

## *Bradyrhizobium japonicum*-Environment Interactions: Nodulation and Interstrain Competition in Soils along an Elevational Transect†

THOMAS GEORGE, B. BEN BOHLOOL,\* AND PAUL W. SINGLETON

*NifTAL Project, University of Hawaii, Paia, Hawaii 96779-9744*

Received 21 November 1986/Accepted 27 January 1987

The effects of temperature and soil type on interstrain competition of *Bradyrhizobium japonicum* and on nodulation and nitrogen accumulation in five soybean varieties belonging to four maturity groups were investigated at three sites devoid of soybean rhizobia along an elevational transect in Hawaii. Competition patterns of the three *B. japonicum* strains were unaffected by soil type or soil temperature. Strain USDA 110 was the best competitor, occupying on the average 81 and 64% of the nodules in the field and greenhouse experiments, respectively. Strain USDA 138 was the least successful in the field (4%), although it formed 34% of the nodules in the greenhouse. Nodule occupancy by *B. japonicum* strains was found to be related to soybean maturity group. Strain USDA 110 formed 61, 71, 88, 88, and 98% of the nodules in the field on Clay (00), Clark (IV), D68-0099 (VI), N77-4262 (VI), and Hardee (VIII), respectively. Strain USDA 136b formed few nodules on Hardee, an *Rj2* soybean variety incompatible with that strain, in both experiments. Nodule number and weight at the 1,050-m site were reduced to 41 and 27%, respectively, of those at the 320-m site because of the decrease in temperature. Nodule number increased with increasing maturity group number at each site; however, there was not a corresponding increase in nodule weight. Nitrogen accumulation decreased from 246 mg of N per plant at the lowest elevation site to 26 mg of N per plant at the highest elevation. While soil type and temperature had no effect on strain competition, temperature had a profound influence on nodule parameters and plant growth.

The establishment of an effective and efficient symbiosis between rhizobia and the host legume is essential to viable legume production. The symbiosis is affected by various environmental factors. An understanding of the effects of environmental factors on the symbiosis may assist in the selection of rhizobial strains that will enhance symbiotic nitrogen fixation and soybean yield in different environments.

*Bradyrhizobium japonicum* strains differ considerably in competitive ability, nodulation, and nitrogen fixation (1, 3, 9). These characteristics have been shown to be influenced by the host genotype (4, 19). Soil type may also influence the competition between strains for nodule sites. Dominance of different *B. japonicum* strains in soybean nodules from different soils has been reported (6, 8, 13). Kosslak and Bohlool (14) reported that, in soils devoid of native *B. japonicum*, the pattern of competition between two introduced strains remained the same, regardless of soil type or soil amendments.

Strain recovery and plant performance in nodulation and nitrogen accumulation were also related to temperature. Caldwell and Weber (5) observed dominance of different serogroups in soybean nodules based on planting dates, and Weber and Miller (27) concluded that the effect was due to differences in soil temperature. Low soil temperatures have also been shown to adversely affect both nodulation and nitrogen fixation (17, 25).

Better understanding of the ecology of rhizobia in relation to the host and its environment may aid in making appropriate management decisions for a wide range of conditions. The Maui Network of Experimental Sites, Hawaii, provided

us with a field laboratory of various edaphic and climatic conditions. The purpose of our investigation was to test selected genotypes of soybean in field environments that differed in soil type and in soil and air temperatures for *B. japonicum* strain competition, nodulation, and early nitrogen accumulation. We controlled other variables by equalizing pH, maintaining soil moisture at  $3.0 \times 10^4$  Pa tension, and applying nutrients at maximum levels.

### MATERIALS AND METHODS

**Selection of sites.** Three sites within an elevational transect of the Maui Experimental Network, Hawaii, were selected. The sites were located along the slopes of volcanic Haleakala mountain on the island of Maui. The relevant characteristics of the sites are given in Table 1. All sites were free of *B. japonicum* as determined by the most probable number technique by using growth pouches (26).

**Soil amendments.** The soils were limed to a pH of between 5.5 and 6.0 by the addition of  $\text{Ca}(\text{OH})_2$ . Other amendments (in kilograms hectare<sup>-1</sup>) were as follows: P as treble superphosphate, 600; K as  $\text{K}_2\text{SO}_4$ , 300; Mg as  $\text{MgSO}_4$ , 75; Zn as  $\text{ZnSO}_4$ , 15; Mo as  $\text{Na}_2\text{Mo}_4$ , 2; and B as  $\text{H}_3\text{BO}_3$ , 3.

