

RESPONSE OF TREE LEGUMES TO RHIZOBIAL INOCULATION IN RELATION TO THE POPULATION DENSITY OF INDIGENOUS RHIZOBIA

DAN TURK,* HAROLD H. KEYSER† and PAUL W. SINGLETON

NifTAL Project, University of Hawaii, 1000 Holomua Road, Paia, HI 96779,

U.S.A. (Accepted 10 July 1992)

Summary—To determine the relationship between leguminous tree yield response to inoculation and indigenous rhizobial population density, an inoculation experiment was conducted in pots using four soils and six tree species: *Acacia auriculiformis* A. Cunn. ex Benth., *A. mangium* Willd., *A. mearnsii* De Wild., *Leucaena diversifolia* (Schlecht.) Benth., *Robinia pseudoacacia* L. and *Sesbania grandiflora* Poir. Densities of indigenous rhizobia were determined by most probable number (MPN) plant-infection assays. Statistically significant increases in shoot N due to inoculation were observed most frequently in soils with < 50 rhizobia g⁻¹ soil. Inoculation resulted in significant increases in shoot N ($P < 0.05$) for *R. pseudoacacia* and *A. mearnsii* in three and one soils, respectively, despite the presence of > 1000 rhizobia g⁻¹ soil. A hyperbolic model best described the relationship between response to inoculation and the density of indigenous rhizobia. Incorporating an index of available soil N into the hyperbolic model reduced residual mean square values, indicating that available mineral N attenuates the response to inoculation.

INTRODUCTION

As natural forests are depleted and fallow periods diminished in shifting agricultural systems of the tropics, fast-growing nitrogen-fixing trees are becoming more important as sources of fuelwood, fodder and nitrogen-rich biomass. Inoculation of seeds or seedlings with rhizobia insures that there are sufficient effective rhizobia to meet the demand from leguminous trees for biological N₂ fixation. Where available mineral N limits growth and appropriate indigenous rhizobia are scarce or absent, inoculation can increase legume yields and monetary returns. However, availability of quality inoculant, distribution infrastructure, and educational constraints often prevent farmers from using rhizobial inoculant in the tropics. Because of the investment required to overcome these constraints, knowledge of where inoculation is likely to increase economic yields can help farmers and regional planners make sound decisions concerning investment in rhizobial inoculant technology.

Quantifying factors regulating legume response to inoculation is an approach proposed to assess the potential magnitude of responses to inoculation with rhizobia without resorting to pot or field experiments (Singleton and Tavares, 1986; Brockwell *et al.*, 1988). Major factors likely to influence response of legumes to inoculation are limitations to plant growth other than N; density of indigenous rhizobia in soil; availability of mineral N;

and effectiveness of indigenous rhizobia (Singleton *et al.*, 1992).

Empirical models describing the response to inoculation of field-growth grain and forage legumes (Thies *et al.*, 1991 b) indicated that when limitations other than mineral N availability are removed, rhizobial density as estimated by the most probable number plant infections (MPN) assay is the primary factor determining the magnitude of response to inoculation. Models that accounted for the effects of mineral N availability in addition to rhizobial density improved the agreement between observed and predicted responses to inoculation. Singleton *et al.* (1992) noted that measures of indigenous rhizobial population effectiveness, except where completely ineffective, have not proven to be good indicators of the magnitude of response to inoculation.

Our experiment was made to determine how the response of six tree legumes to rhizobial inoculation related to the density of their homologous indigenous rhizobial populations and to an index of available soil N. A second purpose was to evaluate how specificity for nodulation and effectiveness of the six tree legumes (Turk and Keyser, 1992) influenced responses to inoculation.

MATERIALS AND METHODS

Experimental design of pot experiment

A pot experiment was conducted in a greenhouse at 110 m elevation at Hamakuapoko, Maui, Hawaii using four soils (Table 1), seven legumes, and two treatments.

*Present address: Department of Forestry, North Carolina State University, Box 8002, Raleigh, NC 27695, U.S.A.

†Author for correspondence.

