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Influence of the Size of Indigenous Rhizobial Populations on Establishment and Symbiotic Performance of Introduced Rhizobia on Field-Grown Legumes†

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Indigenous rhizobia in soil present a competition barrier to the establishment of inoculant strains, possibly leading to inoculation failure. In this study, we used the natural diversity of rhizobial species and numbers in our fields to define, in quantitative terms, the relationship between indigenous rhizobial populations and inoculation response. Eight standardized inoculation trials were conducted at five well-characterized field sites on the island of Maui, Hawaii. Soil rhizobial populations ranged from 0 to over 3.5×10^4 g of soil^{-1} for the different legumes used. At each site, no less than four but as many as seven legume species were planted from among the following: soybean *(Glycine mar),* lima bean *(Phaseolus lunatus),* cowpea *(Vigna unguiculata),* bush bean *(Phaseolus vulgaris),* peanut *(Arachis hypogaea), Leucaena leucocephala,* tinga pea *(Lathyrus tingeatus),* alfalfa *(Medicago saliva),* and clover *(Trifolium repens).* Each legume was (i) inoculated with an equal mixture of three effective strains of homologous rhizobia, (ii) fertilized at high rates with urea, or (iii) left uninoculated. For soybeans, a nonnodulating isoline was used in all trials as the rhizobia-negative control. Inoculation increased economic yield for 22 of the 29 (76%) legume species-site combinations. While the yield increase was greater than 100 kg ha^{-1} in all cases, in only 11 (38%) of the species-site combinations was the increase statistically significant ($P \leq 0.05$). On average, inoculation increased yield by 62%. Soybean *(G. max)* responded to inoculation most frequently, while cowpea (V. *unguiculata)* failed to respond in all trials. Inoculation responses in the other legumes were site dependent. The response to inoculation and the competitive success of inoculant rhizobia were inversely related to numbers of indigenous rhizobia. As few as 50 rhizobia g of soil-' eliminated inoculation response. When fewer than 10 indigenous rhizobia g of soil^{-1} were present, economic yield was significantly increased 85 % of the time. Yield was significantly increased in only 6% of the observations when numbers of indigenous rhizobia were greater than 10 cells g of soil^{-1}. A significant response to N application, significant increases in nodule parameters, and greater than 50% nodule occupancy by inoculant rhizobia did not necessarily coincide with significant inoculation responses. No less than a doubling of nodule mass and 66% nodule occupancy by inoculant rhizobia were required to significantly increase the yield of inoculated crops over that of uninoculated crops. However, lack of an inoculation response was common even when inoculum strains occupied the majority of nodules. In these trials, the symbiotic yield of crops was, on average, only 88 % of the maximum yield potential, as defined by the fertilizer N treatment. The difference between the yield of N-fertilized crops and that of N,-fixing crops indicates a potential for improving inoculation technology, the N_2 fixation capacity of rhizobial strains, and the efficiency of symbiosis. In this study, we show that the probability of enhancing yield with existing inoculation technology decreases dramatically with increasing numbers of indigenous rhizobia.

Inoculation of legumes with exotic strains of rhizobia is a common agricultural practice intended to promote nitrogen fixation and increase crop yield. Despite improvements in inoculation methods (3, 13, 31, 34) and selection of rhizobial strains for increased nitrogen fixation capacity (16), competitive ability (1), and ability to withstand environmental stress (15, 17, 19), inoculation does not always lead to increased plant growth and crop yield.

Plant response to inoculation is determined by a variety of factors. The presence and quality of indigenous rhizobial populations (3, 6, 11, 27), soil nitrogen (N) availability (9, 32), soil physicochemical constraints (12, 24), and climatic conditions (4) all significantly influence the ability to achieve increased crop yield through inoculation.

Population density, effectiveness, and competitive ability are the primary characteristics of indigenous rhizobial populations that affect inoculation responses. In greenhouse studies, Singleton and Tavares (27) demonstrated that statistically significant inoculation responses can be eliminated when there are as few as 20 indigenous rhizobia g of soil^{-1} as long as the population contains some effective strains. Strains within populations of rhizobia differ significantly in their ability to supply the host plant with fixed N (effectiveness) under greenhouse conditions (24, 26, 27). Differences in the effectiveness of inoculant strains can also be demonstrated under field conditions as long as the soil is free of indigenous rhizobia (10). In the presence of an indigenous population, however, improved crop yield through inoculation with more effective inoculant strains is difficult to demonstrate (6, 11, 18).

Successful competition for nodule sites by indigenous rhizobia is one reason for the failure to achieve a response to inoculation with elite rhizobial strains (18, 36). Both pot experiments (2) and field trials (36) demonstrated that to achieve nodule occupancy of greater than 50%, inoculant rhizobia must be applied at a rate at least 1,000 times greater

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TABLE 1. Locations, sites characteristics, and planting dates for eight inoculation trials conducted at five field sites on the island of Maui, Hawaii

Site		Planting date[a]	Elevation (m)	Soil classification[b]	Median annual rainfall (mm/yr)[c]	Mean temp. (°C) of:		Irradiance[d] (W/m²/day)	Legumes present at site
No.	Name					Soil	Air		
1	Hashimoto Farm	3/24/87[e]	37	Torroxic haplustoll	322	30.2	23.5	274	*Leucaena*, *Prosopis*
1a		3/10/88				34.1	24.9	291	
2	Kuiaha	8/15/86	320	Humoxic tropohumult	1,875	25.1	23.4	230	*Desmodium*, *Indigofera*, *Crotalaria*, *Acacia*, *Cassia*
3	Kula Agricultural Park	9/12/86	366	Torroxic haplustoll	375	25.8	22.5	210	*Leucaena*, *Indigofera*,
3a		5/14/87				28.7	23.5	258	*Macroptilium*, *Prosopis*
4	Haleakala Station	6/08/87	660	Humoxic tropohumult	1,800	22.9	21.5	233	*Desmodium*, *Trifolium*, *Acacia*, *Crotalaria*
5	Tengan Farm	10/20/87[f]	670	Torroxic haplustoll	523	22.1[g]	18.9[g]	187[g]	*Medicago*, *Vicia*, *Leucaena*,
5a		1/07/88				22.5	18.6	206	*Acacia*

