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Single-Strain versus Multistrain Inoculation: Effect of Soil Mineral N Availability on Rhizobial Strain Effectiveness and Competition for Nodulation on Chick-Pea, Soybean, and Dry Bean†

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The nitrogen-fixing effectiveness of multistrain inoculants was found to be determined by both the effectiveness of the component strains and the percentage of the nodules occupied by them. Multistrain formulations were always either as good as the most effective single-strain inoculant or intermediate between the most and the least effective. The percentage of nodules occupied and the amount of nitrogen fixed by the component strains of a multistrain inoculant showed highly significant linear correlation. The availability of soil N had a significant influence on the nitrogen fixation potential of each strain. The mineral N status of the soil was clearly a significant factor in affecting the competition pattern of *Rhizobium loti* (chick-pea) and *Bradyrhizobium japonicum* strains. Differences between the effectiveness of strains were masked under conditions of soil N availability. However, when soil N was immobilized with sugarcane bagasse, the differences became significant. In the chick-pea system, *R. loti* TAL 1148 (Nit 27A8) was the most effective but not the most competitive of the three strains used. In the soybean and dry bean systems, *B. japonicum* TAL 102 (USDA 110) and *R. leguminosarum* bv. phaseoli TAL 182, respectively, were consistently the most effective and, more often than not, the most competitive of the strains used for each species.

Many legumes can obtain much of their N requirement through symbiotic nitrogen fixation if the root nodules are formed by effective strains of rhizobia. To establish an effective symbiosis, seeds of legumes are often inoculated with peat-based rhizobial inoculants. Two types of inoculants are produced, namely, single-strain and multistrain inoculants. Multistrain inoculants may contain either rhizobial strains from two distinct inoculation groups, e.g., strains for Trifolium spp. or *Medicago* spp., or a mixture of strains for just one of the two groups (19). In Australia, only single-strain inoculants are produced, to prevent possible dominance and antagonistic effects of a particular strain in the mixture (20), to be able to diagnose loss of effectiveness, and to facilitate quality control (27). However, in the United States multistrain inoculants are produced commercially (5) to provide a compensatory mechanism to theoretically meet the constraints imposed by the host-strain-environment interactions, which is impossible with single-strain inoculants.

Among the many environmental factors, soil N, especially nitrate, has an inhibitory effect on the nodulation and nitrogen fixation of the legume-rhizobium symbiosis (9, 15). Therefore, it is conceivable that the availability and immobilization of soil N has significant effects on strain effectiveness and competition, especially when multistrain inoculants are used for seed inoculation. Generally, very few systematic studies have been carried out to evaluate single-strain and multistrain inoculants, and the study that has provided the limited information available on the soybean-*Bradyrhizobium japonicum* symbiosis (18) did not use strain identification techniques to analyze nodule occupancy data. In research involving the influence of nitrogen on nodulation, it was shown that strains of *B. japonicum* varied in their ability to nodulate soybean in the presence of nitrate (12, 13, 21). Limited information is available demonstrating the effects of N on strain competition. For example, there is evidence indicating differential competition for nodule occupancy between strains of *B. japonicum* in the presence of nitrate in sand cultures (13), but the addition of nitrate did not change the competitiveness between two serogroups of *B. japonicum* in soil (11).

Critical comparisons of single-strain and multistrain inocula axe rare and generally dependent on so few strains that generalizations are not possible (28). Evaluations of singlestrain versus multistrain inoculants are necessary to understand inoculant performance and, from the practical and economic standpoint of the inoculant producer, to justify the formulations of inoculants with regard to single or multiple strains.

In this study we conducted experiments by using pots containing one of two soils to evaluate the effectiveness of single-strain and multistrain inoculants and to study the effect of soil N on the effectiveness and competitiveness of the strains. Both these objectives were investigated in the soybean-B. *japonicum,* dry bean-*Rhizobium leguminosarum* bv. phaseoli and *chick-pea-Rhizobium loti* symbioses; plants were grown in two soils with the soil N available or immobilized by the addition of sugarcane bagasse.