**Soybean varieties.** Soybean varieties belonging to four maturity groups were selected for the study. The varieties were Clay 00 (J. H. Orf, Department of Agronomy and Plant Genetics, University of Minnesota-Minneapolis), Clark IV (P. Cregan, U.S. Department of Agriculture Nitrogen Fixation Laboratory, Beltsville, Md.), and D68-0099 VI and N77-4262 VI, which are determinant and indeterminate selections, respectively, from crosses of varieties Lee (T. E. Carter, Department of Crop Science, North Carolina State University at Raleigh) and Hardee VIII (K. Hinson, Department of Agronomy, University of Florida, Gainesville).

**Rhizobial inoculum strains.** Yeast extract-mannitol (24) agar slants of the serologically distinct rhizobial strains USDA 110, USDA 136b (CB 1809), and USDA 138 were

\* Corresponding author.

† Journal series 3093 of the Hawaii Institute of Tropical Agriculture and Human Resources, University of Hawaii, Honolulu, HI 96779-9744.

TABLE 1. Soil and site characteristics of three locations in an elevational transect on the island of Maui, Hawaii

Site (soil series)	Soil classification	Elevation (m)	Total rainfall (mm) <sup>a</sup>	Avg temp (°C) <sup>a</sup>	
				Soil	Air
Kuiaha (Haiku)	Clayey, ferritic, isohyperthermic Humoxic Tropohumult	320	139	25.3	22.8
Haleakala (Makawao)	Clayey, oxidic, isothermic Humoxic Tropohumult	660	112	22.9	20.9
Olinda (Olinda)	Medial over loamy-skeletal, isomesic Entic Dystrandept	1,050	122	20.7	18.2

<sup>a</sup> During crop growth.

obtained from the NifTAL Project collection, Paia, Hawaii (TAL strains 102, 379, and 377, respectively). Each strain was grown in yeast extract-mannitol broth at 27°C for 5 days. From each broth culture, 50 ml (approximately  $5.0 \times 10^9$  cells ml<sup>-1</sup>) was inoculated into 100 g of gamma-irradiated Australian peat (Agricultural Laboratories Pty. Ltd., Sefton, New South Wales, Australia) in separate polyethylene bags. After 14 days, plate counts were made from serial dilutions of the peat inoculants by the Miles and Misra drop plate method (24). The three inoculants were mixed in proportions that would provide equal numbers of each strain in a mixed inoculant that had a final total population of  $3.2 \times 10^9$  cells g of peat<sup>-1</sup>.

**Inoculation procedure.** Gum arabic (40 g 100 ml of water<sup>-1</sup>) was applied to seeds in the amount of 3 ml 100 g of seed<sup>-1</sup>. The seeds were then coated with the prepared peat inoculum in the amount of  $4.5 \times 10^7$  rhizobia seed<sup>-1</sup>. The seeds were air dried and kept at 4°C overnight. Viable counts of rhizobia at planting were  $1.5 \times 10^7$  seed<sup>-1</sup>.

**Plant culture.** All of the sites were planted on 29 July 1985. Seeds were sown in 60-cm rows, with approximately 3.5 cm between seeds. The final plant population was approximately 400,000 plants ha<sup>-1</sup>. The fields were irrigated on the day of planting to a moisture tension of  $3.0 \times 10^4$  Pa and maintained at that tension during crop growth.

**Greenhouse experiment.** Seven seeds, each coated with inoculum prepared in the same way as those for the field, were planted into 3-liter pots each filled with 2 kg (on an oven dry basis) of soil from the field sites. The greenhouse soils were amended by the same method as the field soils. Nutrients applied (in milligrams kilogram of soil<sup>-1</sup>) were P, 300; K, 150; Mg, 37.5; Zn, 7.5; Mo, 1; and B, 1.5. The pots were watered daily to a tension of  $3.0 \times 10^4$  Pa. The plants were thinned to two per pot after 10 days. Average soil temperature during the experiment was 27°C.

**Sampling procedures.** Twelve plants per replication were harvested at random from the two outer rows of each plot at 45 days after planting. The plant tops were cut at the soil surface, oven dried at 70°C, weighed, and ground, and nitrogen content was determined after H<sub>2</sub>SO<sub>4</sub> digestion followed by the indophenol blue method (12). The roots were excavated, and the nodules were collected after the roots were washed free of soil. The nodules were counted, dried at 70°C in a forced-air oven, and weighed. Nodules from the plants grown in the greenhouse were also collected and dried at 45 days after planting.

