum* (Waseca)

Inoculum	Preexposure time (h)	No. of nodules per plant[a]	Nodule occupancy (% total)[a] by strain(s):			
			USDA 110	USDA 123	USDA 110 + 123	Other[b]
None	None	127^e	2^g	70^k	2^l	26^m
USDA 110 (peat inoculated)	None	127^e	15^h	60^k	9^l	16^m
USDA 110	2	163^f	26^h	46^k	4^l	24^m
USDA 110	48	120^e	51^i	26^j	6^l	17^m
USDA 110	72	75^d	55^i	27^j	7^l	11^m

[a] Numbers followed by the same letters (^{d} to ^{m}) within a given column do not differ significantly ($P = 0.05$). Values are means of four replications.
[b] Percentage of nodules which did not react with two fluorescent antibodies specific for USDA 110 and USDA 123.

vermiculite, USDA 123 was less successful than USDA 110. When 1.0 g of Waseca soil was used to inoculate the vermiculite, the percentages of nodules occupied by the two strains were approximately equal. However, when the vermiculite was inoculated with both the soil and the two strains, the percentage of nodules occupied by USDA 110 increased, whereas the percentage of nodules occupied by USDA 123 decreased.

Only two of the actinomycete isolates from the rhizosphere of soybeans were found to inhibit the growth of USDA 110 or USDA 123 in culture (Table 2). The establishment of four isolates of actinomycetes in the rhizosphere of soybeans grown in vermiculite had no effect on the pattern of competition between USDA 110 and USDA 123 (Table 2).

The addition of bagasse or calcium nitrate to the Waimea, Kawaihae, and Waukegan soils did not change the percentages of nodules occupied by USDA 110 and USDA 123 (Table 3). In the *R.* japonicum-free soils Waimea and Kawaihae, USDA 110 was highly competitive and formed a significantly greater percentage of the nodules than did USDA 123. In contrast, in the Waukegan soil, which contains a complex of indigenous *R.* japonicum strains, USDA 123 formed approximately the same percentage of the nodules as did USDA 110.

Nodule dry weight was decreased by the addition of nitrogen to the two Hawaiian soils (Table 3). In the Waukegan soil, the addition of nitrate had no significant effect on nodule number or dry weight.

The percentages of nodules occupied by five *R.* japonicum strains on soybeans grown at the Mauka Field Station are given in Table 4. Strain USDA 123 occupied a significantly greater percentage of the nodules on cultivars Lee and Chippewa for the first two harvests. On the Davis cultivar, USDA 123 was dominant for Harvest I, but equal to some of the other strains by Harvest II and Harvest III. Under these conditions, USDA 110 was poorly competitive and was present in less than 10% of the nodules on the three cultivars for the three harvest times.

In the Minnesota soil Waseca, preexposure of soybean seedlings to USDA 110 significantly increased nodule occupancy by this strain (Table 5). Even a 2-h preexposure to USDA 110 resulted in a significant increase in nodule occupancy by USDA 110. When seedlings were preexposed to USDA 110 for 48 h or longer, USDA 110 became the dominant strain in the nodules. The majority of nodules on plants which were not preexposed to USDA 110 or which received USDA 110 as a peat inoculum were occupied by *R.* japonicum strain USDA 123.

The patterns of competition between USDA 110, USDA 123, and SM-5 in the Hawaiian soil Waimea are given in Tables 6 and 7. USDA 110 was the most competitive of the three strains and formed 62% of the nodules on plants harvested after 4 weeks (Table 6) and 73% of the nodules on plants harvested at pod-fill (Table 7). The percentage of nodules occupied by USDA 110 was significantly increased when seedlings were preexposed to USDA 110 for 2 h or longer. Similarly, preexposure of young seedlings to USDA 123 caused a significant increase over controls in the percentage of nodules occupied by USDA 123 for both Harvest I and Harvest II (Tables 6 and 7). Preexposure of young seedlings to the ineffective strain SM-5 also resulted in an increase in nodule occupancy by this strain for Harvest I (Table 6).

For the preexposure experiment, changes in nodule occu-

TABLE 6. Effect of preexposure to *R. japonicum* strains on competition pattern in a *Rhizobium*-free soil (Waimea): Harvest I

