

Environmental effects on competition for nodule occupancy between introduced and indigenous rhizobia and among introduced strains¹ 85982

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Understanding the impact of environmental variables on interstrain competition is important to ensure the successful use of rhizobial inoculant. In eight inoculation trials conducted at five diverse sites on Maui, Hawaii, equal numbers of three serologically distinct strains of effective, homologous rhizobia in a peat-based inoculant were applied to seeds of soybean, bush bean, cowpea, lima bean, peanut, leucaena, clover, and tinga pea. We studied the influence of environmental variables on interstrain competition between applied and indigenous rhizobia and among the three strains comprising the inoculum. Although temperature and soil fertility were correlated with nodule occupancy by inoculant strains in a few cases, the most significant environmental variable controlling their competitive success was the size of the indigenous rhizobial population. Nodule occupancy was best described ($r^2 = 0.51, p < 0.001$) by the equation $y = 97.88 - 15.03(\log_{10}(x + 1))$, where y is percent nodule occupancy by inoculant rhizobia and x is the most probable number of indigenous rhizobia per gram soil. For each legume, one of the three inoculant strains was a poor competitor across sites. Competition between the other two strains varied between sites, but was infrequently related to environmental variables. Results indicated that competitive strains could be selected that perform well across a range of environments.

Key words: competition, rhizobial ecology, inoculation response, competitiveness index.

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Pour assurer le succès des inoculations, il importe de connaître l'impact des variables environnementales sur la compétition des souches rhizobiennes. Dans ce but, huit essais d'inoculation ont été faits dans cinq sites différents de l'île Maui, en Hawaï. Trois souches de *Rhizobium*, homologues, efficaces, mais de sérologie distincte, ont été utilisées comme inoculum dans une base de mousse de tourbe et furent appliquées à des graines de soya, haricot nain, pois chiche, haricot de Lima, arachide, *Leucaena*, trèfle et pois « tinga ». L'influence des variables environnementales sur la compétition des souches rhizobiennes a été étudiée entre les rhizobiums d'origine indigène et d'origine appliquée et entre les trois souches constituant l'inoculum. La température et la fertilité du sol ont été corrélées avec l'occupation des nodosités par les souches inoculantes, mais la variable la plus significative, qui contrôle le succès de la compétitivité, a été la dimension de la population rhizobienne indigène. L'occupation des nodosités ($r^2 = 0.51; p < 0,001$) a été mieux décrite par l'équation : $y = 97,88 - 15,03(\log_{10}(x + 1))$, où : y est le pourcentage d'occupation des nodosités par les rhizobiums inoculants et x , le nombre le plus probable de rhizobiums indigènes par gramme de sol. Pour chaque légumineuse, l'une des trois souches inoculantes s'est révélée une pauvre compétitrice dans les divers sites. La compétition entre les deux autres souches a varié entre les sites et, de façon non fréquente, elle fut reliée aux variables de l'environnement. Les résultats indiquent que des souches compétitives, dont la performance serait bonne dans une gamme d'environnements, pourraient être sélectionnées.

Mots clés : compétition, écologie rhizobienne, réponse de l'inoculation, index de compétitivité.

[Traduit par la rédaction]

Introduction

Competition for nodule occupancy between strains of rhizobia is a complex and controversial area in the study of the legume-*Rhizobium* symbiosis. Many environmental variables, intrinsic characteristics of the rhizobia themselves, and genetic determinants of the host contribute to the success or failure of rhizobial strains to occupy a significant proportion of nodules formed under a given set of conditions (for review see Dowling and Broughton 1986; Bottomley 1992).

Much attention has been paid to factors that affect the ability to establish inoculant strains on plants growing in soil with indigenous rhizobia. Emphasis on the competitive ability of inoculant strains results from the expectation that successful nodule establishment by strains superior in N_2 -fixing ability will lead to yield improvement. However, this has been difficult to demonstrate under field conditions (Weaver and Frederick 1974b; Thies *et al.* 1991).

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Environmental factors reported to affect competition for nodule occupancy include presence of indigenous rhizobia (Ireland and Vincent 1968; Bohlool and Schmidt 1973; Weaver and Frederick 1974a, 1974b; Thies *et al.* 1991), soil type (Ham *et al.* 1971), temperature (Kluson *et al.* 1986), moisture (Boonkerd and Weaver 1982), soil pH (Dughri and Bottomley 1983, 1984), nitrogen availability (Abaidoo *et al.* 1990), and microbial antagonism (Triplett and Barta 1987).