**Strain identification.** Occupancy of nodules by the three rhizobial strains was determined by the fluorescent-antibody technique (22) with oven-dried nodules (23). At least 5%, but not fewer than 24 nodules, from the composite sample from each replication for the field experiment and 12 nodules per pot for the greenhouse experiment were selected at random. The nodules were then placed in the wells of microtiter plates and rehydrated in the refrigerator overnight with a few

drops of sterile water. The nodules were then crushed, and smears were heat fixed on glass slides. The smears were treated with gelatin-rhodamine isothiocyanate to control background fluorescence and nonspecific staining (2), and, after drying, they were stained with strain-specific fluorescent antibodies (22). The slides were observed by epifluorescence microscopy for positive reactions. Positive reaction for more than one strain in the same nodule was counted as a mixed infection.

**Experimental design and data analysis.** The field and greenhouse experiments were arranged in a randomized complete block design with three replications. Data from the three sites were subjected to a combined analysis (20). Nodule and plant growth data, as well as arcsine transformations of percent nodule occupancy from both experiments, were subjected to analysis of variance. SAS procedures (SAS Institute, Inc., Cary, N.C.) were used for all analyses.

## RESULTS

There were significant differences in the competitive abilities of the rhizobial strains used (Table 2). USDA 110 was the most competitive strain in both experiments; it formed 81 and 64% of nodules in the field and greenhouse, respectively. Strain USDA 138, which formed few nodules in the field, was able to occupy the same number of nodules as USDA 136b in the greenhouse. Both USDA 138 and USDA 136b were present in higher proportions in the greenhouse study than in the field. However, there was no significant effect of site or soil on nodule occupancy in the two experiments.

Soybean varieties exhibited significant differences in rhizobial strain occupancy under both field and greenhouse conditions (Table 3). USDA 110 formed 61 to 98% of the nodules in the field and 55 to 86% of the nodules in the greenhouse study, depending on variety. There was a positive correlation between maturity group and the proportion of nodules formed by USDA 110. Reduced nodulation by USDA 110 was countered almost entirely by increased

TABLE 2. Proportion of nodules formed by *B. japonicum* strains from soybean grown at three sites<sup>a</sup>

Site	% Nodules formed by:					
	USDA 110		USDA 136b		USDA 138	
	Field	Pot	Field	Pot	Field	Pot
Kuiaha	80.3	62.9	25.8	28.7	3.9	37.4
Haleakala	80.3	61.8	26.9	35.2	4.2	33.6
Olinda	82.0	67.8	30.0	37.4	4.2	31.4
Mean	80.8A	64.2a	27.6B	33.8b	4.1C	34.2b

<sup>a</sup> Data are means of five varieties. Nodules with more than one strain were counted for each strain testing positive. Data followed by the same lower or upper case letter for either field or pot were not found to be significantly different ( $P = 0.05$ ) by Duncan's multiple range test.

TABLE 3. Proportion of nodules formed by *B. japonicum* strains from five soybean varieties grown in the field and the greenhouse<sup>a</sup>

Variety	Maturity group	% Nodules formed by:					
		USDA 110		USDA 136b		USDA 138	
		Field	Pot	Field	Pot	Field	Pot
Clay	00	60.7D	55.1B	40.7A	38.0B	7.4A	47.9A
Clark	IV	70.8C	57.8B	31.9B	28.9B	8.8A	39.8AB
D68-0099	VI	87.5B	61.4B	26.9B	38.9B	0.5B	37.1B
N77-4262	VI	87.5B	60.5B	30.1B	52.4A	2.8B	33.4B
Hardee	VIII	97.7A	85.8A	8.3C	10.8C	0.9B	12.7C

<sup>a</sup> Data are means of three soils. Nodules with more than one strain were counted for each strain testing positive. Data followed by the same letter within a column were not found to be significantly different ( $P = 0.05$ ) by Duncan's multiple range test.

nodulation by USDA 138 in the greenhouse and by USDA 136b in the field.

There was no consistent trends for mixed infections of nodules (data not shown), which varied from 0 to 29% and from 0 to 42% in the field and greenhouse, respectively. The most frequent combinations were those of USDA 110 and USDA 136b in the field and USDA 110 and either USDA 136b or USDA 138 in the greenhouse.

There were significant differences between sites and between soybean varieties in the number of nodules and between sites in dry weight of nodules (Table 4) in the field experiment. Both parameters decreased with decreasing temperature for all of the varieties. Nodule weight was affected by site more than was nodule number. Mean nodule number at Olinda was 42% of that at Kuiaha, while nodule mass at Olinda was only 27% of that at Kuiaha. In general, late-maturing varieties formed more nodules than early-maturing varieties with the exception of Hardee. However, nodule dry weight did not increase with maturity group. There were no significant differences in nodule number and nodule weight among plants grown in soils from the three sites (data not shown) when the varieties grown in the fields were grown in pots under a constant temperature regime.