MATERIALS AND METHODS

Rhizobial cultures. To evaluate single-strain and multistrain inoculations, we selected soybean *(Glycine* max)-B. *japonicum, dry* bean *(Phaseolus* vulgaris)-*R. leguminosarum* bv. phaseoli, and chick-pea (*Cicer arietinum)-R. loti* systems because highly effective strains for the host legumes have been tested in numerous investigations in various laboratories. From these a set of three or more serologically distinct strains for each of the three host legumes have been assembled (10) and tested in inoculation experiments conducted internationally (6). The following cultures were used

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in this study: *B. japonicum* TAL 102 (USDA 110), TAL 377 (USDA 138), and TAL 379 (CB 1809); *R. loti* (chick-pea) TAL 620 (CB 1189), TAL 480 (UASB 67), and TAL 1148 (Nitragin 27A8; USDA 3100); and *R. leguminosarum* bv. phaseoli TAL 182, TAL 943 (Kim 5), TAL 1383 (CIAT 632), TAL 1797 (CIAT 899), and TAL 1865 (Viking-1). Except for *R. leguminosarum* bv. phaseoli TAL 943 and TAL 1865, which were obtained from F. Robert, University of Hawaii, all cultures were from the NifTAL Project and MIRCEN *Rhizobium* germ plasm resource. Cultures were maintained on yeast-mannitol agar (YMA) slants (29) and stored under refrigeration.

Inoculant preparation. The individual strains were cultured separately -in yeast-mannitol broth. Each culture was then injected aseptically into gamma-irradiated peat to prepare pure peat cultures for each strain. After a period of incubation (7 to 14 days, depending on the rhizobial species) in the peat, and on the basis of viable counts of the individual strains, a multistrain inoculant containing equal numbers of the appropriate strains was prepared as described previously (24, 25).

Seeds. Chick-pea seeds (small red-seeded genotype, 6130 ICC 4948, Desi); dry bean (Burpee's Bush Bountiful); and soybean (cv. Lee) were obtained from the NifTAL Project.

Plant culture and inoculation. Seeds were surface sterilized and pregerminated in sterile vermiculite as described previously (24). Six (soybean and chick-pea) or eight (dry bean) pregerminated seeds were planted per pot. Each seed was inoculated at planting with 1 ml (1×10^6 to 2×10^6 rhizobia) of the aqueous peat inoculant suspension.

Soil characteristics and preparation. Two Hawaiian mollisols (Torroxic Haplustoll), the Keahua series (pH 6.8; 0.3% total N) and the Waikoa series (pH 7.5; 0.1% total N), were used. Both soils were free of *B. japonicum* (23) and *R. loti* (chick-pea) (24), but the Keahua soil contained a small population of ineffective *R. leguminosarum* bv. phaseoli.

Soils were sieved and filled into 4.4-liter black plastic pots to give 5.5 kg of soil per pot. Experiments were done with the soil N available or immobilized. To immobilize the soil N, finely milled sugarcane bagasse was mixed with the soil (16) at 10 g of bagasse per kg of soil (K. G. Cassman, Ph.D. dissertation, University of Hawaii, Manoa, 1979). The procedure of Singleton *et al*. (22) was followed for adjusting the soil moisture level and macro- and micronutrient additions.

Experimental treatments, block design, and harvest. There were two main treatments for each experiment, namely, inoculated and noninoculated control. Experiments in the Keahua soil series consisted of three single-strain inoculations and a multistrain inoculation which was a mixture of all the three used as single strains. For example, for soybean, single-strain inoculations were *B. japonicum* TAL 102, TAL 377, and TAL 379 and the multistrain inoculation consisted of these three strains applied as a 1:1:1 aqueous mixture. Soybean, bean and chick-pea rhizobium strains were tested in the Keahua soil under conditions of N availability and immobilization. The Waikoa soil (with N available) was used only with dry bean to test five strains of *R. leguminosarum* bv. phaseoli. As in the Keahua soil, single-strain and multistrain inoculation treatments were instituted. However, there were two multistrain inoculations. In the first, all five strains were equally represented in the multistrain inoculation, whereas in the second, TAL 182 was represented 2 logs lower than the other component strains.

In all experiments, a randomized complete block design was followed and each treatment was set up in quadruplicate. Experiments were conducted in a naturally illuminated greenhouse. Plants were grown for 34 to 60 days before harvest.

At harvest, plants were cut at the soil surface, oven dried (65°C for 48 h), weighed, ground, and analyzed for N content by the method of Mitchell (14) with a Technicon auto analyzer (Technicon Instruments, Tarrytown, N.Y.). Root systems were washed free of soil, and nodules were picked for dry weight determination and strain identification.