TABLE 1. Environmental characteristics of five sites on the island of Maui, Hawaii

Site No.	Soil classification ^a	Elevation (m)	MAR (mm/year) ^b	Native pH ^c	Clay (%)	Soil temp. (°C) ^d		Organic C (%)	Total soil N (%)	Soil N mineralization ($\mu\text{g} \cdot \text{g soil}^{-1} \cdot \text{week}^{-1}$)
						Maximum	Average			
1	Torroxic Haplustoll	37	322	6.8	34	34.9	30.8	1.06	0.08	7.0
1a						34.0	29.8			
2	Humoxic Tropohumult	320	1875	4.7	48	24.9	24.6	2.92	0.25	27.4
3	Torroxic Haplustoll	366	375	7.5	42	35.2	29.4	1.46	0.15	17.5
3a						31.5	24.8			
4	Humoxic Tropohumult	660	1800	5.0	32	22.2	20.5	3.58	0.32	44.1
5	Torroxic Haplustoll	670	523	5.7	26	31.6 ^e	24.7 ^e	1.96	0.19	20.9
5a						29.4	20.7			

^aFrom Soil Conservation Service (1972).

^bMedian annual rainfall (MAR), from Department of Land and Natural Resources (1982).

^cSoils were limed to achieve a minimum pH of 5.8 at sites 2 and 4 prior to planting, hence native pH values relate only to the discussion of size of indigenous populations and is not germane to that on competitive success of inoculant strains.

^dAt 10 cm depth for the first 10 days following planting. From weather stations on location operated by University of Hawaii Maui Soil, Climate, and Land Use Network. Temperature differences at the same site are due to planting at different times of year.

^eFrom MauiNet Pulehu Farm. Site weather station located at the same elevation 0.78 km north.

Characteristics of rhizobia that may influence the outcome of competition include host genotype compatibility (Keyser and Cregan 1987), motility and chemotactic responses (Hunter and Fahring 1980; Wadisirisuk *et al.* 1989), and ability to attach to host roots and initiate nodule formation (Dart 1977). While researchers agree that indigenous rhizobia have a tremendous impact on competition for nodule occupancy by inoculant rhizobia, considerable disparity exists in the literature concerning the influence of other environmental variables.

Some evidence indicates that, in the absence of indigenous rhizobia, competitive ability is a stable characteristic of rhizobial strains as long as plant growth conditions are favorable (George *et al.* 1987; Abaidoo *et al.* 1990). In other words, the competition pattern exhibited among several introduced rhizobial strains remains constant as long as the environmental conditions remain within the ecological amplitude (range of tolerance) of the strains in question. Implicit in this concept is the concept that competitive competence may indeed be influenced by extreme environments, some of which may be within the ecological amplitude of the crop. It is generally thought that crops are more sensitive to environmental adversity than are rhizobia (Lowendorf 1980); however, certain aspects of competition such as bacterial motility, attachment, and nodule initiation may be more sensitive to changes in environmental conditions than either crops or rhizobia living saprophytically.

In this study, the influence of environmental factors on competition for nodule occupancy by rhizobia was investigated from two perspectives: (i) between inoculant and indigenous rhizobia for three legume hosts grown in five environments and (ii) among three select inoculant strains for each of four legume hosts grown in the different environments. Competitive success of inocula on all species was also evaluated in relation to size and competitiveness of indigenous rhizobial populations, inoculant application rate, and yield response to inoculation. We took advantage of the diverse environments present at five well-characterized sites in the Maui Soil, Climate, and Land Use Network (MauiNet) (Soil Conservation Service 1984), which provided a suitable database to correlate environmental factors with competition for nodule occupancy on different legumes. Identification of environmental factors that strongly influence the outcome of competition may enable manipulation of conditions or selection for particular strain characteristics that may give the balance of the advantage to inoculant strains.

Materials and methods

Field inoculation trials

Eight field inoculation trials were conducted at five ecologically diverse sites on the island of Maui, Hawaii, from August 1986 to May 1988 (Table 1). Two to four legumes were grown in each trial, chosen from among the following: soybean, *Glycine max* cv. Clark IV (P. Cregan, USDA Nitrogen Fixation Laboratory, Beltsville, Md.); lima bean, *Phaseolus lunatus* cv. Henderson's Baby; bush bean, *Phaseolus vulgaris* cv. Bush Bountiful; cowpea, *Vigna unguiculata* cv. Big Boy at sites 2 and 3 and cv. Knuckle Purplehull at the other sites; peanut, *Arachis hypogaea* cv. Burpee Spanish at site 1 and cv. McRan Valencia at site 3; leucaena, *Leucaena leucocephala* cv. K-8; tinga pea, *Lathyrus tingeatus*; and clover, *Trifolium repens* cv. Regal Ladino. This protocol yielded 27 legume species by site observations. There were four replications. Design, installation, harvest, and analysis of these trials; enumera-