Shoot dry weight (Table 5) and total shoot nitrogen accumulation (Table 6) decreased with increasing elevation. There were no differences among varieties in shoot dry weight within each site. Total nitrogen content did not vary with varieties except at Kuiaha, where late-maturing varieties (VI and VIII) accumulated less N at 45 days after planting than did maturity groups 00 and IV.

## DISCUSSION

In this study, we investigated the effect of soil type and temperature on nodulation and early growth of soybean varieties from a wide range of maturity groups under a

TABLE 5. Shoot dry weight of five soybean varieties at 45 days after planting at three elevations<sup>a</sup>

Site	Shoot dry wt (g plant <sup>-1</sup> ) from:					Mean
	Clay (00)	Clark (IV)	D68-0099 (VI)	N77-4262 (VI)	Hardee (VIII)	
Kuiaha	7.7	7.7	7.8	6.3	7.0	7.3A
Haleakala	4.8	5.6	5.6	5.6	5.9	5.5B
Olinda	1.0	1.1	1.0	1.0	0.9	1.0C
Mean	4.5a	4.8a	4.8a	4.3a	4.6a	

<sup>a</sup> Data followed by the same letter within a row or column were not found to be significantly different ( $P = 0.05$ ) by Duncan's multiple range test.

constant photoperiodic regime. By introducing equal numbers of three *B. japonicum* strains into soils which were devoid of soybean rhizobia, we were also able to evaluate the effects of soil type, soil temperature, and host genotype on interstrain competition among rhizobia for nodule sites.

The large differences between *B. japonicum* strains in nodule occupancy and the limited effects of temperature and host genotype indicate that these strains have inherent differences in competitive ability. Strain USDA 110 was the predominant strain in the nodules of all of the soybean varieties and at all of the sites. Higher recoveries of USDA 110 in competition with other strains have been reported previously (3, 9). Kosslak and Bohlool (14) found USDA 110 to be highly competitive against USDA 123 in vermiculite and in Hawaiian soils devoid of *B. japonicum*. However, in soils from the north central region of the United States, strains belonging to the USDA 123 serogroup outcompeted USDA 110 (and others) and occupied the majority of the nodules on soybean. Even though the ranking of the strains in field and greenhouse nodules is essentially the same, the higher recoveries of USDA 138 in the greenhouse indicate that the competitive advantage of USDA 110 in the field was affected in the pot-grown plants. It is unlikely that the slightly higher soil temperature (27°C) in the greenhouse caused this shift because even greater temperature differences at the field sites had no effect on the competition pattern.

Soil type did not affect the percent recovery of rhizobial strains from either field or greenhouse nodules. Since there was no difference between soils in strain recovery in the greenhouse, where all of the soils were subjected to the same temperature regime, we concluded that the competitive attributes of these strains are independent of soil type and, within a reasonable range, of soil temperature. This differs from the view that interstrain competition among rhizobia is very much dependent on soil type (6, 8) and soil temperature (16, 27). However, it should be noted that, in contrast to these reports, no indigenous rhizobia were present in our

TABLE 4. Number and dry weight of nodules from five soybean varieties at 45 days after planting at three elevations<sup>a</sup>

Site	No. of nodules plant <sup>-1</sup> (dry wt [mg plant <sup>-1</sup> ]) from:					Mean
	Clay (00)	Clark (IV)	D69-0099 (VI)	N77-4262 (VI)	Hardee (VIII)	
Kuiaha	43 (289)	40 (278)	60 (294)	65 (236)	57 (249)	53A (269A)
Haleakala	20 (180)	32 (216)	31 (137)	35 (134)	28 (146)	29B (162B)
Olinda	18 (75)	24 (76)	24 (73)	28 (73)	18 (66)	22B (73C)
Mean	27d (181a)	32cd (190a)	38ab (168ab)	43a (147b)	34bc (154b)	

<sup>a</sup> Data followed by the same letter within a row or a column were not found to be significantly different ( $P = 0.05$ ) by Duncan's multiple range test.

