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Inoculation Response of Legumes in Relation to the Number and Effectiveness of Indigenous Rhizobium Populations

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The response of legumes to inoculation with rhizobia can be affected by many factors. Little work has been undertaken to examine how indigenous populations of rhizobia affect this response. We conducted a series of inoculation trials in four Hawaiian soils with six legume species (*Glycine max*, *Vigna unguiculata*, *Phaseolus lunatus*, *Leucaena leucocephala*, *Arachis hypogaea*, and *Phaseolus vulgaris*) and characterized the native rhizobial populations for each species in terms of the number and effectiveness of the population for a particular host. Inoculated plants had, on average, 76% of the nodules formed by the inoculum strain, which effectively eliminated competition from native strains as a variable between soils. Rhizobia populations ranged from less than 6×10^0/g of soil to 1×10^4/g of soil. The concentration of nitrogen in shoots of inoculated plants was not higher than that in uninoculated controls when the most probable number MPN counts of rhizobia were at or above 2×10^1/g of soil unless the native population was completely ineffective. Tests of random isolates from nodules of uninoculated plants revealed that within most soil populations there was a wide range of effectiveness for N_2 fixation. All populations had isolates that were ineffective in fixing N_2. The inoculum strains generally did not fix more N_2 than the average isolate from the soil population in single-isolate tests. Even when the inoculum strain proved to be a better symbiont than the soil rhizobia, there was no response to inoculation. Enhanced N_2 fixation after inoculation was related to increased nodule dry weights. Although inoculation generally increased nodule number when there were less than 1×10^2 rhizobia per g of soil, there was no corresponding increase in nodule dry weight when native populations were effective. Most species compensated for reduced nodulation in soils with few rhizobia by increasing the size of nodules and therefore maintaining a nodule dry weight similar to that of inoculated plants with more nodules. Even when competition by native soil strains was overcome with a selected inoculum strain, it was not always possible to enhance N_2 fixation when soil populations were above a threshold number and had some effective strains.

Soils may vary considerably in the nature of their established rhizobial populations. Populations of *Rhizobium* may contain a number of species, and within a species, strains may exhibit a range of symbiotic effectiveness with a legume (2, 5). In addition, the number of naturalized rhizobia varies between soils by many orders of magnitude (10). The number of rhizobia in soil is determined by both physiochemical and biological factors (for a review see Lowendorf [8]); however, no systematic study on the determinants of the number of rhizobia in soil or their effectiveness has been undertaken.

Agricultural legumes are commonly inoculated with selected strains of rhizobia in the expectation that inoculation will increase nitrogen fixation and crop yield. While dramatic increases in yield from inoculation are possible (1), many factors limit the full expression of the inoculation response: (i) failure to establish the inoculum at normal inoculation rates due to competition from native soil strains (4, 7) or reduced inoculum viability due to stresses such as temperature (9) and desiccation (16); (ii) plant yield potential may be limited by environment, so that N demand is met by available soil N or through N_2 fixation by less effective soil rhizobia (13); and (iii) a sufficient population of soil rhizobia may exist to meet host demand for symbiotic N_2 under nonlimiting growth conditions for the host.

While much effort has been expended to identify strains of *Rhizobium* that are superior in N_2 fixation (6) and tolerant to a variety of soil stresses (for a review see Lowendorf [8]). There is little understanding of how native populations of rhizobia determine legume N_2 fixation potential. We conducted a series of inoculation trials with legumes in soils with a diversity of native rhizobial populations. Soil populations were characterized in terms of the number of invasive rhizobia and the effectiveness of the population on a legume host. Other factors which may condition the response to inoculation such as inoculation failure, low plant demand for nitrogen, and available soil N were eliminated as variables in these experiments. We therefore were able to examine how the nature of soil rhizobial populations affects our ability to obtain a response to inoculation with selected rhizobia and define what constitutes a sufficient population of rhizobia to meet host N demands.

MATERIALS AND METHODS

General experimental approach. Greenhouse inoculation trials of different legume species were carried out in four different soils. These experiments had three main components: (i) each species was inoculated with large numbers of a selected strain of *Rhizobium* or not inoculated; (ii) the number of native soil rhizobia for each species was determined at planting; and (iii) the average effectiveness of native soil strains was determined by conducting strain tests of isolates made from the uninoculated control for each species. As a measure of yield potential in the system, one species for each soil was provided with 600 mg of N per kg of soil as a positive N control. The inoculation trials were carried out in N-free soil conditions by immobilizing soil N with sugarcane bagasse.