TABLE 2. Pearson correlation coefficients (r) for environmental factors influencing percent nodule occupancy by inoculant rhizobia and size of indigenous rhizobial populations

Species	Variable	Log R^a	Organic C (%)	Total soil N (%)	Soil N mineralization ($\mu\text{g} \cdot \text{g}^{-1} \cdot \text{week}^{-1}$)	Soil temp. ($^{\circ}\text{C}$) at 10 cm depth		MAR (mm/year) ^b
						Maximum	Average	
<i>P. lunatus</i>	Occupancy ^c	-0.89* ^d	-0.89*	-0.88	-0.93*	0.88*	0.89*	nm ^e
	Log R		0.97*	0.95*	0.93*	-0.98**	-0.98**	0.88*
<i>V. unguiculata</i>	Occupancy	-0.88*	-0.74	-0.64	-0.60	0.84	0.72	nm
	Log R		0.96**	0.92*	0.91*	-0.98**	-0.93*	0.90*
<i>P. vulgaris</i>	Occupancy	0.57	0.29	0.24	0.27	-0.38	-0.47	nm
	Log R		0.94*	0.90*	0.88*	-0.98**	-0.94*	0.89*
All 3 species ($n = 15$)	Occupancy	-0.74**	-0.49	-0.46	-0.46	0.52*	0.47	nm
	Log R		0.79***	0.76***	0.75**	-0.81***	-0.78***	0.73**

^aLog(1 + MPN of indigenous rhizobia) per gram soil.

^bSee Table 1.

^cPercent nodule occupancy by three inoculant rhizobial strains as determined by immunofluorescence microscopy.

^dSignificance of correlation coefficient: *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

^enm, not meaningful as crops were drip irrigated.

tion of indigenous rhizobia; other site characteristics; and collection of climatic data have been described previously (Thies *et al.* 1991).

Inoculum strains and inoculation procedure

Inoculum strains used were USDA 110 (U.S. Department of Agriculture, Beltsville, Md.), USDA 138, and USDA 136b for *G. max*; TAL 22 (NifTAL Project, Paia, Hawaii), Nit 176A22 (Nitragin Co., Madison, Wis.), and CIAT 257 (Centro Internacional Agricultura Tropical, Cali, Columbia) for *P. lunatus*; TAL 182, CIAT 632, and CIAT 899 for *P. vulgaris*; Nit 176A30, TAL 209, and CIAT 71 for *V. unguiculata*; Nit 176A22, Nit 176A30, and CIAT 71 for *A. hypogaea*; TAL 82, CB 81 (Commonwealth Scientific Industrialization Research Organization, Brisbane, Australia), and TAL 1145 for *Leu. leucocephala*; Nit 92A3, Allen 344 (O.N. Allen, University of Wisconsin, Madison), and Nit 128C75 for *La. tingeatus*; S11-6, S11-16, and AR 21 (P.J. Bottomley, Oregon State University, Corvallis, Oreg.) for *T. repens*. All strains used were from the germ-plasm collection of the NifTAL Project, Paia, Hawaii. Seeds were inoculated at a rate of 10^5 – 10^7 cells per seed, depending on seed size, with an equal number of three serologically distinct, effective strains of homologous rhizobia in a peat-based inoculant (Thies *et al.* 1991). A 40% gum arabic solution was used as a sticker and a final coating of finely ground CaCO_3 was applied after seed inoculation to facilitate handling. Seeds were inoculated immediately prior to planting. Subsamples of inoculated seeds were stored at 4°C overnight and total number of rhizobia per seed was counted 24 h after inoculation. Resulting values defined the application rate per seed for each legume.

Nodule harvest and determination of nodule occupancy

Nodules were harvested from the root systems of 10 randomly selected plants per plot at full bloom (generally 35–45 days after planting) for the grain legumes and 71–74 days after planting for the forage legumes. Nodules were dried at 70°C , stored at room temperature, and rehydrated overnight in a few drops of sterile water under refrigerated conditions prior to analysis. Nodule occupancy was determined on 24–36 randomly selected nodules per plot by use of strain-specific fluorescent antibodies, as described by Somasegaran and Hoben (1985). If one or more of the three inoculant strains was detected in a nodule, the nodule was considered to be occupied by introduced rhizobia.