[a] Month/day/year.
[b] From reference 28.
[c] From reference 5.
[d] Averaged across the duration of the longest crop for each planting at a site. From weather stations on location operated by MauiNet.
[e] Soybean was replanted 4/8/87 because of poor emergence.
[f] Lima bean and bush bean were replanted 10/28/87 and cowpea was replanted 11/18/87 because of poor emergence.
[g] From MauiNet Pulehu Farm site weather station located at the same elevation 0.78 km north.

than the estimated number of indigenous rhizobia. However, even when a highly effective inoculum strain forms the majority of nodules, yield improvement due to inoculation is uncommon (6, 36).

High concentrations of soil N also affect the response to inoculation by inhibiting nodulation, thereby decreasing the proportion of plant N that is derived from N_2 fixation (9). Available soil N, therefore, must be less than the legume crop N requirement for an inoculation response to be measured.

Environmental stresses that limit yield potential and, hence, the crop N requirement also affect the nitrogen fixation potential of the symbiotic association (24). Environmental constraints include soil physicochemical factors such as acidity, toxicity, salinity, and low fertility (12, 24, 25); climatic stresses such as low rainfall, inadequate soil and air temperatures, and insufficient solar radiation (4); and insect predation and disease. Consequently , the ability to improve crop yield through inoculation involves an interaction between soil N availability and other environmental conditions affecting crop yield potential.

The natural diversity in rhizobial population size and composition present at five sites on the island of Maui, Hawaii (38), was used to examine the role of the size of indigenous rhizobial populations in obtaining a legume yield increase from rhizobial inoculation. We tested the hypothesis that the magnitude of the legume inoculation response is an inverse function of the size of the indigenous rhizobial population and soil N availability in relation to crop N demand. Sites in the University of Hawaii's Maui Soil, Climate and Land Use Network (MauiNet) (29) provided a unique opportunity to study this relationship, as the sites lacked indigenous rhizobia for some legumes but provided a range of from less than 10^1 to more than 3.5×10^4 indigenous rhizobia g of soil^{-1} for other legumes. MauiNet sites also have a diversity of soils and climates, allowing measurement of the impact of various crop yield potentials and soil N availabilities on the interaction between indigenous rhizobial population size and legume inoculation response. A quantitative understanding of the role of indigenous rhizobial populations in determining host response to inoculation should help to identify locations at which inoculation will succeed in improving crop yield. Such knowledge can help determine where and when to use inoculants, appropriate locations for inoculum production facilities, and production requirements.

MATERIALS AND METHODS

General experimental approach. A series of standardized field inoculation trials was set up at five ecologically diverse sites on the island of Maui, Hawaii (Table 1), with legume species for which the number of soil rhizobia varied between sites (Table 2). Each legume species received three N source treatments: (i) uninoculated, no N applied; (ii) inoculated at 10^6 to 10^1 rhizobia per seed; and (iii) fertilizer N applied as urea at a rate of 100 kg of N ha^{-1} week^{-1} beginning at planting for sites 1, 2, 3, and 3a and at week 2 for sites 4, 5, and 5a for a total of 800 to 1,800 kg of N ha^{-1} over the cropping cycle, depending on crop duration. The yield of the fertilizer N treatment estimated the maximum yield potential of each legume species at each site. The uninoculated treatment measured both soil N available for crop growth and the effect of indigenous rhizobial populations (when present). Rates of inoculation used ranged from 11 to 68 times recommended farmer rates (8) and represented the maximum rhizobial numbers that could be successfully applied to the seed. A nonnodulating isoline of soybean was also planted at each site to provide a biological measurement of soil N available for plant growth during the cropping cycle. Each site was equipped with a CR-21 micrologger (Campbell Scientific, Inc., Logan, Utah) to record climate and soil data.

Soil amendments. Soils were limed at sites 2 and 4 (Table 1) with $Ca(OH)_2$ 1 week prior to planting to achieve a pH of between 5.5 and 5.9. Nutrients were applied in nonlimiting amounts based on soil test values. Compounds used and ranges of application rates were as follows (in kilograms hectare-1): P as treble superphosphate, 300 to 610; K as K_2S0_4, 285 to 352; Mg as $MgS0_4 \cdot 7H_20$, 60 to 77; Zn as $ZnS0_4 - 7H_20$, 5 to 15; B as H_3B0_3, 5; and Mo as $Na_2MoO_4 - 2H_20$, 2.