Site selection, soil sampling, and preparation. Soils from four sites on the island of Maui, Hawaii, were selected for

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TABLE 1. Site and characteristics of four Hawaiian soils

Site	Soil type	Elevation (m)	Annual rainfall (cm)	Mean soil temp (°C)	pH	Legume species at site
Haiku	Humoxic tropohumult	305	165	21	4.8	*Desmodium, Indigofera, Crotalaria*
Makena	Aridic haplustoll	30	38	24	6.5	*Prosopis*
Iao	Typic huplustoll	121	83	23	6.5	*Desmodium, Crotalaria, Leucaena*
Keahua	Typic torrox	420	51	23	7.1	*Desmodium, Leucaena, Prosopis*

these experiments (Table 1). None of the sites are used for agriculture, and all the legumes at the sites were growing wild. The top 2 cm of soil was removed from a sample site, and the soil was mined to a depth of 20 cm and passed through a 6.5-mm screen. The screened soil was removed in plastic buckets for storage and used as soon as possible.

Bulk soil was mixed with screened sugarcane bagasse (6.5-mm screen) at 10 g/kg of soil (oven dry basis, 65°C). The Haiku soil was amended with 3.0 g of $Ca(OH)_2$ per kg of soil to bring the pH to 5.8. The other soils remained at their existing pH with 50 mg of Ca as $CaSO_4$ added as a nutrient.

Air-dried soil (7.0 kg of Haiku, 7.3 kg of Makena, 7.0 kg of Iao, and 7.0 kg of Keahua, oven-dried weight basis, 110°C) was placed in 11.4-liter plastic pots. Soils were brought to 0.01 MPa tension (corresponding to 370, 410, 370, and 320 g of H_2O per kg of soil for Haiku, Makena, Iao, and Keahua soils, respectively) by adding liquid nutrient stocks and water. Nutrients added (in milligrams per kilogram of soil): P, 600; K, 750; Mg, 100; S, 133; Fe, 7.5; Fn, 2.5; B, 1.8; Mo, 0.2; Cu, 0.8; Mn, 2.3; Co, 0.2. Sources were K_2HPO_4, $MgSO_4 \cdot 7H_2O$, and micronutrients from a liquid concentrate (Monterey Chemical Co.). Soil was allowed to equilibrate for 2 to 3 days before planting.

Plant culture. Seeds of four legume species were surface sterilized in 2% sodium hypochlorite for 2 min, rinsed, and planted in sterile vermiculite. Planting began when the radicles were 2 to 3 cm long. Ten planting holes were made in the wet soil to a depth of 2 to 3 cm with a glass rod; 1 ml of a turbid YEM broth culture of the inoculant strain (15) was placed in each planting hole. Radicles (2 cm) were placed in the planting hole, and enough sterile water was added to each hole to ensure root-soil contact. The surface of the pots was then covered with 1 cm of sterile aquarium gravel. Pots were maintained at field capacity (0.001 mPa) on a daily basis by applying water passed through a 200-molecular-weight reverse osmosis system.

The positive nitrogen control for each soil type received a total of 600 mg of N per kg of soil as NH_4NO_3 in water in equal applications at planting and 21 days later.

Plants were thinned to seven per pot 1 week after planting. Pots were arranged in the greenhouse in a randomized complete block design.

Early harvest. An early harvest of three plants was performed between 16 and 26 days after planting, depending on the time of year and the species being tested. Three random plants were cut at the soil surface, and the leaf area of the most recently expanded trifoliolate was determined. For plants in Haiku and Makena soils, a leaf area meter was used. For the Keahua and Iao soils, the plants were kept turgid by being placed in plastic bags. The trifoliolates were photocopied, the leaf area was cut out, and the area was determined by weighing the paper cut-out. Plants were dried (65°C), weighed, ground, and digested in H_2SO_4, and NH_4 was determined by the micro-Kjeldahl test.