Data analysis

Pearson correlation and multiple linear and stepwise regression analyses were used to evaluate the relationship between nodule occupancy by inoculant strains and details of the environment. Soil variables used in the analyses were most probable number (MPN)

of indigenous rhizobia; organic C and N content (percent); C:N ratio, N mineralization (micrograms N per gram soil per week) from an incubation assay conducted at 40°C for 7 days under water-logged conditions (Keeney 1982); sum of extractable base nutrient ions (milliequivalents per 100 g soil dry weight); clay, silt, and sand content (percent); P retention (percent); bulk density; water-holding capacity; and pH. Climate variables used were median annual rainfall; maximum, minimum, and average soil temperature at 10 cm depth for the first 10 days following planting, and for the interval between planting and nodule harvest; maximum and minimum air temperature for the first 10 days after planting; average soil temperature at 50 cm depth during the interval between planting and nodule harvest; and the Julian date of planting.

Significance of differences in interstrain competition for nodule occupancy by inoculant rhizobia was determined by a χ^2 test for deviation from a 1:1:1 ratio. Significance of differences in nodule occupancy by the two more similar of the three inoculant strains was determined using a paired t -test. In these analyses, double occupancy by inoculant strains was scored as positive for each strain. Therefore, total nodule occupancy exceeded 100% in some cases. However, nodule occupancy by inoculant strains for each legume species was adjusted to total 100% prior to correlation analysis.

Multiple linear regression analysis was performed using the MGLH module of SYSTAT v 4.0 (Wilkinson 1988). All other analyses were performed using SAS procedures (SAS Institute 1986).

Results and discussion

Environmental effects on competition for nodule occupancy between inoculant and indigenous rhizobia

The influence of environmental factors on inoculant strain competition for nodule occupancy in the presence of indigenous populations of rhizobia was studied for the legume hosts: lima bean, bush bean, and cowpea. Nodule occupancy by inoculant strains on all three species was most significantly correlated with $\log_{10}(1 + \text{MPN of indigenous rhizobia})$ ($\log R$) whether the species were analyzed individually or as a group (Table 2). These two variables were inversely related for lima bean and cowpea and positively correlated for bush bean. Decreasing nodule occupancy by inoculant strains with increasing number of indigenous rhizobia, as was observed for lima bean and cowpea, is consistent with other reports (Ireland and Vincent 1968; Weaver and Frederick 1974a). Positive correlation

between these variables observed for bush bean may have resulted from the presence of noncompetitive indigenous populations of *Rhizobium leguminosarum* biovar *phaseoli* or an overestimation of the size of the effective population, as discussed in Singleton and Tavares (1986) and Thies *et al.* (1991).

For cowpea and bush bean no other variables in the environmental database were significantly correlated with nodule occupancy by inoculant strains. Occupancy by inoculant strains on lima bean was related to several soil fertility and temperature variables (Table 2). Successful nodule occupancy by these inoculant strains was inversely related to soil organic C content and measures of soil N availability and positively correlated with soil temperature. This is contrary to the expected effect of these variables on biological activity in soil. In contrast, log *R* was always positively related to measures of soil fertility, including P retention ($r = 0.54$, $p = 0.038$), and median annual rainfall; and inversely correlated with soil temperature variables, pH ($r = -0.75$, $p = 0.001$), and base saturation ($r = -0.58$, $p = 0.024$). All of these relationships are consistent with the anticipated effects of these variables on the activity of microorganisms in soil and are consistent with other reports (Woomer *et al.* 1988). The high significance of correlations between log *R* and these environmental variables most likely reflects the impact of these variables on the incidence of legumes and the ability of indigenous rhizobia to persist at these sites.

Correlation coefficients between environmental variables and nodule occupancy by inoculant strains for lima bean and cowpea were the converse of those observed for log *R* in all instances (Table 2). In accord with the positive correlation observed between nodule occupancy by inoculant bush bean rhizobia and log *R*, correlation coefficients between environmental variables and bush bean nodule occupancy coincided with those observed for log *R*. In a stepwise regression procedure (SAS Institute 1986) performed for the dependent variable, percent nodule occupancy by inoculant strains, and all environmental variables measured, log *R* was the only variable that met the 0.15 significance level for entry into the model. This model takes the form

$$[1] \quad y = 97.88 - 15.03(\log_{10}(x + 1))$$

where *y* is percent nodule occupancy by inoculant rhizobia and *x* is the MPN of indigenous rhizobia ($r^2 = 0.51$, $p < 0.001$). These results indicate that the number of indigenous rhizobia present at a site is the primary environmental factor influencing total nodule occupancy by inoculant strains. Other environmental factors appear to exert their influence on nodule occupancy by inoculant strains indirectly through their effect on the size of the indigenous rhizobial population.