Legume cultivars. The legume species and cultivars used were as follows: *Glycine max* cv. Clark IV (nodulating and

TABLE 2. MPN counts of indigenous homologous rhizobia for the legume species grown in eight inoculation trials at five sites on the island of Maui, Hawaii

Site		MPN of rhizobia/g of soil for the following legumes[a]:								
No.	Name	*G. max*	*P. lunatus*	*V. unguiculata*	*P. vulgaris*	*A. hypogaea*	*L. leucocephala*	*M. sativa*	*T. repens*	*L. tingeatus*
1	Hashimoto Farm	0	<1	54	7					
1a						5[b]	>1,650			
2	Kuiaha	0	61[c]	2,306[c]	93[c]					
3	Kula Agricultural Park	0	<1[d]	18[b]	2[d]					
3a		0			211[d]	5[b]	>5,938[d]			
4	Haleakala Station	0	311[d]	35,900[c]	437[d]					
5	Tengan Farm	0	23	283	31					
5a								1,038	<1	15

a Upper and lower fiducial limits can be determined by dividing or multiplying by 2.7, respectively, unless otherwise noted. A value of 0 means that no indigenous rhizobia were present; a blank space means that the legume was not grown as implied in the caption. [b] Upper and lower fiducial limits can be determined by dividing or multiplying by 2.0, respectively. [c] Upper and lower fiducial limits can be determined by dividing or multiplying by 3.8, respectively. [d] Upper and lower fiducial limits can be determined by dividing or multiplying by 2.9, respectively.

nonnodulating isolines; P. Cregan, Nitrogen Fixation Laboratory, U.S. Department of Agriculture, Beltsville, Md.); *Phaseolus lunatus* cv. Henderson's Baby; *Phaseolus vulgaris* cv. Bush Bountiful; *Vigna unguiculata* cv. Big Boy at sites 2 and 3 and cv. Knuckle Purplehull at the remaining sites; *Arachis hypogaea* cv. McRan Valencia at site 3a and cv. Burpee Spanish at site la; *Leucaeua leucocephala cv.* K-8; *Lathyrus tingeatus* (tinga pea); *Medicago sativa cv.* Florida 77; and *Trifolium repens* cv. Regal Ladino. All were from the seed germplasm collection of the University of Hawaii, NifTAL Project, Paia.

Inoculum strains and inoculation procedure. Three serologically distinct rhizobial strains were used to inoculate each legume species. The strains used and their sources are listed in Table 3. All strains were grown separately in yeast extract-mannitol broth cultures (35) to a concentration of 10^9 cells ml-1. For all trials, except those at sites 2 and 3 (Table 1), 50 ml of each broth culture was injected into 100 g of gamma-irradiated peat in separate polyethylene bags (Agricultural Laboratories Pty. Ltd., Sefton, New South Wales, Australia). Peat inoculants were incubated for 14 days at 26°C, counted, and kept at 4°C until used. Rhizobial numbers in each inoculant were determined by the drop plate method (30). The three peat inoculants for each legume species were combined to provide equal numbers of each strain in a mixed inoculant. For trials conducted at sites 2 and 3, broth cultures of the three strains for each legume species were combined in equal volumes. Fifty milliliters of these combined broth cultures was injected into 100 g of gamma-irradiated peat. These inoculants were incubated, counted, and stored as described above. Rhizobial numbers gram of peat-1 averaged 3.16×10^9, with a minimum of 4.03×10^8. Immediately before planting, seeds were coated with 0.4 to 2.8 ml of a 40% gum arabic solution per 100 g of seed (based on seed size). Inoculant was applied to the coated seeds in amounts sufficient to provide 10' rhizobia seed-1 for largeseeded legumes and 10^5 rhizobia seed-1 for small-seeded legumes. A final coating of $CaC0_3$ was applied to all seeds to facilitate handling. Viable counts of rhizobia on pelleted seeds averaged 2.47×10^7 seed^{-1} for large-seeded legumes and 1.13×10^5 seed^{-1} for small-seeded legumes.

Enumeration of indigenous soil rhizobial populations. Immediately prior to planting, field soils were sampled to determine the most probable number (MPN) of indigenous soil rhizobia capable of nodulating the selected host legumes

TABLE 3. Strain designations and sources of inoculant rhizobia used in the Maui inoculation trials

Legume host	NifTAL designation	Original designation and other names	Source[a]
G. max	TAL 102	USDA 110	1
	TAL 377	USDA 138	1
	TAL 379	USDA 136b, CB 1809	1
P. lunatus	TAL 22	NifTAL original	2
	TAL 169	Nit 176A22	3
	TAL 644	CIAT 257	4
P. vulgaris	TAL 182	NifTAL original	2
	TAL 1383	CIAT 632	4
	TAL 1797	CIAT 899	4
V. unguiculata	TAL 173	Nit 176A30	3
	TAL 209	NifTAL original	2
	TAL 658	CIAT 71	4
A. hypogaea	TAL 169	Nit 176A22	3
	TAL 173	Nit 176A30	3
	TAL 658	CIAT 71	4
L. leucocephala	TAL 82	NifTAL original	2
	TAL 582	CB 81	5
	TAL 1145	CIAT 1967	4
L. tingeatus	TAL 634	Nit 92A3	3
	TAL 1236	Allen 344	6
	TAL 1402	Nit 128C75	3
T. repens	TAL 1826	S11-6	7
	TAL 1827	S11-16	7
	TAL 1828	AR 21	7
M. sativa	TAL 380	SU 47	8
	TAL 1372	POA 116	9
	TAL 1373	POA 135	9

[a] 1, U.S. Department of Agriculture, Beltsville, Md.; 2, NifTAL Project, Paia, Hawaii; 3, Nitragin Co., Madison, Wis.; 4, Centro Internacional Agricultural Tropical, Cali, Columbia; 5, Commonwealth Scientific Industrialization Research Organization, Brisbane, Australia; 6, O. N. Allen, University of Wisconsin, Madison; 7, P. J. Bottomley, Oregon State University, Corvallis; 8, University of Sydney, New South Wales, Australia; 9, Universidade Federal Rio Grande do Sul, Porto Alegre, Brazil.