Nodule identification. Nodule occupancy by the inoculant strains was determined by immunofiuorescence (2) with oven-dried nodules (26). Twenty-five nodules were typed from each replication of the multistrain inoculation treatments to determine interstrain competition. Fifteen nodules per replication were typed for the single-strain inoculations.

Relationship between the amount of N fixed and nodule occupancy. To determine how each of the component strains contributed to the amount of N fixed (difference between milligrams of shoot N per inoculated plant and milligrams of shoot N per noninoculated plant) when applied as a multistrain inoculant, we calculated arcsine-transformed percentages of the nodule occupancies by each strain and the corresponding value of milligrams of N fixed by the same strain. For example, to calculate the contribution to the milligrams of N fixed (N_c,) by chick-pea rhizobium strain TAL 480, the following parameters must be determined: milligrams of N fixed (N_s) by TAL 480 when applied as a single-strain inoculant, percent (P_s) nodule occupancy when applied as a single-strain inoculant (100%), and percent (P_m) nodule occupancy when applied as a multistrain inoculant. The general expression for N_c, is as follows: $N_c = (P_m/P_s) \times N_s$. *By* using the above expression, the N_c, and P_m values for all the component strains in the mixed inoculation treatment for chick-pea were calculated and pooled. The relationship between N_c, and P_m was then analyzed by regression.

Statistical analysis. Shoot and nodule dry weights and total N were analyzed for differences by analysis of variance. Nodule identification data were converted to percentages, arcsine transformed, and analyzed by analysis of variance. Noninoculated controls were not included in the analysis.

RESULTS AND DISCUSSION

In the chick-pea-*R. loti* symbiosis, inoculation with singlestrain or multistrain inoculants did not cause a differential plant response, as is evident from the shoot, nodule, and N parameters of plants grown in the Keahua soil under N availability (Table 1). All three strains of *R. loti* that were applied as single-strain inoculants were of similar low effectiveness as observed in the result for milligrams of N fixed. In contrast, when soil N was immobilized, inoculation responses and strain differences were pronounced. The most effective (milligrams of N fixed) treatment was inoculation with TAL 1148, whereas multistrain inoculation was similar to single-strain inoculation with TAL 480 or TAL 620. Plants grown under N availability were larger (shoot dry weight) and poorly nodulated (nodule dry weight) and fixed low levels of N. Immobilization of N stimulated better modulation and higher N fixation, although plants were smaller (shoot dry weight).

The availability of N in the soil was significant in influencing the competition pattern of the three *R. loti* strains (see Table 4). The order of competitiveness in untreated soil was TAL 480 > TAL 1148 > TAL 620. However, when soil N was immobilized, the differences in nodule occupancy by the three strains were insignificant ($P > 0.05$), although numerically TAL 620 formed the highest percentage of

TABLE 1. Influence of soil mineral N availability on the effectiveness of three strains of *R. loti* on chick-pea grown in Keahua soil

Inoculation	Soil mineral N available				Soil mineral N immobilized			
	Shoot dry wt (g)[a]	Nodule dry wt (g)[a]	Shoot N (mg)[a]	N fixed (mg)[a]	Shoot dry wt (g)[b]	Nodule dry wt (g)[b]	Shoot N (mg)[b]	N fixed (mg)[b]
TAL 480 (UASB 67)	2.6	0.10	55.7	6	1.2	0.31	23.3	22.3
TAL 620 (CB 1189)	2.5	0.06	52.2	4	1.6	0.30	27.0	26
TAL 1148 (Nit. 27A8)	2.5	0.07	52.5	4	2.0	0.38	35.2	34.2
Multistrain	2.6	0.07	52.5	4	1.4	0.35	25.4	24.4
Uninoculated	2.7	0	48.2	0	0.2	0	1	0
LSD ($P = 0.05$)[c]	NS[d]	NS	NS	NS	0.2	NS	4.7	4.7

[a] Values are given per plant. Plants were 58 days old at harvest.
[b] Values are given per plant. Plants were 59 days old at harvest.
[c] LSD, Least significant difference.
[d] NS, Not significant.

nodules. It was interesting that although TAL 1148 showed consistently similar nodule occupancy under both soil N conditions, TAL 480 nodule occupancy decreased from 59% (mineral N available) to 35% (mineral N immobilized) and TAL 620 nodule occupancy increased from 13% (mineral N available) to 40% (mineral N immobilized).