Late harvest. The remaining plants were harvested between 30 and 43 days after planting, depending on the time of the year and the species. Plants were cut at the soil surface, oven dried (65°C), ground, and digested in H_2SO_4, and NH_4 was determined by a micro-Kjeldahl assay. Roots were removed from the pots at harvest, placed in 2-liter plastic containers, and incubated for 0.5 h in 5% C_2H_2 (vol/vol) in air. Ethylene production was determined by gas chromatography. Root systems were washed free of soil, and nodules were removed and counted. Random subsamples of nodules from uninoculated pots were selected for isolation. Subsamples had at least 25 to 60 nodules per species from the four replications. At least 50% of the total nodules were sampled when the total number was below 50. All nodules were sampled for plants with fewer than 25 nodules. The weight of fresh nodules and subsamples was recorded. All nodules except subsamples for isolation were dried (65°C) and weighed.

Inoculum strains and plant cultivars. Strains used for inoculation were *Glycine max* USDA110 (U.S. Department of Agriculture, Beltsville, Md.), *Phaseolus lunatus* TAL22 (NifTAL Project, Paia, Hawaii), *Phaseolus vulgaris* TAL182 (NifTAL Project), *Vigna unguiculata* TAL209 (NifTAL Project), *Leucaena leucocephala* CIAT1967 (Centro Internacional Agricultural Tropical, Cali, Colombia), *Arachis hypogaea* TAL169 in Iao soil (NifTAL Project), and TAL1000 for others (NifTAL Project). Cultivars used were *G. max* cv. Davis, *P. lunatus* cv. Henderson Baby, *L. leucocephala* cv. K-8, *P. vulgaris* cv. Bluelake, *V. unguiculatia* cv. Vita-5 (from International Institute for Tropical Agriculture, Ibadan, Nigeria) for Haiku soil and cv. California Blackeye for the remaining soils, and *A. hypogaea* cv. Burpee Spanish.

Characterization of native soil populations. Before planting, soil cores (2 cm) were taken from random pots, mixed, and subsampled for moisture determination. A 50-g (oven-dried basis, 110°C) subsample of soil was diluted in 450 ml of sterile water, except Keahua soil for *P. lunatus*, which was diluted in 150 ml of water. Serial 1:10 dilutions (1:4 for *P. lunatus* in Keahua soil) were then made.

Most probable number (MPN) determinations for the number of rhizobia in the soil (15) for each species were made by applying a 1-ml portion of each dilution to four replicate plants growing in plastic growth pouches (American Scientific Products) (17) with 30 ml of an N-free nutrient

TABLE 2. Proportion of nodules formed by inoculated strains[a]

Soil	% of total nodules					
	G. max	*A. hypogaea*	*P. lunatus*	*V. unguiculata*	*P. vulgaris*	*L. leucocephala*
Haiku	100	79	97	57		
Makena	100	98	100	96		
Iao	100	94		53	100	
Keahua	100		88	88		76

[a] Nodulating strains were identified by immunofluorescence microscopy. Nodules having both inoculum and soil strains counted as nodules formed by the inoculum.

TABLE 3. Total shoot N content

Soil	Shoot N content (mg/plant)[a]															
	G. max		*A. hypogaea*		*P. lunatus*		*V. unguiculata*		*P. vulgaris*		*L. leucocephala*		Controls (N supplied)			
	I	U	I	U	I	U	I	U	I	U	I	U	*G. max*	*P. lunatus*	*A. hypogaea*	*L. leucocephala*
Haiku	49[b]	14	67	61	55	59	67	75					449			
Makena	83[b]	10	65[b]	18	45[b]	7	98[b]	31						682		
Iao	54[b]	6	74	77			59	71	115[b]	10					122	
Keahua	77[b]	14			78[c]	62	118	108			14	16				35

[a] I, Inoculated; U, uninoculated.
[b] Significant increase ($P = 0.001$).
[c] Significant increase ($P = 0.05$).

solution (12). Pouches were scored for nodulation 14 to 21 days after inoculation.

Isolates from randomly selected nodules of uninoculated plants were made by surface sterilizing nodules in 2.75% sodium hypochlorite for 1 to 3 min (depending on nodule size) and rinsing them in sterile water. Nodules were crushed in sterile water and streaked onto a plate of YEM agar containing bromthymol blue (15). Single-colony isolates were picked from plates, numbered, and stored on YEM agar slants. Random isolates (15 to 23 or 25% of the isolates, whichever was less) were selected and tested for effectiveness in comparison to the inoculum strains for each test plant grown in a soil with invasive rhizobia. The one exception was *P. vulgaris* in Iao soil, for which the MPN analysis indicated that invasive rhizobia were present but the host failed to nodulate effectively (as determined by C_2H_2 reduction) in the soil pots. Isolates from nodules of the MPN plants indicated that these rhizobia were slow-growing, alkali-producing organisms not characteristic of *Rhizobium phaseoli*.