(Table 2). Thirty 2.54-cm-diameter soil cores to a depth of 25 cm were taken in a grid pattern across each experimental area. Soil cores were pooled, mixed, subsampled for determination of moisture content, and stored at 4°C overnight. Serial 1:2, 1:4, 1:5, or 1:10 soil dilutions were prepared as described by Somasegaran and Hoben (30) with no less than 50 g (oven-dried basis, 100°C) of soil for the first dilution step. Prior estimations of soil rhizobial populations performed by Woomer et al. (38) were used as a guideline for the appropriate dilution ratio to use for each legume species at each site. Test plants were inoculated as described by Somasegaran and Hoben (30) and kept supplied with an adequate volume of an N-free nutrient solution (23). Plants were scored for nodulation 21 to 28 days after inoculation, and the MPN of indigenous rhizobia was determined by use of a computer with the Most Probable Number Enumeration System (MPNES) (37). The reliability of MPN results was ascertained with the criteria outlined by Woomer et al. (39).

Plant culture. Seeds of all cultivars except the forage legumes were sown in rows 60 cm apart. Seeds were spaced to provide a planting density (plants hectare^{-1}) of 416,667 for G. max, 333,333 for P. lunatus, P. vulgaris, and V. unguiculata, 166,667 for A. hypogaea, 125,000 for *L.* leucocephala at site 3a, and 333,333 for *L.* leucocephala at site la. Seeds of *M.* sativa and T. repens were sown in rows 30 cm apart. Seeds were broadcast along the rows at a rate of 22 kg of seed ha^{-1} for *M.* sativa and 10 kg of seed ha^{-1} for T. repens. *L.* tingeatus was sown in rows 40 cm apart. Seeds were spaced to provide a planting density of 500,000 plants ha^{-1}. All fields were irrigated to 0.03 MPa (field capacity) at planting and maintained near that tension for the duration of each trial with the aid of tensiometers. Planting dates for each site are given in Table 1.

Early harvest. Grain legumes were harvested at or near full bloom. Forage crops were harvested 71 to 74 days after planting. Plants were cut at the soil surface from a 1.8- to 3.6-m^{2}area, depending on the species. Outside rows were used for plot borders, with a minimum border of 50 cm at the end of each plot. The fresh weight of samples was determined immediately. Subsamples of 10 to 20 plants were taken, and the fresh weight was recorded in the field. Subsamples were dried at 70°C to a constant weight, weighed, and ground to pass through a 2-mm-pore-diameter sieve. Ground samples (0.25 g) were digested in 6 ml of H_2S0_4 containing 0.25 g of salicylic acid liter-' after pretreatment with 3 ml of H_2O_2 (30%) (21). Ammonium in the digests was determined by the indophenol blue method (14).

Ten randomly selected rootstocks were excavated from each plot. Nodules were removed, counted, dried at 70°C, and weighed. Plant density was determined in each plot. Nodule number plant-1 and nodule mass plant-l in the sample were multiplied by the plant stand hectare-1 to determine the numbers and kilograms of nodules hectare-1. Nodule occupancy by inoculum strains was determined on 24 to 36 randomly selected nodules from each plot with strain-specific fluorescent antibodies as described by Soma-segaran and Hoben (30). The indirect immunofiuorescence method was used for *L.* tingeatus and T. repens, and the direct method was used for the remaining legume species.

Late harvest. G. max, P. vulgaris, and A. hypogaea were harvested at harvest mdturity (7). P. lunatus and V. unguiculata were harvested When the majority of the first flush of pods were dry. *L.* leucdcephala was harvested 118 days after planting at Kula Agricultural Park and 166 days after planting at Hashimoto Farm. The forage legumes were harvested 112 to 117 days after planting. Plants were harvested from a 3.6- to 6.0-m2 area, depending on the species. Subsamples of 10 to 15 plants were taken, dried, and analyzed for N content as described above.

Experimental design and analysis. Inoculation trials were set up in a split-plot design with four replications. Legume species were assigned to main plots, and N source treatments confined to subplots. All plant growth and nodulation data were analyzed by site, except for the yield data for *L.* leucocephala at sites la and 3a and P. vulgaris at site 1 and the nodulation data for V. unguiculata at site 1, which were analyzed as separate randomized complete block experiments because of the nonhomogeneity of variance with the other legume species grown at these sites. Nodulation data for G. max were also excluded from the analyses because the uninoculated (nonnodulated) plants lacked any variance. Means of nodule mass and nodule number on inoculated soybean were considered to be significantly different from zero as long as their 95% confidence intervals did not include zero. Analysis of variance procedures (22) were used for all other analyses.