TABLE 6. Shoot nitrogen content of five soybean varieties grown at three elevations at 45 days after planting<sup>a</sup>

Site	Shoot N content (mg plant <sup>-1</sup> ) from:					Mean
	Clay (00)	Clark (IV)	D68-0099 (VI)	N77-4262 (VI)	Hardee (VIII)	
Kuiaha	294a	290a	232b	195b	218b	246A
Haleakala	182a	198a	181a	178a	198a	187B
Olinda	26a	28a	25a	25a	25a	26C
Mean	167a	171a	146ab	133b	147ab	

<sup>a</sup> Data followed by the same lower case letter within a row or by the same upper case letter within a column were not found to be significantly different ( $P = 0.05$ ) by Duncan's multiple range test.

soils. Our results agree with those of Kamicker and Brill (11), who reported that predominant indigenous strains nodulating soybean plants were not related to soil type.

The varietal differences in rhizobial strain recovery were related to soybean maturity group. Physiological development of the plant is not a contributing factor since plants within a maturity group were at the same growth stage in the three soils in the greenhouse but were at different stages of development in the three field sites, where decreasing temperature delayed development. The variety Hardee was an exception to other varieties, forming the maximum number of nodules with USDA 110 and very few nodules with USDA 136b and USDA 138. This result may be partly explained by the fact that strain USDA 136b is incompatible with the variety Hardee, which carries the gene *Rj2* (19).

Mixed infection of nodules, although not related to site or to soybean variety, was considerable. The high inoculum density ( $1.5 \times 10^7$  viable rhizobia seed<sup>-1</sup>) may have been one reason for mixed occupancy of nodules. According to Lindeman et al. (18), mixed-strain inoculant with equal numbers of each strain resulted in mixed infections of soybean nodules at all inoculum densities above  $10^3$  rhizobia plant<sup>-1</sup>.

The reduction in nodule number and mass may be the direct consequence of decreasing soil and air temperatures on plant growth. Decreased nodulation in soybeans due to soil temperatures below 24°C was reported as early as in 1921 by Jones and Tisdale (10). Gibson (7) observed considerable delay in nodulation and a general decline in the rate of nodule formation by *Trifolium subterraneum* when root temperatures were below 22°C. Enhanced plant development and increased nodulation in soybeans with increasing soil temperatures within the range of 15 to 25°C have been reported previously (16).

Despite a large decrease in nodule number between sites, the three strains occupied the same percentage of nodules in all of the sites. It has been suggested that infection of roots by rhizobia is less sensitive to low temperatures than is nodule development (7). Our results indicate that competition among rhizobial strains for nodule sites may not be related to the final number of nodules formed.

The increase in nodule number with maturity group suggests that late-maturing varieties favor the development of more nodules in early growth. This increased nodule number may be required to support an extended growth period. Increased nodule number did not result in increased nodule dry weight within a site and may be explained by the fact that there were no large differences in shoot N within a site. Therefore, late-maturing varieties are capable of initiating more nodules in early growth, but their subsequent development is determined by later plant growth and demand for nitrogen.

Large differences in shoot dry weight and nitrogen accumulation between sites may be attributed to the effects of soil and air temperatures on nodulation and plant growth. Plant growth was severely affected by a 5°C decline in average temperature from the lowest to the highest elevation. There was also considerable delay in plant development with decreasing temperatures (data not shown). At the lowest site, all varieties were beyond the flowering stage at sampling, with the early varieties Clay and Clark already forming seeds. At the highest site, plants had not flowered, with the exception of Clay and Clark. In addition to the direct effect of temperature on plant growth, there may be an indirect effect through the effects of temperature on nitrogen assimilation and nitrogen fixation, which also contributed to reduction in growth and delay in plant development. The average percent N in the shoot at Olinda was only 2.6%, compared with 3.4% at both Kuiaha and Haleakala. The low percentage of nitrogen in shoots at Olinda was further evidence for nitrogen stress at that site. Root temperatures of 24 to 27°C are reported to be optimum for nitrogen fixation and nitrate assimilation in soybean (15, 21). The soil temperature at the lowest site was in the vicinity of the reported optimum. The highest nitrogen accumulation and growth at that site is, therefore, in agreement with the previous findings.

Our experiments identified USDA 110 as the best competitor compared with USDA 136b and USDA 138 for nodule occupancy, regardless of large differences in soil type, soil temperature, or maturity group of soybean. Nodulation and plant growth at 45 days after planting were drastically reduced by a 5°C decline in both soil and air temperatures. Large differences in soybean plant growth between sites had little effect on competition between rhizobial strains. None of the nodulation and plant growth parameters were related to occupancy of strains in the nodules. Our results indicate that rhizobial strains superior in competitiveness can be identified for a broad range of host genotypes, soil types, and temperatures.

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