Table 1. Characteristics of soils used in inoculation experiments and MPN assays

Soil series ^a	Soil classification ^b	Maui net site	Median annual rainfall ^c (mm year ⁻¹)	Mineralized N (g pot ⁻¹) ^d	pH ^e	Legumes present at site
Pane	Typic Hapludand	Pasture Keko	1060	5.35	6.2	<i>Desmodium</i> sp., <i>Medicago</i> sp., <i>T. repens</i> , <i>V. sativa</i>
Makawao	Humoxic Tropohumult	Haleakala Station	1800	2.32	5.4	<i>Desmodium</i> sp., <i>T. repens</i> , <i>L. leucocephala</i>
Keahua	Aridic Haplustoll	Pasture Waiakoa	380	2.21	6.2	<i>Crotalaria</i> spp., <i>Prosopis</i> sp.
Waiakoa	Aridic Haplustoll	Hashimoto Farm	322	0.94	6.3	<i>L. leucocephala</i> , <i>Crotalaria</i> spp., <i>Indigofera</i> sp.

^aSoil Conservation Service (1972).^bSoil Conservation Service (1972) and Ikawa *et al.* (1985).^cDepartment of Land and Natural Resources (1982).^dCalculated as g N g⁻¹ soil week⁻¹ 9 weeks g soil pot⁻¹.^e1:1 with water.

Treatments were inoculation with rhizobia or no inoculation. In addition, two species in each soil received mineral N to measure yield potential in the growth system. Pots were arranged in a randomized complete-block design, with four replicates. The experiment was divided into two halves: the first half was conducted with the Pane and Makawao soils from 7 December 1990 to 8 February 1991 and the second half with the Keahua and Waiakoa soils from 1 February 1991 to 5 April 1991.

Description of tree species

Six tree species were selected. *Leucaena diversifolia* (Schlecht.) Benth., *Robinia pseudoacacia* L. and *Sesbania grandiflora* Poir., were from genera known to nodulate effectively with rhizobia belonging to distinct effectiveness groups within the genus *Rhizobium* Frank (Turk and Keyser, 1992a). The other species, *Acacia auriculiformis* A. Cunn. ex Benth., *A. mangium* Willd. and *A. mearnsii* De Wild., have a range of specificity within the genus *Bradyrhizobium* Jordan (Turk and Keyser, 1992). *Macroptilium atropurpureum* Urb. cv. siratro, which nodulates effectively with many strains of *Bradyrhizobium* (Vincent, 1970), was included for comparison with the *Acacia* spp. All seeds were obtained from either the Nitrogen Fixing Tree Association (Box 680, Waimanalo, HI 96795, U.S.A.) or NifTAL Project collections.

Establishment and maintenance of pot experiment

Four soils (Table 1) were selected based on the diversity of their respective rhizobial populations (Woomer *et al.*, 1988). Soils were excavated to a depth of 20 cm and sieved (< 5 mm). Black plastic pots (5 litres) lined with polyethylene bags were filled with 2000, 3300, 3500 or 4520 g of soil (oven dry weight basis) per pot for the Pane, Makawao, Keahua and Waiakoa soils, respectively, to give equivalent soil volumes. The following fertilizers were added (kg⁻¹ soil): 1.07 g K₂HPO₄, 0.25 g MgSO₄ · 7H₂O, and 0.5 ml of a liquid micronutrient mix (Hawaiian

horticultural mix, Monterey Chemical Co.). A total of 350 mg N kg⁻¹ soil as NH₄NO₃ were added to the mineral N controls as follows: 50 mg kg⁻¹ soil at the time of inoculation, 150 mg kg⁻¹ soil 3 weeks after inoculation, and 150 mg kg⁻¹ soil 6 weeks after inoculation.

Seeds were appropriately scarified and surface sterilized. Imbibed seeds were planted in expanded horticultural vermiculite (Grace and Co.) 4–10 days prior to transplanting into pots. Four to eight seedlings were planted and thinned to four plants per pot after 3 weeks.