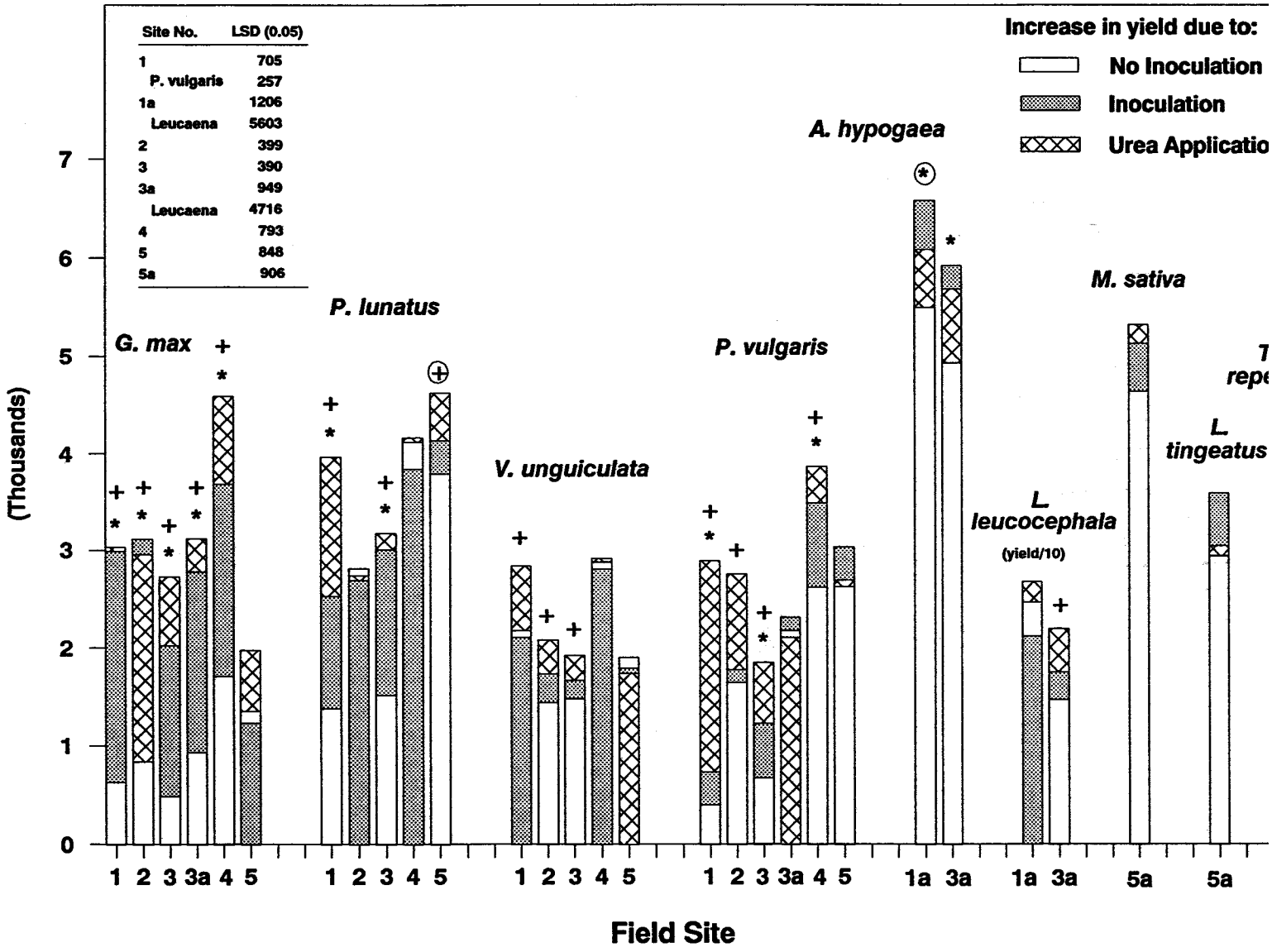
RESULTS

The yield of nine legumes grown under uninoculated, inoculated, and fertilizer N conditions in eight field inoculation trials is presented in Fig. 1. Seed yield for the grain legumes and above-ground biomass for the forage legumes represent the reported economic yields. For the grain legumes, economic yield was highly significantly correlated with above-ground biomass *(r* = 0.91) and N accumulation *(r* = 0.90) (data not shown). Economic yield for the forage legumes was also highly significantly correlated with N accumulation *(r* = 0.97). Inoculation increased economic yield for 22 of the 29 (76%) legume species-site combinations. While the yield increase was greater than 100 kg ha^{-1} in all cases, in only 11(38%) of the species-site combinations was the increase statistically significant ($P < 0.05$).

Response to inoculation varied between both sites and legume species. Inoculation response was most frequent at sites 1 and 3. No response to inoculation was obtained at sites 5 and 5a. Soybean *(G. max)* responded to inoculation in five of six trials (83%), with the yield of inoculated crops being at least double that of uninoculated crops. While lima bean *(P. lunatus),* peanut *(A. hypogaea),* and cowpea (V. *unguiculata)* all are expected to nodulate with *Bradyrhizobium* sp., lima bean and peanut responded to inoculation at sites 1 and 3, whereas cowpea failed to respond in all trials. Bush bean *(P. vulgaris)* responded to inoculation 50% of the time. No significant inoculation response was obtained with the forage legumes.

N application, improved yield over the uninoculated condition 90% of the lime; however, only 52% of the observations were significant ($P < 0.05$) (Fig. 1). A significant increase in yield due to N fertilization was accompanied by a significant inoculation response only 67% of the time. In 8 of 29 cases there was S significant increase in yield due to N application over that due to inoculation. Of these, only half also had a significant inoculation response.

Biomass at early harvest was highly significantly correlated *(r* = 0.97) with total N accumulation at early harvest (data not shown). However, there Was no significant correlation between biomass and N accumulation measured at early harvest and any of the yield parameters measured at late harvest. Consequently, significant responses to inoculation or N application at final harvest could not be reliably



3. 1. Increase in economic yield due to rhizobial inoculation and urea application. Symbols: *, Significant response to inoculation ($P < 0.05$); +, significant respon application ($P < 0.05$); ⊛, significant response to inoculation ($P < 0.10$); ⊕, significant response to urea application ($P < 0.10$). Least significant difference (LSD) v used to compare N source treatments within a species at a site. Field sites: 1 and 1a, Hashimoto Farm; 2, Kuiaha; 3 and 3a, Kula Agricultural Park; 4, Haleakala Sta 5a, Tengan Farm.

TABLE 4. Incidence of significant biomass increases due to inoculation at early harvest and observed economic yield increases due to inoculation and N application at late harvest

Significant increase in biomass due to inoculation (n) at early harvest	Significant increase (n, $P < 0.10$) in economic yield due to:	
	Inoculation	N application
Yes (7)	4	4
No (22)	8	12

predicted from yield measurements made at early harvest (Table 4).