Soil inoculated with strain(s):	Preexposure treatment:		Nodule occupancy (% total)[a] by strain(s):					
	Strain	Time (h)	USDA 110	USDA 123	SM-5	USDA 110 + USDA 123	USDA 110 + SM-5	USDA 123 + SM-5
USDA 110	None	0	100	0	0	0	0	0
USDA 123	None	0	0	100	0	0	0	0
SM-5	None	0	0	0	100	0	0	0
110 + 123 + SM-5	None	0	62^b	7^e	13^k	7^{mn}	8^s	3^t
110 + 123 + SM-5	USDA 123	2	39^b	33^d	11^k	0^n	13^s	4^t
110 + 123 + SM-5	USDA 123	24	40^b	25^d	17^k	8^m	7^s	3^t
110 + 123 + SM-5	USDA 123	48	31^b	31^d	13^k	17^m	7^s	1^t
110 + 123 + SM-5	USDA 123	72	22^c	35^d	11^k	22^m	6^s	4^t
110 + 123 + SM-5	SM-5	2	52^b	6^e	27^k	1^n	13^s	1^t
110 + 123 + SM-5	SM-5	24	55^b	0^g	33^k	1^n	10^s	1^t
110 + 123 + SM-5	SM-5	48	44^b	4^{ef}	32^k	0^n	18^s	2^t
110 + 123 + SM-5	SM-5	72	37^b	1^{fa}	43^k	0^n	18^s	1^t

[a] Values are means of three replicates. Numbers followed by the same letter (*b*–*t*) do not differ significantly ($P = 0.05$) within a given column. The Duncan multiple range test was used to distinguish between treatments within a strain. Data for preexposure to USDA 110 were not determined for Harvest I.

TABLE 7. Effect of preexposure of soybean roots to *R. japonicum* strains on interstrain competition in an *R. japonicum*-free soil (Waimea) inoculated with 3 strains: Harvests I and II[a]

Inoculum strain(s)	Preexposure strain	Treatment time (h)	No. of nodules[b] containing strain(s): Total		USDA 110		USDA 123		SM-5		Multistrain	
			I	II	I	II	I	II	I	II	I	II
USDA 110	None	0	38^{c}	94^{i}	38	94	0	0	0	0	0	0
USDA 123	None	0	63^{cd}	198^{jh}	0	0	63	198	0	0	0	0
SM-5	None	0	132^{cde}	215^{jk}	0	0	0	0	132	215	0	0
USDA 110 + USDA 123 + SM-5	None	0	73^{cde}	141^{jk}	45	102	5	10	9	10	14	19
USDA 110 + USDA 123 + SM-5	USDA 123	2	69^{cdef}	97^{i}	27	66	23	16	8	3	11	12
USDA 110 + USDA 123 + SM-5	USDA 123	24	82^{cdef}	157^{jk}	33	97	21	24	14	16	14	20
USDA 110 + USDA 123 + SM-5	USDA 123	48	95^{defg}	118^{ij}	29	55	29	26	12	22	25	15
USDA 110 + USDA 123 + SM-5	USDA 123	72	107^{defgh}	141^{jk}	24	78	37	25	12	20	34	18
USDA 110 + USDA 123 + SM-5	SM-5	2	85^{def}	144^{jk}	44	102	5	6	23	9	13	27
USDA 110 + USDA 123 + SM-5	SM-5	24	122^{defgh}	176^{jk}	67	123	0	7	40	19	15	27
USDA 110 + USDA 123 + SM-5	SM-5	48	142^{h}	160^{jk}	62	90	6	3	45	24	29	43
USDA 110 + USDA 123 + SM-5	SM-5	72	113^{defgh}	165^{jk}	42	102	1	8	49	28	21	27
USDA 110 + USDA 123 + SM-5	USDA 110	2	ND	83^{i}	ND	78	ND	0	ND	2	ND	3
USDA 110 + USDA 123 + SM-5	USDA 110	24	ND	100^{ij}	ND	85	ND	5	ND	4	ND	6
USDA 110 + USDA 123 + SM-5	USDA 110	48	ND	76^{i}	ND	68	ND	0	ND	2	ND	6
USDA 110 + USDA 123 + SM-5	USDA 110	72	ND	79^{i}	ND	71	ND	2	ND	0	ND	6

[a] Harvest: I, 4 weeks after planting; II, 9 weeks after planting.
[b] Values, in actual numbers of nodules, are means of three replicates. Numbers followed by the same letter (*c–k*) do not differ significantly ($P = 0.05$) within the same column. The Duncan multiple range test was used to establish significance. Each harvest was analyzed separately. ND, Not determined.

pancy from Harvest I (4 weeks from planting) to Harvest II (9 weeks) are shown in Table 7. Data are shown in actual numbers of nodules to indicate how one strain, USDA 110, continued to form nodules from Harvest I to II, whereas USDA 123 and SM-5 nodules increased in numbers substantially only when these strains were used singly.

DISCUSSION

Under growth chamber conditions, strain USDA 123 occupied the majority of the nodules on soybean plants grown in Waseca soil. However, the addition of Waseca soil to vermiculite did not alter the pattern of competition between USDA 123 against another strain, USDA 110. The pattern of competition between the two strains was not altered by the presence of antibiotic-producing actinomycetes isolated from the rhizosphere of soybeans. Changes in available nitrogen in three different soils also had no effect on the percentages of nodules occupied by the two strains. However, the pattern of competition between USDA 110 and USDA 123 could be changed by preexposing young seedlings to either strain before transplantation into soil. In the Minnesota soil Waseca, nodule occupancy by USDA 110 was significantly increased after soybean seedlings were preexposed to USDA 110 for 2 h or more (Table 5). Likewise, in the Hawaiian soil Waimea, preexposure of seedlings to either USDA 123 or the ineffective strain SM-5 increased the percentages of nodules occupied by these two strains significantly (Tables 6 and 7).