Within 2 days after planting, each pot received 5 ml of a 10⁻³ dilution of an appropriate three-strain mixture of rhizobia (Table 2). The inoculant was prepared by growing each of the three strains separately for 6–10 days in either yeast-extract mannitol broth (Vincent, 1970) or arabinose-gluconate

Table 2. Strains used in inoculation experiment and MPN assays

Species	TAL No.	Strain synonyms ^a	Original host ^a
<i>A. auriculiformis</i>	569 ^b	MAR 472	<i>D. uncinatum</i>
	1388		<i>A. mearnsii</i>
	1446 ^b		<i>A. auriculiformis</i>
<i>A. mangium</i>	569	MAR 472	<i>D. uncinatum</i>
	1388		<i>A. mearnsii</i>
	1867 ^b	LB 5	<i>A. mangium</i>
<i>A. mearnsii</i>	132		<i>A. mearnsii</i>
	940	NUM 777	<i>A. mearnsii</i>
	941 ^b	NUM 778	<i>A. mearnsii</i>
<i>L. diversifolia</i>	583	NGR 8	<i>L. leucocephala</i>
	1145 ^b	CIAT 1967	<i>L. leucocephala</i>
	1887	MS 111	<i>L. leucocephala</i>
<i>R. pseudoacacia</i>	183	NIT 137A4	<i>R. pseudoacacia</i>
	1889 ^b	USDA 3436	<i>R. pseudoacacia</i>
	1907	USDA 3112	<i>R. pseudoacacia</i>
<i>S. grandiflora</i>	1113	IC 70	<i>Sesbania</i> sp.
	1114	IC 71	<i>Sesbania</i> sp.
	1119 ^b	IC 91	<i>Sesbania</i> sp.
<i>M. atropurpureum</i>	169	NIT 176A22	<i>V. unguiculata</i>
	209 ^b		<i>V. radiata</i>
	1000		<i>A. hypogaea</i>

^aNifTAL strain collection.^bStrain used for comparison of pure culture MPN estimates with plate counts.

Table 3. Rhizobial densities of six tree legumes and *M. atropurpureum* in four tropical soils and associated comparisons of pure culture MPN estimates to plate counts

Species	Growth system	Soil				Pure rhizobial culture	
		Pane	Makawao (Rhizobia g ⁻¹ soil)	Keahua	Waiakoa	Greenhouse Trial 1 (Plate count:MPN ratio)–	Greenhouse Trial 2
<i>L. diversifolia</i>	Tube	ND ^a	> 338,000 ^b	6.1	9670	1.0	3.0
<i>S. grandiflora</i>	Pouch	ND	ND	1.0	ND	6.5	i.f. ^c
<i>R. pseudoacacia</i>	Pouch	21,600	> 21,600	39.5	6480	2.0	15.4
<i>A. auriculiformis</i>	Tube	> 2510	> 26,300	8.6	366	5.0	5.2
<i>A. mearnsii</i>	Tube	49,400	> 227,000	135	320	0.6	3.0
<i>A. mangium</i>	Tube	21.9	> 35,600	41.3	6.8	17.9	5.0
<i>M. atropurpureum</i>	Pouch	18,400	10,500	6.5	27.2	i.f.	14.9

^aND = below the detection limits of the MPN assay: <0.2 rhizobia g⁻¹ soil for Pane and Makawao soils and <1.1 rhizobia g⁻¹ soil for Waiakoa soil.

^b> = above the detection limits of the MPN assay.

^ci.f. = inoculant failure: colonies formed on plates but plants did not nodulate.

medium (Sadowsky *et al.*, 1987). The inoculum was applied evenly around the roots of each seedling to give ca 5 x 10⁶ rhizobia per pot. The inoculant was washed into the soil with 50 ml of sterile water. The soil surface was then covered with 850 g of sterile washed gravel.

Pots with *M. atropurpureum* were provided with sterile stakes to accommodate their climbing habit. Soil was maintained at, or near, field capacity throughout the course of the experiment.