Inoculation enhanced modulation in 25 of 28 (89%) legume species-site combinations (Fig. 2). Increases were significant ($P \leq 0.05$) in only 14 cases for nodule number and 17 cases for nodule mass. Significantly enhanced nodule number and nodule mass led to a significant inoculation response 71 and 65% of the time, respectively. No indigenous *Bradyrhizobium japonicum* was present at any of the sites (Table 2); consequently, inoculation enhanced modulation of soybean at all sites. Nodule number on soybean was relatively consistent among sites 1 through 4; however, at site 5 nodule number was less than half that obtained on average at the other sites. Nodule mass on soybean was inversely correlated (r = -0.60) with the economic yield of uninoculated (nonnodulated) soybean, which depended solely on soil N for growth (Fig. 1). In general, at sites at which the yield of the uninoculated crop was low (sites 2, 3, and 3a), the nodule mass of the inoculated crop was high. Conversely, at sites at which the yield of the uninoculated crop was high (sites 4 and 5), the nodule mass of the inoculated crop was low. Nodule mass for the other species grown at these sites followed the same relative pattern, indicating that environmental factors, primarily soil N availability, were controlling modulation.

Indigenous rhizobia capable of modulating legume species other than soybean were present in various numbers at each of the sites (Table 2). Nodulation of uninoculated plants was closely related to the size of the indigenous homologous rhizobial population (Table 5). On average, when less than 10 rhizobia g of $soil^{-1}$ were present, inoculation increased nodule number and nodule mass many fold. When the number of indigenous rhizobia was between 10 and 100 g of $soil^{-1}$, inoculation roughly doubled nodule mass and tripled nodule number. However, nodule number and nodule mass in the inoculated and uninoculated treatments were not significantly different when the number of soil rhizobia was greater than 100 g of $soil^{-1}$. Notable exceptions were bush bean at sites 2 and 4, peanut at sites la and 3a, and clover at site Sa.

Nodule occupancy by inoculant strains ranged from 7 to 100% (Table 6) and was inversely related to numbers of indigenous rhizobia (Table 5). Inoculant strains were, in general, very successful in competing with indigenous rhizobia for nodule occupancy. Nodule occupancy by inoculant strains of no less than 66% was required for a significant increase in economic yield to be realized. However, the lack of an inoculation response was common even when inoculant rhizobia occupied the majority of nodules.

DISCUSSION

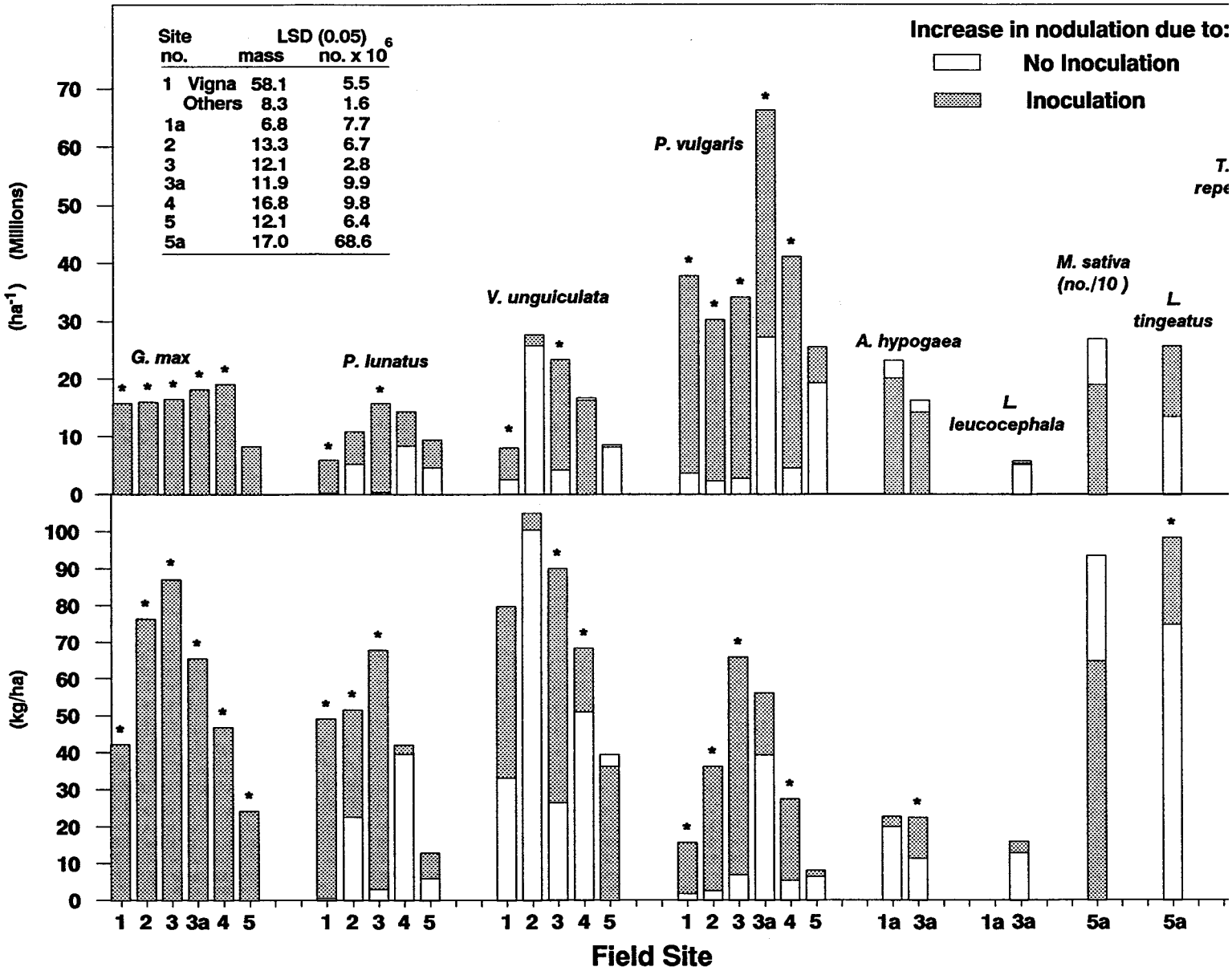
Legume response to rhizobial inoculation is measured as the increase in the yield of inoculated over uninoculated crops. The goal of many inoculation programs is to maximize this increase. In these inoculation trials, we examined the effect of indigenous rhizobial population size, in relation to crop yield potential and available soil N, on the ability to improve legume yield through inoculation.