Effectiveness tests were carried out in 1-liter plastic pots (Lab Tek Products) filled with wet vermiculite. The lid of the pot had four 1.25-cm holes punched in it. The hole in the center was to receive a weighted drip irrigation emitter, and there were three planting holes around the center hole. A similar hole was punched in the bottom of each vessel. A planting hole was made in the vermiculite and inoculated with a 2-ml suspension of culture from agar slants of the appropriate isolate or inoculum strain or with an invasive, nonfixing strain for controls. Pots were planted with germinated seedlings (radicle length, 2 to 3 cm), a drip irrigation emitter was placed in the center hole, and the holes were covered with sterile aquarium gravel. Plants were thinned to two per pot 1 week after planting, harvested 28 to 40 days after planting (depending on species), dried, and weighed.

TABLE 4. MPN[a] of rhizobia

Soil	No. of rhizobia/g of soil					
	G. max	*A. hypogaea*	*P. lunatus*	*V. unguiculata*	*P. vulgaris*	*L. leucocephala*
Haiku	0^b	6×10^1	1×10^2	2.0×10^1		
Makena	0^b	$<6 \times 10^{0b}$	$<6 \times 10^{0b}$	1.0×10^{0b}		
Iao	0^b	1×10^2		5.8×10^3	1×10^{4b}	
Keahua	0^b		7.2×10^{0c}	3.0×10^1		3×10^3

[a] Fiducial limits are determined by dividing or multiplying numbers by 3.8 for all plants except *P. lunatus* in Keahua soil, which had a factor of 2.7.
[b] Significant increase due to inoculation ($P = 0.001$).
[c] Significant increase due to inoculation ($P = 0.05$).

Each pot received 100 ml of N-free nutrient solution (12) per day by timed pump from a 335-liter reservoir for the first 3 weeks. After 3 weeks, pots received two 100-ml nutrient solution applications per day. All isolates selected for testing nodulated their hosts. There were three replications for each isolate.

Success of inoculum strain. Dried nodules from inoculated plants (at least 35 nodules or 5% of the total, whichever was greater) in the soil pot tests were selected at random for serological identification. Nodules were rehydrated for 2 to 3 h in sterile water, a toothpick was inserted into the bacteroid zone, and a small smear was heat-fixed on a glass slide. Nodule smears were treated with gelatin-rhodamine isothiocyanate (3) and then with a drop of rabbit antiserum specific for the inoculum strain. Homologous controls were from YEM agar slants (15). Slides were incubated for 30 min in a moist chamber, rinsed in saline, and then treated with goat anti-rabbit fluorescent antibody conjugated by the method of Schmidt et al. (11). Positive reactions were determined by epifluorescence microscopy. From 20 to 30 nodules from uninoculated pots were tested for cross-reaction with antisera against the inoculum strains. Cross-reaction was less than 14%, except for *P. vulgaris* in Iao soil; however, the only nodulating organisms in that soil were ineffective cowpea rhizobia.

Data analysis. Data were analyzed by analysis of variance; each soil was treated as a separate randomized complete block design.

RESULTS AND DISCUSSION

The nature of soil rhizobial populations may affect the N_2 fixation potential of legumes. First, the number of available invasive rhizobia may be insufficient to nodulate the host adequately. Second, the average effectiveness of the population may be inadequate to support the host's fixed-N_2 requirements. When one or both conditions are present, we might reasonably expect that successful inoculation with an effective *Rhizobium* strain would enhance N_2 fixation.

By increasing the number of rhizobia inoculated to soybean plants (*G. max*), Weaver and Frederick (18, 19) increased nodule occupancy by the inoculum strain. Nodule number was increased in their experiments by increasing the inoculum when there were less than 10^3 rhizobia per g of soil. However, Weaver and Frederick (19) did not observe an increase in plant yield with inoculation even when there were less than 1.1×10^1 soil rhizobia per g of soil and the inoculum strain formed the majority of nodules. They attributed the lack of inoculation response to restricted growth conditions in which available soil N was not a limiting factor. However, the native populations may have been sufficient to meet the yield potential under their experimental conditions.