The model proposed above agrees closely with that proposed by Weaver and Frederick (1974a). However, in their equation, Weaver and Frederick include the additional independent variable, inoculant application rate. Because inoculant was applied at consistently high rates in these trials, the coefficient for inoculant application rate generated by regression analysis with these field data was not significant. Ability of the equation proposed by Weaver and Frederick (1974a) to describe field results ($r^2 = 0.52$, $p < 0.001$) was, therefore, not different from that of the equation described above (eq. 1). Results of regression analyses using the models proposed by Amarger and Lobreau (1982) and Beattie *et al.* (1989) ($r^2 = 0.18$, $p < 0.05$), although

significant, did not explain as much of the observed variation in percent nodule occupancy by inoculant rhizobia as that described above (eq. 1).

Competitive success of inoculant strains and response to inoculation

Although nodule occupancy by inoculant strains declined as numbers of indigenous rhizobia increased, inoculant strains were, in general, quite competitive (Table 3). Weaver and Frederick (1974b) reported that for inoculant rhizobia to occupy greater than 50% of the nodules formed in the presence of indigenous rhizobia, they must be applied at a rate 1000 times higher than the estimated size of the indigenous population (per gram soil). Greater than 50% nodule occupancy by inoculant strains was achieved in 76% of the observations where inoculant rhizobia were applied at a rate less than 1000 times the size of the indigenous rhizobial population (Table 3). This result demonstrates the tremendous inoculation success, as measured by nodule occupancy by inoculant rhizobia, achieved in these trials across a wide range of environments. Inoculant was applied at realistic economic rates, which indicates that existing inoculation technology may be adequate for successful nodule establishment by inoculant rhizobia.

Nodule occupancy by inoculant rhizobia was significantly correlated ($r = 0.43$, $p < 0.02$) with percent increase in yield due to inoculation. However, trials where inoculant strains occupied greater than 50% of the nodules formed did not consistently result in significant yield responses to inoculation (Table 3). This could be due to a high average effectiveness of indigenous rhizobial populations. A significant inoculation response was achieved in all trials where the ratio of applied to indigenous rhizobia exceeded 1000 to 1, and in only three trials, all with bush bean, where this ratio was less. These results support conclusions reached previously (Thies *et al.* 1991) that, where yield is limited by insufficient soil N, size and effectiveness, rather than competitive success, of the indigenous homologous rhizobial population are the primary environmental factors determining the ability of inoculation to increase yield.

In these trials, competitiveness of indigenous rhizobial populations could be expressed as the ratio of nodule occupancy by indigenous rhizobia to their number in the soil in relation to the inoculant application rate (percent occupancy by indigenous rhizobia $\cdot (\log R)^{-1} \cdot (\log_{10}$ number of rhizobia applied per seed) $^{-1}$). This value provided both a measure of the strength of the competition barrier presented by the indigenous population and a means to compare the relative competitiveness of rhizobial populations across sites (Table 3). For example, at the two sites where peanut was grown (sites 1a and 3a), numbers of indigenous rhizobia were equal, yet inoculant strains occupied less than half the number of nodules at site 1a than they did at site 3a. Using the ratio defined above, it can be seen that indigenous rhizobia at site 1a were more than twice as competitive, under local conditions, as those at site 3a and presented a much stronger competitive barrier to nodule occupancy by inoculant strains. Indeed, the *Bradyrhizobium* sp. population present at site 1 was also more competitive on cowpea and on lima bean than that at any other site (Table 3). With the exception of indigenous rhizobia nodulating cowpea, the next most competitive indigenous population was that present at site 3. Environmental conditions at sites 1 and 3 are harsher than at the other sites (higher soil temperatures and

TABLE 3. Competitive success of inoculant rhizobia in relation to indices of the size and competitive strength of indigenous rhizobial populations