For inoculation to improve crop yield, there must be a demand for fixed N in the cropping system not met by soil N or N_2fixed by indigenous rhizobia. In the absence of indigenous rhizobia, the demand for fixed N is the difference between the quantity of soil N available for crop uptake and the amount of N required for the crop to meet its yield potential. Yield potential can be defined as the maximum yield attainable under a given set of growth conditions. If the yield potential of the crop is limited by a nutrient deficiency other than N or environmental stress, N demand will be reduced accordingly (20). If the quantity of N_2 fixed by indigenous rhizobia is adequate to meet crop N demand, inoculation with more elite inoculant strains will not result in increased yield regardless of their effectiveness or competitive ability.

The ability of the indigenous rhizobial population to meet crop N demand is determined by the number of invasive rhizobia present in the soil and their effectiveness. Soil rhizobia incapable of fixing N_2 in symbiosis with the host will do little to meet crop N demand. However, Singleton Tavares (27) have shown that indigenous rhizobial populations with a range of effectiveness from ineffective to highly effective are capable of meeting crop N demand as long as they are present in sufficient numbers to adequately modulate the host. This capability may be due to a mechanism by which products of photosynthesis are selectively partitioned to effective nodules (26). The results of these trials support the findings of Singleton and Tavares (27) and indicate that relatively small indigenous populations of rhizobia are sufficient to meet host N demand as long as there are some effective strains in the population.

Since B. *japonicum* was absent from all sites and since soybean main plots were randomized over each field, the measurement of soil N available to the crop at each site was possible. The yield of N-fertilized soybean estimated the maximum yield potential of the crop at each site under non-N-limiting conditions. The difference between the yield of uninoculated (nonnodulated) and N-fertilized soybeans defined the crop symbiotic N demand. For other indices of the crop symbiotic N demand and soil N status at these sites, see Thies et al. (33). Demand for fixed N was highest at site 3 and lowest at site 5, at which 18 and 68%, respectively, of the maximum yield potential was met by soil N (Fig. 1). While soil N contributed most toward realizing the maximum yield potential of soybean at site 5, maximum yield was lowest at this site. Impacts of low soil and air temperatures and solar radiation (Table 1) were most likely responsible for the decreased yield potential at this site and the consequent failure to achieve a significant response to either inoculation or N application. At the remaining sites, at which there was a demand for fixed N, both soybean inoculation and N application resulted in significant increases in economic yield.

Results from these soybean trials indicated that a failure to respond to applied N in the remaining crops grown at sites 5 and 5a could be primarily attributed to a soil N supply adequate to meet crop N demand. This condition would preclude obtaining an inoculation response on any of the species grown at these sites regardless of the presence of indigenous rhizobia. Reduced modulation in both inoculated and uninoculated clover and the grain legumes grown at sites



G. 2. Increase in nodulation due to rhizobial inoculation. *, Significant increase in nodulation due to inoculation ($P < 0.05$). Least significant difference (LSD) val used to compare N source treatments within a species at a site. Field sites: 1 and 1a, Hashimoto Farm; 2, Kuiaha; 3 and 3a, Kula Agricultural Park; 4, Haleakala Stat 1 5a, Tengan Farm.

TABLE 5. Nodulation responses to inoculation in relation to the MPN of indigenous rhizobia

MPN of soil rhizobia	No. of cases	No. of trials with a significant increase due to inoculation in nodule:		Ratio (fold increase) of inoculated to uninoculated yields of nodule:		Avg nodule occupancy by inoculant strains (%)
		Mass	No.	Mass	No.	
0–10	13	11	9	17.6[a]	36.2[a]	89
10–100	7	4	3	2.1[b]	2.7[b]	86
>100	9	2	2	1.1[c]	1.3[c]	53

[a] Excludes soybean data.
[b] Excludes bush bean at site 2.
[c] Excludes bush bean at site 4.

TABLE 7. Yield responses to inoculation and N application in relation to the MPN of indigenous rhizobia

MPN of soil rhizobia	No. of cases	No. of trials with a significant increase ($P < 0.10$) in economic yield due to:		% Maximum yield of inoculated and uninoculated treatments relative to N fertilizer treatment[a]		Avg yield increase due to inoculation (%)[b]
		Inoculation	N application	Uninoculated	Inoculated	
0–10	13	11	9	46	82	128
10–100	7	0	4	85	92	9
>100	9	1	3	88	92	7

[a] Arithmetic average of (mean yield of uninoculated crops/mean yield of N-fertilized crops) × 100 for all cases within an MPN group.
[b] Arithmetic average of (mean yield of inoculated crop − mean yield of uninoculated crop)/mean yield of uninoculated crop for all cases within an MPN group.

5 and 5a, as compared with other sites, supports this interpretation.