TABLE 5. Effectiveness of native populations of rhizobia[a]

Soil	*A. hypogaea*		*P. lunatus*		*V. unguiculata*		*L. leucocephala*	
	SD	Range	SD	Range	SD	Range	SD	Range
Haiku	+0.63	3.3	−1.79	3.1	−0.07	1.5		
Makena	−0.24	4.4	+0.32	1.7	+0.76	7.7		
Iao	−0.66	3.1			−0.23	4.6		
Keahua			+0.51	6.4	−0.36	1.6	+1.47	1.6

[a] The effectiveness of the inoculum strain was compared with the average effectiveness of the native soil populations. SD is the difference (in standard deviations) of shoot dry weight between plants receiving an inoculum strain and plants receiving single cultures of random nodule isolates from the soil experiments. Range was calculated by dividing the shoot dry weight of the highest-yielding soil isolate by that of the lowest-yielding isolate. *P. vulgaris* was ineffective in Iao soil.

The experiments presented in this paper attempt to further define a sufficient rhizobial population in terms of number and ability to meet host N requirements. Our experimental approach has the advantage of eliminating other potentially confounding variables such as restricted growth, site-dependent soil N availability, and site-specific competition from native soil strains.

Table 2 indicates the high frequency of nodules in inoculated plants that were formed by the inoculum strain. This success rate is much greater than that observed in the field with normal inoculation rates in the presence of native soil rhizobia (7, 19). Our inoculation methods effectively eliminate competition from native rhizobia as a variable in these experiments.

Inoculation significantly increased shoot N in 50% of the species-soil combinations (Table 3). Soybean responded to inoculation in every soil with at least a fourfold increase in shoot N. All other species tested responded in Makena soil, whereas only one other species in the Iao and Keahua series responded to inoculation. The response to inoculation of lima bean (*P. lunatus*) in Keahua soil was significant but substantially less than in Makena soil. The response of inoculated cowpea (*V. unguiculata*) in Keahua soil was slightly greater than that of the uninoculated control ($P \leq 0.1$), whereas in Makena soil the inoculated cowpea had three times more N than the uninoculated plant. Kidney bean (*P. vulgaris*) responded significantly to inoculation in Iao soil.

Essentially all shoot N was derived from fixation in these experiments, since soil N was immobilized by the incorporation of ground sugarcane bagasse (K. G. Cassman, Ph.D. thesis, University of Hawaii, Honolulu, 1979). Uninoculated soybean generally remained unnodulated and was N deficient in all soils (less than 18 g of N per kg of dry tissue; data not shown).

One species was selected for each soil to serve as a positive nitrogen control to demonstrate the lack of other limiting factors in the growth system. These controls remained unnodulated whether or not soil rhizobia were present. These four legumes (Table 3) have a substantial advantage in early growth when supplied with large amounts of mineral N compared with inoculated plants relying completely on symbiosis for nitrogen nutrition.

Early-harvest data for shoot N and leaf area of the most recent fully expanded trifoliolate (data not shown) were generally consistent with the responses to inoculation and applied N at the final harvest. One exception was in the Haiku soil. Cowpea, peanut (*A. hypogaea*), and lima bean had increased shoot N and leaf area at 18 days after planting in this soil, but their responses were not sustained until harvest. Visible differences between inoculated and uninoculated plants disappeared within 2 to 3 days after the early harvests. There were no early responses to inoculation in the Iao soil.

Native soil populations of rhizobia can be characterized functionally for N_2 fixation potential by determining their species composition, number, and ability to fix N_2. MPN counts of native soil rhizobia are given for each species and soil in Table 4. There were no *Rhizobium japonicum* organisms in any of the soils. The other soils ranged from less than 6×10^0 to 1×10^4 rhizobia per gram of soil.