In the soybean-B. *japonicum* symbiosis, under conditions of soil mineral N availability differences due to single-strain or multistrain inoculations were not significant in the shoot and nodule dry weights (Table 2). However, significant differences were seen in the shoot N and milligrams of N fixed, for which inoculation with TAL 102 alone was highly effective although not significantly different from singlestrain inoculations with TAL 377 and the multistrain inoculant. The multistrain inoculant was of intermediate effectiveness (6.2% lower value for milligrams of N fixed than with TAL 102), whereas inoculation with TAL 379 ranked lowest of all treatments. When soil mineral N was immobilized, multistrain inoculation was also of intermediate effectiveness (milligrams of N fixed) and inoculation with TAL 102 was again the most effective. Under low N conditions, single-strain inoculations with TAL 377 and TAL 379 were of similar effectiveness and TAL 377 became less effective.

Both the availability and immobilization of soil mineral N affected the competition for nodulation by the three strains of B. *japonicum* (see Table 4). When soil N was available, nodule occupancies by TAL 102 and TAL 377 were almost equal, with only 4% of the nodules occupied by TAL 379. However, when soil N was immobilized TAL 102 remained the most competitive strain, occupying 55% of the nodules. It was interesting that TAL 379 increased in nodule occupancy from 4 to 20% and ranked similar in nodule occupancy to TAL 377 (which showed a decrease from 48 to 22% in nodule occupancy). These data indicated that TAL 102 was a highly competitive strain and that its competitiveness, compared with TAL 377 and TAL 379, was not influenced by the soil N status.

In the bean-R. *leguminosarum* bv. phaseoli symbiosis, measurements of the shoot dry weight did not indicate significant differences due to single-strain or multistrain inoculation in plants grown in soil in which soil N was available (Table 3). Low levels of N were fixed in the single-strain inoculation with TAL 182 and the multistrain inoculation, whereas inoculation with TAL 1383 and TAL 1797 did not result in any fixation. Uninoculated treatments were nodulated but nonfixing. The nodule smears from the uninoculated controls did not react with the fluorescent antibody of the inoculant strains, thus indicating nodulation by indigenous ineffective strains.

Immobilization of soil N did not change the symbiotic potential of TAL 1382 or TAL 1797, as both remained poorly effective. Single-strain inoculation with TAL 182 was highly effective. The multistrain inoculation was of intermediate effectiveness and resulted in approximately 37% lower N fixation than did inoculation with TAL 182, although the difference was not statistically significant. Nodulation by the ineffective indigenous strains was also observed when soil N was immobilized.

The N status of the Keahua soil was not a significant factor in the competitive ability of TAL 182 in the multistrain inoculant (Table 4). TAL 182 showed 70 and 85% nodule occupancy under conditions of N availability and immobili-

TABLE 2. Influence of soil mineral N availability on the effectiveness of three strains of *B. japonicum* on soybean grown in Keahua soil

Inoculation	Soil mineral N available				Soil mineral N immobilized			
	Shoot dry wt (g)[a]	Nodule dry wt (g)[a]	Shoot N (mg)[a]	N fixed (mg)[a]	Shoot dry wt (g)[b]	Nodule dry wt (g)[b]	Shoot N (mg)[b]	N fixed (mg)[b]
TAL 102 (USDA 110)	7.0	0.32	142.3	84.5	2.8	0.26	76.8	73.0
TAL 377 (USDA 138)	7.0	0.43	135.1	77.3	2.3	0.30	63.6	59.6
TAL 379 (CB 1809)	6.5	0.32	115.2	57.4	2.2	0.25	62.3	58.4
Multistrain	7.1	0.33	136.8	78.9	2.7	0.26	72.5	68.5
Uninoculated	6.2	0	57.8	0	0.6	0	4.0	0
LSD ($P = 0.05$)[c]	NS[d]	NS	15.1	13.4	0.3	NS	8.5	8.4

[a] Values are given per plant. Plants were 60 days old at harvest.
[b] Values are given per plant. Plants were 43 days old at harvest.
[c] LSD, Least significant difference.
[d] NS, Not significant.