The presence of antagonistic microflora has been postulated to affect the outcome of competition between strains of rhizobia. Although some studies show that inhibition of *Rhizobium* by soil microorganisms may occur under laboratory conditions, the presence of inhibitory microorganisms in the legume rhizosphere (in soil) has lttle effect on the nodulating ability of strains of rhizobia (9, 12, 25). In this study, the outcome of competition between *R. japonicum* strains USDA 110 and USDA 123 was not affected by the presence of antagonistic microorganisms in the soybean rhizosphere (Table 2).

Reports on the effect of combined nitrogen on the competitive ability of strains are equivocal (11, 15, 21, 24). In our studies, depletion of soil nitrogen by the addition of bagasse or replenishment of soil nitrogen by the addition of calcium nitrate had no effect on the percentages of nodules occupied by USDA 110 and USDA 123 in the three soils (Table 3). In the two Hawaiian soils, USDA 110 outcompeted USDA 123, whereas in the Minnesota soil, USDA 123 was the dominant strain in the nodules.

Although no significant strain-nitrate interactions were observed, significant strain-soil interactions were detected (Table 3). Significant strain-soil interactions have been reported for the *Rhizobium leguminosarum-Lens esculenta* (20), *R. leguminosarum-Vicia faba* (2), *R. japonicurraGlycine max (1, 8),* and *Rhizobium meliloti-Medicago sativa* (26) associations. As observed by others (11, 15, 21), the addition of nitrate reduced nodule numbers and dry weight in the two Hawaiian soils (Table 3).

The results from the field study (Table *4)* showed that the host cultivar and plant age affected the nodule occupancy of five *R. japonicum* strains. USDA 123 was highly competitive and occupied a greater percentage of the nodules than either of the four inocula strains or the indigenous soybean rhizobia (Table *4).* Nodulating abilities of *R. leguminosarum* (20), *R. meliloti* (26), and *R. japonicum* (10, 21) were also affected by the host cultivar. Changes in nodule occupancy over successive harvest times by strains of *R. japonicum* have also been reported (9).

In soils from the north central region of the United States, the use of highly effective strains as inocula has not always resulted in an increase in soybean yields when other indigenous strains of *R. japonicum* are present. In particular, strains belonging to the USDA 123 serogroup form the majority of the nodules on soybeans grown in this area (see reference 13). In the Minnesota soil Waseca, USDA 123 outcompeted USDA 110 and formed the majority of the nodules (Table 5). However, when young seedlings were preexposed to USDA 110 and transplanted into pots containing the Waseca soil, as little as 2 h of preexposure to USDA 110 was adequate to significantly increase the percentage of nodules formed by this strain. Longer periods *(48* h or more) of preexposure resulted in USDA 110 in the majority of nodules.

In the Hawaiian soil Waimea, USDA 110 was highly competitive and outcompeted USDA 123 and the ineffective strain SM-5 (Tables 6 and 7). Preexposure of seedlings to USDA 123 significantly increased nodule occupancy by this strain. Likewise, when seedlings were preexposed to USDA 110, its nodule occupancy increased even more, to 85 to 94% of the nodules. The percentage of nodules occupied by the strain to which the host was preexposed appeared to be related to the effectiveness of that strain. Nodule occupancy by the ineffective strain SM-5 decreased on plants harvested at pod-fill, whereas the percentage of nodules containing one of the two effective strains, USDA 110 or USDA 123, remained significantly greater (Table 7).

The data in Table 7 also show the differences in the abilities of strains to continue forming nodules over time when confronted with competition from other strains. All three strains were able to increase their nodule occupancy when they were present by themselves. However, only USDA 110 was able to continue forming significantly more nodules from Harvest I to II when all three strains were present. Nodule occupancy by USDA 123 and SM-5, in the multistrain treatments, either remained approximately the same over the two harvests or decreased, perhaps due to nodule senescence.

We have previously shown that preexposure of young soybean seedlings to a poorly competitive strain enhanced nodule occupancy by that strain in vermiculite (19). Furthermore, we have reported that in a split-root system of soybeans, inoculation of one-half of the root system caused suppression of nodulation on the opposite half inoculated later (18). The results from the present study confirm our earlier findings (18, 19) that interactions during the early period of nodulation are critical in determining the outcome of competition between strains of R. japonicum. Changes in the pattern of competition between USDA 110 and USDA 123 occurred only when young seedlings were preexposed to either strain before transplantation into soil. Neither the presence of antibiotic-producing actinomycetes nor changes in available soil nitrogen had any effect on nodule occupancy by the two strains in either vermiculite or soil.

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