Harvest and analysis

Plants were harvested 9 weeks after inoculation. Tops were severed at gravel level, dried at 70°C, weighed, ground and analyzed for N content using a Leco 600 C-H-N analyzer. The root systems of three replicates were cleaned by washing over 1 mm mesh screens. Nodules were removed and counted, and roots and nodules weighed separately after drying at 70°C.

Results were analyzed using SYSTAT, version 5.0 (Wilkinson, 1990). Dependent 1-tailed *t*-tests for each species-soil combination were used to test for significant increases due to inoculation. In addition, Tukey's HSD was used to analyze species-soil combinations where mineral N was applied.

Soil N availability

N mineralization values for each soil were determined following the anaerobic incubation method of Keeney (1982) using standards prepared by steam distilling aliquots of NH₄SO₄. N mineralization values were multiplied by the dry weight of soil per pot and the number of weeks the plants were grown to yield an index of N mineralized per pot over the course of the experiment.

MPN assays

MPN assays for each species-soil combination were made according to Turk and Keyser (1993), using the best growth system identified in that study. For each of the two sets of greenhouse experiments, MPN assays of pure rhizobial cultures were compared with plate counts (Table 3) to provide an evaluation of the growth

systems used, in accordance with the recommendations of Thompson and Vincent (1967), Scott and Porter (1986) and Singleton *et al.* (1991).

Soil for the MPN assays was taken from extra pots just prior to planting. 50 g of soil (dry weight equivalent) was used for the initial dilutions. Dilution ratios were 4.0 for *L. diversifolia*, *R. pseudoacacia* and *S. grandiflora* in the Pane and Makawao soils and 5.0 for all other species-soil combinations. The amount of soil applied at the lowest dilution level was 1 g for the Pane and Makawao soils, and 0.2 g for the other two soils. Eight uninoculated controls were interspersed among the growth units of each MPN assay.

Nodulation was assessed at 7 weeks after inoculation. The MPN was determined with a computer program (Woomer *et al.*, 1990).

Models of response to inoculation

Models relating the response to inoculation to the density of indigenous rhizobia were fit to the data using the "nonlin" module of the SYSTAT program version 5.0 (Wilkinson, 1990). Response to inoculation for each species-soil combination was defined as $R = 100 (N_1 - N_u)/N_u$ where R = response to inoculation, N_1 = average shoot N in inoculated pots, and N_u = average shoot N in uninoculated pots. An MPN value of 0 was assigned to those observations where no rhizobia were detected in the MPN assay. In cases where the MPN assay did not go to extinction, the density used was that generated by the computer program assuming no nodulation occurred at subsequent dilution steps. The index of available mineral N was incorporated into the model with the lowest residual mean square by replacing the y intercept of this model with functions of the available mineral N index values.

RESULTS

MPN assays

Indigenous rhizobial densities varied for all species and in each soil (Table 3). No rhizobia infective for *S. grandiflora* or *L. diversifolia* were detected in the

Table 4. Shoot dry weight of tree species for tree-soil combinations having mineral N treatments

Species	Soil	+N	Inoculated	Uninoculated
			(g Pot ⁻¹)	
<i>A. mearnsii</i>	Pane	24.5 a	9.1 b	6.5 b
<i>S. grandiflora</i>	Pane	23.5 a	17.5 b	6.7 c
<i>L. diversifolia</i>	Makawao	25.2 a	12.1 b	11.0 b
<i>A. auriculiformis</i>	Keahua	14.3 a	5.4 b	5.7 b
<i>R. pseudoacacia</i>	Keahua	20.4 a	8.8 b	2.4 c
<i>A. mangium</i>	Waiakoa	14.5 a	1.0 b	1.2 b
<i>S. grandiflora</i>	Waiakoa	40.2 a	21.9 b	3.6 c

For each species-soil combination, means followed by the same letter are not significantly different by Tukey's HSD.