Site No.	Legume species	Nodule occupancy by inoculant rhizobia (%)	Log(1 + MPN indigenous rhizobia)	Indigenous competition barrier ^a	Ratio of applied to indigenous ^b	Inoculation response ^c
1	<i>P. lunatus</i>	92	0.26	235	6002	**** ^d
2	<i>P. lunatus</i>	80	1.79	84	137	
3	<i>P. lunatus</i>	94	0.26	180	10016	****
4	<i>P. lunatus</i>	49	2.49	146	18	
5	<i>P. lunatus</i>	85	1.38	77	132	
1	<i>V. unguiculata</i>	67	1.74	141	94	
2	<i>V. unguiculata</i>	54	3.36	101	2	
3	<i>V. unguiculata</i>	96	1.28	24	227	
4	<i>V. unguiculata</i>	48	4.56	81	<1	
5	<i>V. unguiculata</i>	67	2.45	90	3	
1	<i>P. vulgaris</i>	94	0.91	49	443	**
2	<i>P. vulgaris</i>	89	1.97	40	17	
3	<i>P. vulgaris</i>	83	0.52	225	877	***
4	<i>P. vulgaris</i>	96	2.64	12	24	**
5	<i>P. vulgaris</i>	95	1.51	22	43	
1	<i>G. max</i>	100	0	0	nm ^e	****
2	<i>G. max</i>	100	0	0	nm	****
3	<i>G. max</i>	100	0	0	nm	****
3a	<i>G. max</i>	100	0	0	nm	****
4	<i>G. max</i>	100	0	0	nm	****
5	<i>G. max</i>	100	0	0	nm	
1a	<i>A. hypogaea</i>	31	0.78	715	2479	*
3a	<i>A. hypogaea</i>	65	0.78	267	1892	**
1a	<i>Leu. leucocephala</i>	7	3.22	210	2	
3a	<i>Leu. leucocephala</i>	8	3.77	184	1	
5a	<i>La. tingeatus</i>	88	1.20	66	130	
5a	<i>T. repens</i>	96	0.26	73	294	

^aPercent nodule occupancy by indigenous rhizobia · log(1 + MPN of indigenous rhizobia per gram soil)⁻¹ · log(number of rhizobia applied per seed)⁻¹.

^bNumber of inoculant rhizobia applied per MPN of indigenous rhizobia (both on a per hectare basis).

^cPercent increase in mean yield of inoculated (*I*) over uninoculated (*U*) crops: (*I* - *U*)/*U* × 100.

^dSignificance of inoculation response: *, *p* < 0.10; **, *p* < 0.05; ***, *p* < 0.01; ****, *p* < 0.001.

^enm, not meaningful.

lower median annual rainfall) (Table 1). This may indicate that better adaptation to prevailing environmental conditions by indigenous rhizobia is contributing to their competitiveness. With the exception of site 3, indigenous populations of *Rhizobium leguminosarum* bv. *phaseoli* presented a comparatively weak competition barrier across sites. This may help to explain the consistently anomalous results obtained with bush bean (discussed above).

Competition for nodule occupancy among inoculant rhizobia

To investigate the effects of environmental variation on interstrain competition, four to eight legumes grown in as many as five environments were inoculated with an equal mixture of three serologically distinct strains of homologous rhizobia. For each of the legume species except clover, one of the three inoculant strains was shown to be a poor competitor in all environments (Table 4). Competition for nodule occupancy between the remaining two strains for each species varied between sites and, in some cases, was related to climatic and soil variables. Multiple occupancy by inoculant strains was observed for most legume species (Table 4).

Competition for nodule occupancy on soybean was exclusively between inoculant strains as there were no indigenous *Bradyrhizobium japonicum* at any of the sites. USDA 110

and USDA 138 were the more successful competitors on soybean, occupying on average across sites 42 and 50% of the nodules formed, respectively. Competitive success of these strains did, however, vary according to site (Table 4). In comparison, USDA 136b was a relatively weak competitor. Nodule occupancy by this strain was always significantly less than that of USDA 138 and only at site 5 was not significantly less than that of USDA 110.

George *et al.* (1987) and Abaidoo *et al.* (1990) investigated interstrain competition among USDA 110, USDA 138, and USDA 136b at three and two field sites, respectively. In agreement with the results reported here, these authors found USDA 110 to be a strong competitor for nodule occupancy across sites. However, in contrast with these results, George *et al.* (1987) found USDA 138 to be an extremely poor competitor, occupying less than 5% of nodules formed across sites. USDA 110 was found to consistently occupy greater than two-thirds of nodules formed, while USDA 136b occupied the remainder. Abaidoo *et al.* (1990) found the competitive ability of USDA 138 to be equivalent to that of USDA 136b (33 and 37%, respectively) across sites. However, both strains occupied significantly fewer nodules than USDA 110 (68%).