TABLE 3. Influence of soil mineral N availability on the effectiveness of three strains of *R. leguminosarum* bv. phaseoli on dry bean grown in Keahua soil

Inoculation	Soil mineral N available				Soil mineral N immobilized			
	Shoot dry wt (g)[a]	Nodule dry wt (g)[a]	Shoot N (mg)[a]	N fixed (mg)[a]	Shoot dry wt (g)[b]	Nodule dry wt (g)[b]	Shoot N (mg)[a]	N fixed (mg)[a]
TAL 182	3.4	0.13	54.1	8.1	1.9	0.27	46	36.9
TAL 1383 (CIAT 632)	3.4	0.06	44.2	0	1.0	0.09	14.8	5.8
TAL 1797 (CIAT 899)	4.3	0.05	44.3	0	1.1	0.10	17.7	8.6
Multistrain	3.4	0.15	55.5	9.4	1.5	0.21	32.4	23.3
Uninoculated	3.2	0.05	46.1	0	1.0	0.06	9.1	0
LSD (P = 0.05)[b]	NS[c]	0.04	4.3	2.9	0.1	0.07	13.6	13.5

[a] Values are given per plant. Plants were 34 days old at harvest.
[b] LSD, Least significant difference.
[c] NS, Not significant.

zation, respectively. Strains TAL 1383 and TAL 1797 were poor competitors under both soil N conditions, with TAL 182 and indigenous strains being significantly better competitors. Only when TAL 1383 and TAL 1797 were inoculated as single strains did they occupy a large percentage of the nodules.

In the Waikoa soil, significant differences were seen in the effectiveness of the five strains of R. *leguminosarum* bv. phaseoli applied as single-strain inoculants (Table 5). The most effective strain was again TAL 182, whereas TAL 1865 was the least effective in terms of the milligrams of N fixed. TAL 943, TAL 1383, and TAL 1797 were intermediate; their results were similar. Multistrain 1 (with equal number of strains in the mixture) was effective but showed significantly fewer milligrams of N fixed than did single-strain inoculation of TAL 182. Multistrain 2 (with TAL 182 present at 100-fold fewer cells than other strains) was less effective than multistrain 1 and showed less N fixed, but the differences were not significant at the $P = 0.05$ level. Multistrain 1 and multistrain 2 showed 29.8 and 48.2% lower N fixed, respectively, than single-strain inoculation with TAL 182. The five strains had large differences ($P < 0.001$) in their competitive abilities (TAL 182 > TAL 1865 > TAL 943 > TAL 1383 = TAL 1797) when they were equally represented as in multistrain 1 inoculation (Table 6). The most competitive strain was TAL 182, which formed 43.5% of the nodules, whereas TAL 1865 formed 29.4%. The lowering of TAL 182 by 100-fold relative to the other strains in multistrain 2 significantly altered the competition pattern between TAL 182 and TAL 1865, and this was reflected by a significant ($P < 0.001$) interaction between the strains and their numerical representation in the inoculant. In multistrain 2, nodulation by TAL 182 was zero, while nodulation by TAL 1865 increased to 58.3%.

Several desirable qualities required of rhizobial strains for use in inoculants have been listed, of which competitiveness and effectiveness over a range of environmental conditions are frequently mentioned (3, 4, 27). Since soil N is a major environmental factor limiting nitrogen fixation by the symbiotic association between legumes and rhizobia, our study addressed competitiveness and effectiveness under conditions of soil mineral N availability and immobilization.

Recognizing that the inhibition of the legume-rhizobium symbiosis by mineral N is a stress (8), we found that the *B. japonicum*-soybean symbiosis was the most tolerant of the symbiotic systems studied in this investigation. It was clear that of the three *B. japonicum* strains, TAL 102 (USDA 110) was consistently the most effective under both mineral N conditions of the Keahua soil, whereas TAL 377 became less

TABLE 4. Effect of soil mineral N availability on the nodule occupancy by strains of *R. loti*, *B. japonicum*, and *R. leguminosarum* bv. phaseoli

Strain[a]	% Nodule occupancy[b] with soil mineral N:	
	Available	Immobilized
R. loti		
TAL 480	59a	35a
TAL 620	13c	40a
TAL 1148	28b	25a
B. japonicum		
TAL 102	47a	55a
TAL 377	48a	22b
TAL 379	4b	20b
R. leguminosarum		
TAL 182	70a	85a
TAL 1383	1c	1c
TAL 1797	4c	2c
Indigenous	25b	12b

[a] Set of three strains of each rhizobial species in the multistrain inoculant.
[b] Means in the same column followed by the same letter are significantly different by Duncan's multiple range test (P = 0.05) within each rhizobial species.