Pane soil where the MPN estimate for *R. pseudoacacia* was over 10⁶ rhizobia g⁻¹ soil. No rhizobia for *S. grandiflora* were detected in the Makawao soil where the rhizobial densities of *R. pseudoacacia* and *L. diversifolia* were both > 10⁶ rhizobia g⁻¹ soil. All pure culture MPN estimates had plate count: MPN ratios < 18.

Response to mineral N

Mineral N significantly increased shoot dry weight over uninoculated and inoculated treatments in all species-soil combinations where N was applied (Table 4).

Response to inoculation

Response to inoculation as evaluated by total shoot N was also species and soil specific (Fig. 1). Of those tree-soil combinations involving species that nodulate with *Rhizobium*, a significant ($P < 0.05$) increase in N accumulation due to inoculation was observed in nine of 12 cases, but was observed in only four of 12 combinations involving species that nodulate with *Bradyrhizobium*. Inoculation did not significantly

increase shoot N of *M. atropurpureum* in any soil and did not increase shoot N of *L. diversifolia* in the two soils from sites where *L. leucocephala* occurred.

Where indigenous rhizobial density was <50 rhizobia g⁻¹ soil, the mean shoot N in inoculated treatments was 4.75 times greater than in uninoculated treatments. Where the density was > 50 rhizobia g⁻¹ soil, the mean shoot N in inoculated treatments was only 1.22 times greater than in uninoculated treatments. *R. pseudoacacia*, *A. mearnsii* and *A. auriculiformis* had significant increases due to inoculation despite the presence of > 50 rhizobia g⁻¹ soil in three, two and one of the soils, respectively.

Shoot dry weight and nodule dry weight response to inoculation (Fig. 2) were similar to shoot N response. Fewer significant increases due to inoculation were observed with root weight and nodule number data. Coefficients of linear correlation (r) between shoot N and shoot mass, nodule mass, root mass and nodule number were 0.98, 0.88, 0.51 and 0.43, respectively, all of which were significant at $P < 0.05$.

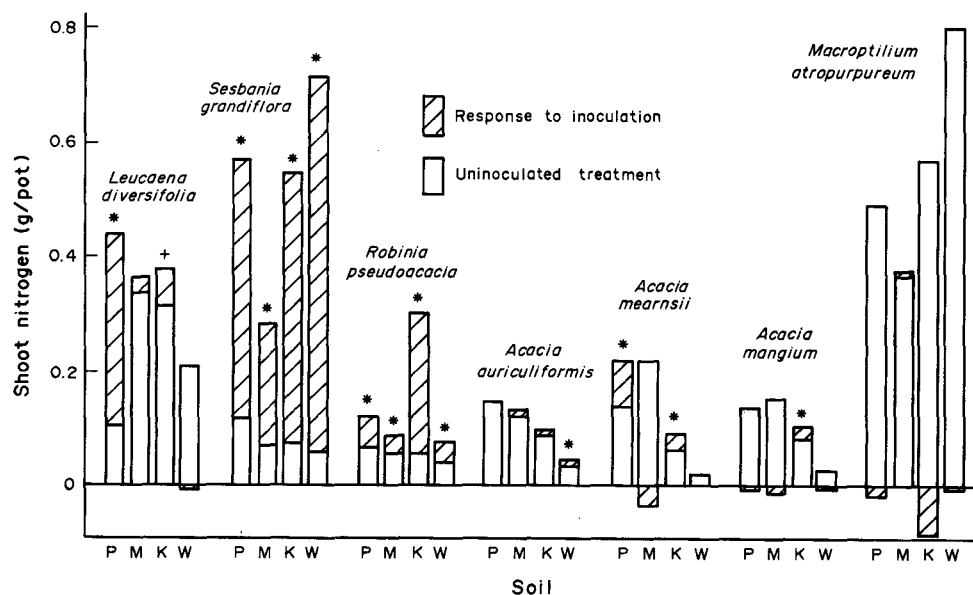


Fig. 1. Shoot nitrogen yield of uninoculated treatments and response due to inoculation. Symbols: *, +; significant increase in shoot N due to inoculation at $P < 0.05$ and $P < 0.10$, respectively. Soils: P, Pane; M, Makawao; K, Keahua; W, Waiakoa.