Nodule occupancy by all three *B. japonicum* strains was significantly correlated with soil minimum temperature at 10 cm depth for the interval between planting and nodule

TABLE 4. Percent nodule occupancy by indigenous and introduced rhizobial strains

Site No.	Legume species	Nodule occupancy (%) ^a					χ^2 ^b	<i>t</i> ^c
		A	B	C	Multiple strains	Indigenous rhizobia		
1	<i>P. lunatus</i>	75	11	0	6	8	115.3	5.9 **
2	<i>P. lunatus</i>	6	67	0	7	20	121.5	11.0 **
3	<i>P. lunatus</i>	14	72	0	8	6	99.0	5.2 **
4	<i>P. lunatus</i>	8	35	0	6	51	83.4	4.5 *
5	<i>P. lunatus</i>	15	58	0	12	15	80.0	4.8 *
1	<i>V. unguiculata</i>	50	17	0	0	33	87.5	4.8 *
2	<i>V. unguiculata</i>	7	32	0	15	46	121.5	5.5 **
3	<i>V. unguiculata</i>	16	68	0	12	4	84.7	4.3 *
4	<i>V. unguiculata</i>	46	0	0	2	52	176.0	10.2 **
5	<i>V. unguiculata</i>	59	4	0	4	33	137.7	5.6 **
1	<i>P. vulgaris</i>	28	50	0	16	6	50.4	1.8 ns
2	<i>P. vulgaris</i>	62	21	0	6	11	77.3	2.1 ns
3	<i>P. vulgaris</i>	47	33	0	3	17	54.2	0.7 ns
4	<i>P. vulgaris</i>	50	40	1	5	4	48.6	1.4 ns
5	<i>P. vulgaris</i>	32	60	0	3	5	61.9	1.8 ns
1	<i>G. max</i>	40	45	2	13	0	35.4	0.6 ns
2	<i>G. max</i>	55	19	4	22	0	53.7	7.0 **
3	<i>G. max</i>	53	33	0	14	0	54.6	3.1 *
3a	<i>G. max</i>	23	59	7	11	0	42.1	3.6 *
4	<i>G. max</i>	31	47	10	12	0	17.3	1.2 ns
5	<i>G. max</i>	17	60	14	9	0	36.5	1.9 ns
1a	<i>Leu. leucocephala</i>	7	0	0	0	93	200.0	
3a	<i>Leu. leucocephala</i>	4	4	0	0	92	55.0	0.4 ns
1a	<i>A. hypogaea</i>	0	31	0	0	69	200.0	
3a	<i>A. hypogaea</i>	4	39	1	21	35	50.0	2.1 ns
5a	<i>La. tingeatus</i>	41	4	37	6	12	25.4	0.2 ns
5a	<i>T. repens</i>	17	35	40	4	4	12.7	0.4 ns

^aFor *G. max* strain, A, USDA 110; B, USDA 138; and C, USDA 136b; for *P. lunatus* strain A, TAL 22; B, CIAT 257; and C, Nit 176A22; for *V. unguiculata* strain A, Nit 176A30; B, TAL 209; and C, CIAT 71; for *P. vulgaris* strain A, TAL 182; B, CIAT 632; and C, CIAT 899; for *Leu. leucocephala* strain A, TAL 82; B, TAL 1145; and C, CB 81; for *A. hypogaea* strain A, Nit 176A30; B, CIAT 71; and C, Nit 176A22; for *La. tingeatus* strain A, Nit 92A3; B, Allen 344; and C, Nit 128C75; for *T. repens* strain A, S11-6; B, S11-16; and C, AR-21.

^b χ^2 for deviation from a 1:1:1 ratio of nodule occupancy by three inoculant strains is 13.82 (2 df, $p < 0.001$).

^c*t*-test is for significance of difference in percent nodule occupancy between the two strains numerically most similar. *, $p < 0.05$; **, $p < 0.01$; ns, not significant.

harvest ($r = 0.94$, $p = 0.006$; $r = 0.85$, $p = 0.034$; and $r = -0.91$, $p = 0.011$ for USDA 110, USDA 138 and USDA 136b, respectively). USDA 110 was the more successful competitor at the warmer sites, whereas USDA 138 and USDA 136b performed better at sites that were consistently cool. Nodule occupancy by USDA 136b was also related to soil water-holding capacity ($r = 0.85$, $p = 0.034$), having higher nodule occupancy in the wetter soils. None of the other environmental variables were significantly correlated with strain competitive ability; however, nodule occupancy by USDA 110 and USDA 136b showed some relationship to soil clay content ($r = 0.72$, $p = 0.10$ and $r = -0.75$, $p = 0.087$, respectively).