TABLE 5. Influence of soil mineral N and inoculant composition on the effectiveness of five strains of *R. leguminosarum* bv. phaseoli inoculated on dry bean grown in Waikoa soil

Inoculation	Shoot dry wt (g)[a]	Nodule dry wt (g)[a]	Shoot total N (mg)[a]	N fixed (mg)[a]
TAL 182	3.2	0.32	138.2	116.6
TAL 943 (Kim-5)	2.4	0.44	104.6	77.9
TAL 1383 (CIAT 632)	2.7	0.21	112.1	85.5
TAL 1797 (CIAT 899)	2.3	0.29	96.8	70.1
TAL 1865 (Viking-1)	1.8	0.28	72.9	46.3
Multistrain 1[b]	2.7	0.30	108.4	81.8
Multistrain 2[c]	2.1	0.28	87.1	60.4
Uninoculated	1.3	0.07	26.6	0
LSD (P = 0.05)[d]	0.5	0.12	20.5	20.5

[a] Values are given per plant.
[b] TAL 182 represented in equal numbers with other strains.
[c] TAL 182 represented at a 100-fold-lower level than other strains.
[d] LSD, Least significant difference.

TABLE 6. Competition for nodule occupancy by five strains of *R. leguminosarum* bv. phaseoli on dry bean grown in a low-N mollisol (Waikoa series)

Inoculant strain	% Nodule occupancy[a] by:	
	Multistrain 1[b]	Multistrain 2[c]
TAL 182	43 a	0 d
TAL 943 (Kim-5)	20 c	21 b
TAL 1383 (CIAT 632)	2 d	10 c
TAL 1797 (CIAT 899)	0.3 d	0.3 d
TAL 1865 (Viking-1)	29 b	58 a

[a] Data for single-strain inoculation not shown. A 100% nodule occupancy was recorded for each strain used as a single-strain inoculant. Means in the same column followed by the same letter are significantly different by Duncan's multiple range test ($P = 0.05$) within each rhizobial species.
[b] TAL 182 represented in equal numbers with other strains.
[c] TAL 182 represented at a 100-fold-lower level than other strains.

TABLE 7. Linear correlations between milligrams of N fixed per plant and percent nodule occupancy by rhizobial strains in multistrain inoculants

Symbiotic system	*r* (significance level) with soil mineral N:	
	Available	Immobilized
Chick-pea, *R. loti*	0.98 ($P < 0.001$)	0.64 ($P < 0.05$)
Soybean, *B. japonicum*	0.99 ($P < 0.001$)	0.99 ($P < 0.001$)
Dry bean, *R. leguminosarum* bv. phaseoli	NA[a]	0.97 ($P < 0.001$)
Dry bean, *R. leguminosarum* bv. phaseoli[b]	0.77 ($P < 0.001$)	ND[c]
Dry bean, *R. leguminosarum* bv. phaseoli[d]	0.76 ($P < 0.001$)	ND

[a] NA, Not applicable since nodulated plants did not fix N.
[b] Dry bean grown in the Waikoa soil given multistrain 1 inoculations.
[c] ND, Not done in Waikoa soil.
[d] Dry bean grown in the Waikoa soil given multistrain 2 inoculations.

effective under conditions of N immobilization. Consistency in the effectiveness (milligrams of N fixed) and competitiveness (Table 4) of TAL 102 qualified it to be a superior strain for these two soils. Our data on *B. japonicum* TAL 102 complements those of George et al. (7), who showed that TAL 102 was the best competitor for nodulation on five soybean varieties grown at three elevations where significant differences in soil types and root temperatures were encountered. Abaidoo *et al.* (1) also found TAL 102 to be the superior competitor on one variety of soybean grown at two sites with three levels of added fertilizer N.