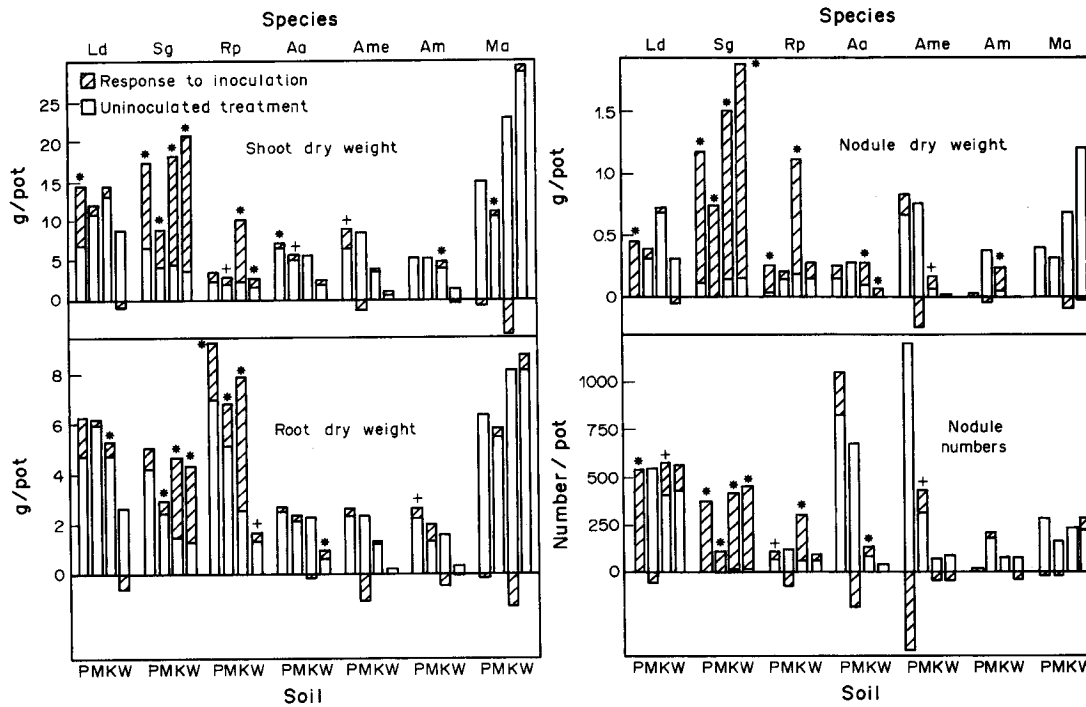


Fig. 2. Shoot dry weight, root dry weight, nodule dry weight, and nodule numbers of uninoculated treatments, and their respective responses due to inoculation. Symbols: *, +; significant increase in shoot N due to inoculation at $P < 0.05$ and $P < 0.10$ respectively. Species: Ld, *L. diversifolia*; Sg, *S. grandiflora*; Rp, *R. pseudoacacia*; Aa, *A. auriculiformis*; Ame, *A. mearnsii*; Am, *A. mangium*; Ma, *M. atropurpureum*. Soils: P, Pane; M, Makawao; K, Keahua; W, Waiakoa.

A hyperbolic model taking the form: $y = 596.3 / (1 + \text{MPN})$, where y = shoot N response to inoculation, best described the relationship between rhizobial density and inoculation response (Table 5). The residual mean square for this model was lower than that of linear, quadratic and power models. Models where the y-intercept of the hyperbolic model was replaced with functions incorporating the measure of soil N availability had even lower residual mean square values (Table 5).