These results differ from those of Weber and Miller (1972), who found nodule occupancy by serogroup 110 on soybean cv. Lee to decrease with increasing soil temperature. Kvien and Ham (1985), however, reported that USDA 138 and USDA 110 were equally successful competitors at both high (30°C) and low (15°C) soil temperatures on four soybean cultivars. Both of these experiments were conducted in controlled-environment chambers. Under field conditions, George *et al.* (1987) and Abaidoo *et al.* (1990) found no significant relationship between either soil temperature or type

and interstrain competition for nodule occupancy among USDA 110, USDA 138, and USDA 136b. Average soil temperatures in the experiment of George *et al.* (1987) ranged from 20.7 to 25.3°C and were 22 and 25°C at the two sites used by Abaidoo *et al.* (1990). Average soil temperatures in the first 10 days following planting in these trials ranged from 20.5 to 30.8°C and were not different from average soil temperatures recorded across the crop duration (Thies *et al.* 1991). Perhaps the more extreme temperatures recorded in these experiments provided more environmentally challenging conditions for these microorganisms, and resulted in the observed temperature-related differences in nodule occupancy by these strains.

In competition for nodule occupancy on lima bean, Nit 176A22 failed to occupy any of the nodules formed at the five sites. This strain was subsequently shown also to be ineffective on this host. Nodule occupancy by the other strains used, TAL 22 and CIAT 257, differed significantly at all sites (Table 4) but was not significantly correlated with any of the environmental variables examined.

For cowpea, CIAT 71 was not detected in any of the nodules examined from any of the sites. Nodule occupancy by Nit 176A30 and TAL 209 differed significantly at all sites

(Table 4). Two genotypes of cowpea were used in these trials, and rather than being consistently related to details of the environment, nodule occupancy by these strains was more closely related to cowpea genotype. Nit 176A30 was the more successful competitor on *V. unguiculata* cv. Big Boy, whereas, TAL 209 occupied a significantly greater proportion of nodules on *V. unguiculata* cv. Knuckle Purplehull.

CIAT 899 was identified as a poor competitor in these trials as it was not detected in bush bean nodules from sites 2, 3, or 5 and occupied less than 6% of nodules examined from the other sites (Table 4). Nodule occupancy by TAL 182 and CIAT 632 did not differ significantly at any of the sites. However, occupancy by these two strains was significantly correlated with soil sodium content ($r = -0.89$, $p = 0.043$ and $r = 0.91$, $p = 0.032$ for TAL 182 and CIAT 632, respectively) and soil clay content ($r = 0.89$, $p = 0.045$ and $r = -0.89$, $p = 0.043$ for TAL 182 and CIAT 632, respectively). TAL 182 was more successful in higher clay soils, whereas CIAT 632 performed somewhat better in soils with higher sodium concentrations.

Competition for nodule occupancy between inoculant strains on peanut, leucaena, tinga pea, and white clover could not be correlated with the environmental database because these crops were grown only at a few sites. However, significant differences in nodule occupancy between inoculant strains on these species were observed (Table 4).

For the legume species, soybean, cowpea, and bush bean, nodule occupancy by most of the inoculant strains was correlated with minimum soil temperature and clay content at $p = 0.10$ or lower. Although higher soil temperatures may result in differential survival of rhizobia in the rhizosphere, neither Abaidoo *et al.* (1990) nor Moawad *et al.* (1984) found a significant relationship between size of the rhizosphere population of different rhizobial strains and their corresponding nodule occupancy. Both soil temperature and clay content may influence nodule occupancy by inoculant rhizobia through effects on bacterial motility, chemotaxis, or hormone production. All of these activities are intrinsic microbial characteristics that have been suggested as mechanisms that may enhance the ability of rhizobial strains to initiate root infections (Bauer 1981). With few exceptions, the other environmental variables examined were not significantly correlated with competition among inoculant strains for nodule occupancy. Soil acidity has been correlated with nodule occupancy in other studies (Dughri and Bottomley 1983, 1984). This relationship and effects of moisture stress could not be evaluated in this study because more acidic soils were limed and fields irrigated to remove these variables as limitations to maximum yield. However, considering the extent of differences among the five environments (Table 1), it is remarkable that so few variables were found to significantly influence competition for nodule occupancy between strains of inoculant rhizobia. This result supports the suggestion of George *et al.* (1987) that highly competitive inoculant strains can be identified that will perform well across a range of environments.

In summary, competition between applied and indigenous rhizobia was most strongly influenced by the size of indigenous rhizobial populations. Results indicate that other environmental factors do not play a major role in determining the outcome of competition except as they influence the size of indigenous rhizobial populations. Remarkably few variables were found to significantly influence the ability of

introduced strains to compete for nodule occupancy among themselves when present in equal numbers in the inoculum. This indicates that intrinsic characteristics of the bacteria, rather than environmental factors, more likely determine the competitive ability of inoculant strains.

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