In Makena soil, responses to inoculation with slow-growing cowpea-type organisms were consistent, and the number of invasive rhizobia was a full log lower than in Kaehua soil, in which the magnitude of the response to inoculation was greatly attenuated. Except for bean (*P. vulgaris*) in Iao soil, there was no response to inoculation when there were more than 2×10^1 rhizobia per gram of soil. The MPN count for bean in Iao soil is an artifact of the MPN system, since all rhizobia forming nodules on these plants in growth pouches were slow growing and produced an alkaline reaction in YEM agar with bromthymol blue not characteristic of *R. phaseoli* (15) and uninoculated plants in Iao soil were not well nodulated. Lima bean had a greater response to inoculation in Keahua soil than did cowpea. The MPN count for lima bean was 1 log lower than that for cowpea and

TABLE 6. Effect of inoculation on number of nodules

Soil	No. of nodules/plant[a]											
	G. max		*A. hypogaea*		*P. lunatus*		*V. unguiculata*		*P. vulgaris*		*L. leucocephala*	
	I	U	I	U	I	U	I	U	I	U	I	U
Haiku	42[b]	1	70[c]	45	62	69	30	30				
Makena	39[b]	2	62[b]	25	90[b]	2	71[b]	14				
Iao	33[b]	2	68[c]	86			55	64	75[b]	8		
Keahua	64[b]	0			142[b]	41	138[b]	72			43	44

[a] I, Inoculated; U, uninoculated.
[b] Significant increase ($P = 0.001$).
[c] Significant increase ($P = 0.05$).

TABLE 7. Effect of inoculation on nodules dry weight

Soil	Dry wt of nodules (mg/plant)[a]											
	G. max		*A. hypogaea*		*P. lunatus*		*V. unguiculata*		*P. vulgaris*		*L. leucocephala*	
	I	U	I	U	I	U	I	U	I	U	I	U
Haiku	127[b]	10	66	63	85	80	109	110				
Makena	174[b]	9	61	43	172[b]	26	427[b]	69				
Iao	140[b]	14	49	61			159	159	184[b]	17		
Keahua	165[b]	0			181[c]	138	386[b]	205			15	13

[a] I, Inoculated; U, uninoculated.
[b] Significant increase ($P = 0.001$).
[c] Significant increase ($P = 0.05$).

below what appeared to be the minimum threshold number (2×10^1) required for maximum N_2 fixation in these experiments.

The strains of rhizobia that make up a native soil population may vary considerably in their effectiveness with a particular host. Effectiveness of soil populations has been reported to follow a normal distribution (2). Nodule isolates from uninoculated plants in soils with native rhizobia ranged considerably in effectiveness with their respective host plants (Table 5). Low-N_2-fixing isolates were found in every soil and produced shoot dry matter yields similar to those of ineffectively nodulated and uninoculated control plants. Although peanut, lima bean, and cowpea cross-inoculate, the range of effectiveness for the three species within a soil was different. Inoculum strains for the most part were not more effective than the average of the soil population (Table 5). Only the inoculum strain for *L. leucocephala* in Keahua soil proved to be better in N_2 fixation than the average soil isolate. The low relative effectiveness of the inoculum strains for peanut and cowpea in Iao soil and for lima bean and cowpea in Haiku soil may explain the slight reduction in N_2 fixed by the inoculated plants in these soils. These two soils had sufficient native rhizobia to meet the yield potential of symbiotic plants. Because the great majority of nodules were occupied in these experiments with a moderately effective inoculum strain, certain highly effective soil strains may not have been able to establish themselves, and therefore yield was reduced slightly. It has been suggested that the host selectively apportions photosynthate to effective nodules (14). This mechanism is functional in uninoculated plants nodulated by soil rhizobia with a range of effectiveness but not in plants nodulated almost entirely by a moderately effective inoculum strain. *L. leucocephala* did not respond to inoculation in Keahua soil despite the superiority of the inoculum strain over the soil population and the fact that the inoculum formed 76% of the nodules.

Even moderately effective strains can elicit large responses when native soil populations are extremely low in number, as in Makena soil, or when the population is completely ineffective, as it was for bean in Iao soil. It appears that very high occupancy of nodule sites by strains of less than average effectiveness may slightly suppress N_2 fixation when there are more than 2×10^1 rhizobia per g of soil. The converse of this situation, high frequency of nodule formation by a superior inoculum strain, does not guarantee a response to inoculation when the soil population is sufficient and contains some highly effective strains. These two observations can be reconciled by examining the nodulation data generated by these experiments. Except for the anomalous results with bean in Iao soil, inoculation did not enhance nodule number when the number of invasive native rhizobia was $\geq 1 \times 10^2$ per g of soil, whereas inoculation always increased nodule number when the number was below 6×10^0 per g of soil (Table 6). Nodules in uninoculated soybean plants were shown by serological identification to be due to contaminants from inoculated pots. Increased nodule formation due to inoculation was variable and dependent on soil and species when the number of rhizobia was about 10^1/g of soil. There were more nodules in uninoculated lima beans and cowpea in Keahua soil than in Makena soil, whereas the number of invasive rhizobia in Keahua soil was approximately 1 log greater than in Makena soil. The additional 30 to 40 rhizobia per g of soil available to lima bean and cowpea root systems resulted in a 5- to 20-fold increase in nodule number.