DISCUSSION

The results of our experiment indicate that response to inoculation declines precipitously as

rhizobial density increases from 0 to 50 rhizobia g^{-1} soil. The relationship observed is very similar to those reported by Thies *et al.* (1991 a, b) and Singleton and Tavares (1986) for grain and forage legumes. As in our experiments, Thies *et al.* (1991b), found that a hyperbolic model best described the relationship between rhizobial density and increase in economic yield of grain and forage legumes due to inoculation. Measures of soil N availability improved the fit of their predictive models to observe inoculation responses, as was achieved with the model developed from our data. Therefore, our data indicate that the relationship between rhizobial density and response to inoculation is fundamentally the same with trees as it is with grain and forage legumes and that

Table 5. Models of shoot N response to inoculation using measures of rhizobial density, mineralized N, and BNF potential

Model ^a	Coefficients			Residual mean square
	<i>a</i>	<i>b</i>	<i>c</i>	
<i>Models with rhizobial density alone</i>				
$y = a + bx$	191.0	-0.001		77,550
$y = ax^{-b}$	550.3	20.84		54,137
$y = a + b(\log x) + c(\log x)^2$	525.8	-129.7	7.368	41,861
$y = a/x$	596.3			38,467
<i>Models with rhizobial density and mineralized N pot^{-1}</i>				
$y = (a + bm)/x$	1105	-149.1		24,883
$y = (ae^{-bm})/x$	1461	0.322		22,184
$y = (am^{-b})/x$	1106	0.755		19,630
$y = (a + b/m)/x$	155.4	947.9		19,360

^a y = shoot nitrogen response to inoculation expressed as a % of the uninoculated treatment; a, b, c = constants; $x = 1 + \text{MPN estimate}$; m = mineralized N pot^{-1} .

availability of mineral N has a similar effect on woody and non-woody legumes.

Species differences in MPN results and response to inoculation (Table 3, Fig. 1) are consistent with what is known about the effectiveness groups of the trees used in our study. The data we obtained support the general view (Dommergues, 1987; Dreyfus and Dommergues, 1981; Peoples *et al.*, 1989) that species nodulating with *Rhizobium* respond more often to inoculation than species nodulating with *Bradyrhizobium*. Our data also support the separation of *Sesbania*, *Leucaena* and *Robinia* into distinct effectiveness groups (Turk and Keyser, 1992) since both the density of indigenous rhizobia and the magnitude of response to inoculation were species dependent.

Effective nodulation of *L. diversifolia* and of *Vicia* sp. and *Trifolium repens* L. when inoculated with rhizobial isolates from nodules formed on *R. pseudoacacia* grown in the Makawao and Pane soils, respectively (data not shown), indicated that *R. pseudoacacia* was nodulating with rhizobia from other effectiveness groups as we found using pure cultures (Turk and Keyser, 1992). These indigenous rhizobia were relatively ineffective at N₂ fixation with *R. pseudoacacia*, as significant inoculation response was obtained in each of the four soils despite MPN estimates of up to >21,600 rhizobia g⁻¹ soil (Table 3). Similarly, the inoculation response of *A. mearnsii* in the Pane soil despite an MPN estimate of over 10⁴ rhizobia g⁻¹ soil supports our conclusion (Turk and Keyser, 1992) that *A. mearnsii* is promiscuous for nodulation but specific for effectiveness.

Due to the lack of precision in MPN estimations (Scott and Porter, 1986), a conservative recommendation would be to inoculate trees where MPN population estimates are < 10³ rhizobia g⁻¹ soil. If this recommendation is applied to our observed results, inoculation would have been recommended for 14 of 24 tree-soil combinations, 10 of which had significant increases due to inoculation. But, no inoculation would have been recommended for four species-soil combinations where, in fact, there were significant increases due to inoculation: *R. pseudoacacia* in the Pane, Makawao and Waiakoa soils and *A. mearnsii* in the Pane soil.

When effectiveness groups of rhizobia are taken into consideration, inoculation would be expected to increase shoot N of *R. pseudoacacia* in each soil investigated because no legumes within its effectiveness group are present at any of the sites. In the same way inoculation would be expected to increase shoot N content of *A. mearnsii* in the Pane soil. With the Makawao soil, although no *A. mearnsii* trees were present at the collection site, they do grow naturally in the general vicinity, which may explain the lack of an increase in shoot N due to inoculation in soil from this site.