Crops grown at the remaining sites, at which there was an N limitation to maximum yield, required either fixed N or applied N to meet their yield potential. For crops other than soybean, a portion of this N demand was satisfied by symbiotic association with indigenous rhizobia. The size of the indigenous rhizobial population was the major determinant of whether the crop symbiotic N demand was met by indigenous rhizobia. Significant responses to both inoculation and N application indicated that the indigenous rhizobial population was unable to meet crop N demand. These responses occurred when counts of indigenous rhizobia were. below 7 cells g of soil^{-1}. A significant inoculation response was observed in only one species-site combination (bush bean at site 4), in which indigenous rhizobia were present in excess of 54 cells g of soil^{-1} (Fig. 1 and Table 2). Low nodulation of uninoculated plants at this site, a highly significant increase in both nodule number and nodule mass due to inoculation, and 96% nodule occupancy by inoculant strains indicated that either the population size was overestimated (27) or indigenous rhizobia were highly noncompetitive. Dramatic increases in yield were observed when less than 10 rhizobia were present g of soil^{-1} (Table 7). When indigenous rhizobia numbered greater than 10 cells g of soil^{-1}, yield was increased only 7 to 9% on average.

Five species-site combinations had significant increases in economic yield due to N application yet failed to respond to inoculation. Three of these had significantly increased economic yield compared with that obtained in the inoculated treatments. These were cowpea at sites 1 and 3 and bush bean at site 2. In these cases, symbiosis between our best available inoculant strains and their legume hosts did not fix enough N_2 to meet maximum yield potential. In all three cases, nodulation was significantly increased by inoculation, soil rhizobial numbers were below 100 g of soil^{-1}, and soil N was insufficient to meet maximum yield potential, but all failed to respond to inoculation. In the remaining two cases, available soil N plus the N_2 fixed by indigenous rhizobia was adequate to achieve an economic yield that did not differ significantly from that of inoculated crops. The indigenous rhizobial population was in excess of 103 cells g of soil^{-1} in both cases.

Results obtained with peanut were atypical. Economic yield was significantly increased by inoculation at both site 3a ($P < 0.05$) and site la ($P < 0.10$); the number of indigenous rhizobia was approximately 5 cells g of soil^{-1} at both sites. However, the economic yield of peanut was not increased by N application at either site. N fertilization did significantly increase above-ground biomass in both cases, however. Failure to enhance seed yield through large applications of fertilizer N while above-ground biomass is greatly increased has also been consistently observed with groundnuts in India (13a).

Crops relying on soil N alone or a combination of soil and fixed N for their N requirement were not able to achieve their maximum yield potential in these trials. On average,, the economic yield of inoculated crops was only 88% that of N-supplied crops (Table 7). This percentage was fairly

TABLE 6. Proportion of nodules formed by inoculant strains on legumes grown in eight inoculation trials at five sites on the island of Maui, Hawaii

Site		% Total nodules formed on[a]:								
No.	Name	*G. max*	*P. lunatus*	*V. unguiculata*	*P. vulgaris*	*A. hypogaea*	*L. leucocephala*	*M. sativa*	*T. repens*	*L. tingeatus*
1	Hashimoto Farm	100	92	67	94					
1a						31	7			
2	Kuiaha	100	80	54	89					
3	Kula Agricultural Park	100	94	96	83					
3a		100			96	66	8			
4	Haleakala Station	100	49	48	96					
5	Tengan Farm	100	85	67	95					
5a								ND[b]	96	88

[a] Determined by immunofluorescence microscopy. See Table 2, footnote *a*, for definition of blank spaces.
[b] ND, Not determined.

consistent regardless of the size of the indigenous rhizobial population. Failure of crops relying on fixed N to achieve their maximum yield potential may reflect basic inefficiencies in the N_2 fixation process. The proportion of maximum yield potential attained by uninoculated crops was dependent upon the indigenous rhizobial population size. On average, when indigenous rhizobia were below 10 cells g of soil^{-1}, uninoculated crops produced only 46% of their maximum yield potential. Nonnodulated soybean, which depended solely upon soil N, met only 34% of its maximum yield potential in these trials. Indigenous rhizobial populations in excess of 10 cells g of soil^{-1} were, on average, able to supply nearly as much fixed N for economic yield as was present in inoculated crops. The gap between the yields of N-fertilized and inoculated crops indicates a potential for improving inoculation technology, the N_2 fixation capacity of rhizobial strains, and efficiency of the symbiosis.

In summary, the relationship between inoculation response and size of the indigenous rhizobial population was consistent regardless of whether inoculation response was measured in terms of enhanced economic yield, above ground biomass, or total N accumulation. Inoculation response in these trials was first dependent upon there being a demand for fixed N by the legume crop. When soil N was insufficient to meet crop N demand, inoculation response was dependent upon whether the sum of available soil N plus N_2 fixed by the indigenous rhizobial population was sufficient to meet demand. In these trials, an indigenous rhizobial population in excess of 7 cells g of soil^{-1} was sufficient to achieve yields not significantly different from those of inoculated crops, except when populations were mostly noncompetitive. Inoculation succeeded in significantly increasing ($P < 0.05$) economic yield in 38% of the trials. When soil rhizobia numbered less than 10 cells g of soil^{-1}, yield was significantly improved 85% of the time. Inoculation significantly increased yield only 6% of the time when indigenous rhizobial populations numbered greater than 10 cells g of soil-1. The yield of inoculated crops was, on average, only 88% of the yield potential, which was defined by the yield of the fertilizer N control. Significantly increased modulation due to inoculation did not guarantee a significant increase in economic yield. No less than a doubling of nodule mass was required to obtain a significant response to inoculation. However, in 7 of the 17 (41%) species-site combinations in which nodule mass was at least doubled, a significant inoculation response was still not obtained. Nodule occupancy by inoculant strains of greater than 50% did not ensure a significant inoculation response. No less than 66% nodule occupancy by inoculant strains was required to achieve a significant inoculation response. In this study, competition from indigenous rhizobia for nodule occupancy was not necessarily the major determining factor for failure to obtain a significant response to inoculation. These results suggest that presence of an adequate soil rhizobial population to meet the N_2 fixation requirements of the host was the primary reason for failure of crops to respond to inoculation.

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