An effective symbiosis was established between the chickpea and the three R. loti strains when soil mineral N was immobilized in the Keahua soil. However, in the same soil and under similar mineral N status, *R. leguminosarum* bv. phaseoli TAL 1383 and TAL 1797 were ineffective compared with TAL 182, which was highly effective. It appears that soil mineral N immobilization was inhibitory to the modulation and nitrogen fixation by strains TAL 1383 and TAL 1797. The ineffectiveness of TAL 1383 (CIAT 632) was surprising, as it was previously shown to be one of the effective strains, but not as highly effective as TAL 182, on three cultivars of *P. vulgaris* grown in autoclaved perlite (17). That the availability of soil mineral N was a factor needed for expressing the symbiotic potential of TAL 1383 was demonstrated in the Waikoa soil (Table 5), in which the soil mineral N was available.

Although our data clearly indicate that a single-strain inoculum of the appropriate strain is the most effective in all the three species, they do not necessarily invalidate the use of multistrain inoculants. It appears that the effectiveness of mixed inoculants is dependent on the effectiveness (Tables 1, 2, 3, and 5) and competitiveness (Tables 4 and 6) of the strains in the mixture. This was true in all three systems used in this study.

The significance and importance of maintaining a balance in the numbers of the component rhizobial strains of the same species in a multistrain inoculant was demonstrated in the difference in the effectiveness between multistrain 1 and multistrain 2 applied on dry bean (Table 5). The number of milligrams of N fixed by multistrain 2 was 51.8% lower than that of the most effective treatment, which was single-strain inoculation of TAL 182. The lowering of the effectiveness of multistrain 2 was attributed to the competitive dominance of the relatively ineffective TAL 1865 (Viking-1), which had an advantage in being present at a 100-fold-higher level than TAL 182 in the inoculant at the time of application (Tables 5 and 6). These results illustrate that relative changes in the population levels among component strains of multistrain inoculants can lessen inoculant effectiveness if an ineffective strain becomes dominant.

The mineral N status of the soil was clearly a significant factor in the competition pattern of the three *R. loti* (chickpea) and *B. japonicum* strains (Table 4). Immobilization of soil mineral N by incorporation of ground bagasse or addition of calcium nitrate had no effect on the percentage of nodules occupied by *B. japonicum* USDA 110 and USDA 123 nodulating soybean cultivars Lee and Davis grown in three soils (11). There were indications that USDA 110 and CB 1809 (TAL 379) competed differently in sand culture when nitrate was present (13). In the dry bean-*R. leguminosarum* bv. phaseoli symbiosis, the soil mineral N status did not appear to influence interstrain competition among inoculant strains although the indigenous population showed a 50% reduction in nodule occupancy when soil mineral N was immobilized. Overall, TAL 182 was the most competitive irrespective of the soil N status and soil type on *P. vulgaris* cv. Bountiful. Abaidoo *et al.* (1) have also found TAL 182 to be the dominant strain on beans grown at two field sites with three levels of added N.

Although much work has been documented in rhizobial ecology in evaluating the success of inoculant strains (measured as percent nodule occupancy) in numerous competition experiments, meaningful interpretations of nodule occupancies in relation to the nitrogen fixed by the nodule occupants have apparently not been quantified previously. We have shown in this work that the percentage of nodules occupied (P_m) and the milligrams of N fixed (N_c) by the component strains of a mixed inoculant bear a simple linear relationship (Table 7). Thus, the proportion of nodules occupied by an individual strain in a mixed inoculation is indicative of its N-fixing contribution to the host legume. For example, *R. leguminosarum* bv. phaseoli TAL 182 showed the highest percentage of nodule occupancy under both conditions of soil mineral N availability (Table 4), and therefore the effectiveness of the multistrain inoculant was attributed to TAL 182. This further highlights the importance of competitiveness of effective strains chosen for inoculant formulations.

Our conclusions are supported by the highly significant values of r (Table 7) in all cases except with chick-pea inoculation in Keahua soil in which soil mineral N was immobilized (Table 1) and $r = 0.64$ ($P < 0.05$) (Table 7). The significant relationship between percent nodule occupancy and milligrams of N fixed remained even when the milligrams of N fixed were small, as was the case with chick-pea (Table 1) and dry bean (Table 3) grown in the Keahua soil after soil mineral N was immobilized. However, this relationship was demonstrated only in soils in which either native rhizobia for the test legume were lacking or native rhizobia were present but nodulation was of low frequency and did not fix nitrogen as seen in the case of dry bean-R. *leguminosarum* bv. phaseoli symbiosis. This relationship warrants further investigation in inoculation studies conducted in the presence of effective native rhizobia nodulating the test legume.

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