A significant increase in nodule number did not necessarily mean better N_2 fixation. Inoculation significantly increased nodule number for peanut in Haiku soil and cowpea in Keahua soil without a corresponding increase in N_2 fixation. In general, inoculation increased N_2 fixation only when inoculated plants had 2.5 times more nodules than the uninoculated control, as was the case with peanut in Makena soil.

Nodule dry weight measurements were better related to inoculation response than nodule number (Table 7), except for cowpea in Keahua soil and peanut in Makena soil. It may

TABLE 8. Effect of inoculation on average nodule weight

Soil	Avg nodule wt (mg)[a]											
	G. max		*A. hypogaea*		*P. lunatus*		*V. unguiculata*		*P. vulgaris*		*L. leucocephala*	
	I	U	I	U	I	U	I	U	I	U	I	U
Haiku	3.0	10.0	0.9	1.4	1.4	1.2	3.6	3.7				
Makena	4.5	9.0	1.0	1.7	1.9	13.0	6.0	4.9				
Iao	4.2	7.0	0.7	0.7			2.9	2.5	2.5	2.1		
Keahua	2.6				1.3	3.4	2.8	2.8			0.3	0.3

[a] I, Inoculated; U, uninoculated.

be that low numbers of native rhizobia delayed nodulation in uninoculated plants. The delayed onset of N_2 fixation as evidenced by shoot N concentration in early harvests (data not shown) was significant in 88% of the soils-species combinations when native rhizobia populations were at or below 1×10^2/g of soil. Early responses to inoculation were not observed when there were 10^3 or more rhizobia. The fact that early responses were not always sustained in later harvests even when low numbers of soil rhizobia reduced nodulation below that in inoculated plants suggests that the host may compensate for low numbers of soil rhizobia.

Singleton and Stockinger (14) demonstrated that when competition from ineffective strains of *R. japonicum* reduced the number of nodules formed by effective rhizobia, soybean compensated for this by selectively apportioning photosynthate to the effective nodules for growth and development. This mechanism tends to keep effective nodule dry weight constant as the number of nodules declines. A similar effect was seen in these experiments. When available rhizobia were few, individual nodules of uninoculated soybean, peanut, and lima bean plants tended to be larger than the nodules on inoculated plants when large numbers of rhizobia were applied to the roots (Table 8). Cowpea did not respond in a similar fashion.

From these experiments we conclude that native rhizobial populations vary considerably between soils. Populations can be defined in terms of number and effectiveness for a particular host, and these characteristics determine whether inoculation will enhance N_2 fixation. The number of invasive native rhizobia counted in these experiments varied by many orders of magnitude and was dependent on both the soil and the species used in the MPN count. The number of rhizobia in these soils tended to be positively associated with annual rainfall. Early response by legumes to inoculation with rhizobia in these soils depended mainly on the number of invasive rhizobia of the test species. Significant ($P \leq 0.05$) responses to inoculation were not obtained when the number of native soil rhizobia was $\geq 2 \times 10^1$/g of soil unless the soil population was completely ineffective.

The strains in these soil populations ranged widely in their symbiotic effectiveness with the test hosts. All populations had some ineffective strains. The effectiveness of many inoculum strains used proved to be no different than that of the average native strain found in effective populations. Even when the inoculum strain was superior to soil strains and formed the vast majority of nodules, there was no response to inoculation when the number of rhizobia was above 2×10^1/g of soil and the population had some effective strains.

Increasing nodule weight by inoculation was generally a prerequisite for increasing N_2 fixation. Although inoculation frequently increased nodule number, there was not necessarily a corresponding increase in nodule weight. Plant-controlled mechanisms appear to compensate for reduced nodulation when there are few rhizobia in the soil by increasing the average size of nodules above that of inoculated plants with more nodules. Other mechanisms, such as selective partitioning of photosynthate to highly effective nodules on uninoculated plants nodulated by soil rhizobia having a range of effectiveness, may also explain why even successful inoculation with superior strains does not necessarily result in enhanced N_2 fixation.

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