Our results show that, assuming mineral N availability limits growth, inoculation can be expected to increase shoot N content of leguminous trees where

rhizobial densities are low. Investors in inoculant technology for tree legumes should focus on areas where rhizobial densities are generally low, and on species that are more specific in their rhizobial requirements. When assessing whether or not inoculation is required, tree planters should take into consideration factors related to rhizobial density in the soil, particularly median annual rainfall (Woomer *et al.*, 1988) and the rhizobial effectiveness groups of legumes growing at the plantation site. On a regional basis, the MPN assay can help identify areas where inoculation is likely to result in improved tree growth. Lastly, the MPN assay should be used in association with an effectiveness test for species such as *R. pseudoacacia* and *A. mearnsii* that are relatively promiscuous for nodulation but specific for effectiveness in their rhizobial requirements.

Acknowledgements—Thanks to Geoff Haines and Kevin Keane for assistance in soil preparation and the tedious task of nodule picking, to Joe Rourke and Patty Nakao for help with the MPN assays and maintenance of the pot experiment, and to Haleakala Ranch Co. and Mr T. Hashimoto for use of soil from their properties. This publication was made possible through support provided under cooperative agreements by the United States Agency for International Development, Office of Agriculture, Bureau of Research and Development. Conclusions in this paper and mention of product names do not constitute an endorsement by USAID. Journal series No. 3662 of the Hawaii Institute of Tropical Agriculture and Human Resources.

REFERENCES

- Brockwell J., Holliday R. A. and Pilka A. (1988) Evaluation of the symbiotic nitrogen-fixing potential of soils by direct microbiological means. *Plant and Soil* 108, 163-170. Department of Land and Natural Resources (1982) Median Rainfall. Circular C88, Department of Natural Resources, Division of Water and Land Development, State of Hawaii, Honolulu.
- Dommergues Y. R. (1987) The role of biological nitrogen fixation in agroforestry. In *Agroforestry, a Decade of Development* (H. A. Steppeler and P. K. R. Nair, Eds), pp. 245-271. International Council for Research in Agroforestry, Nairobi.
- Dreyfus B. L. and Dommergues Y. R. (1981) Nodulation of Acacia species by fast- and slow-growing tropical strains of *Rhizobium*. *Applied and Environmental Microbiology* 41, 97-99.
- Ikawa H., Eswaran H., Sato H. H. and Uehara G. (1985) A critical evaluation of the placement of the Andepts of Hawaii according to the proposed key for Andisols. In *Proceedings of the Sixth International Soil Classification Workshop*, Chile and Ecuador, Part 1 (F. H. Beinroth, W. L. Luzio, F. P. Maldonado and H. Eswaran, Eds), pp. 277-289. Sociedad Chilena de la Ciencia de Suelo, Santiago.
- Keeney D. R. (1982) Nitrogen-availability indices. *Agronomy* 9, 711-733.
- Peoples M. B., Faizah A. W., Rerkasem B. and Herridge D. F. (1989) Methods for Evaluating Nitrogen Fixation by Nodulated Legumes in the Field. Australian Centre for International Agricultural Research, Canberra.

- Sadowsky M. J., Tully R. E., Cregan P. B. and Keyser H. H. (1987) Genetic diversity in *Bradyrhizobium japonicum* serogroup 123 and its relation to genotype-specific nodulation of soybean. *Applied and Environmental Microbiology* 53, 2624-2630.
- Scott J. M. and Porter F. E. (1986) An analysis of the accuracy of a plant infection technique for counting rhizobia. *Soil Biology & Biochemistry* 18, 355-362.
- Singleton P. W. and Tavares J. W. (1986) Inoculation response of legumes in relation to the number and effectiveness of indigenous rhizobium populations. *Applied and Environmental Microbiology* 51, 1013-1018.
- Singleton P. W., Bohlool B. B. and Nakao P. L. (1992) Legume response to rhizobial inoculation in the tropics: myths and realities. In *Myths and Science of Soils in the Tropics* (R. Lal and P. Sanchez, Eds). American Society of Agronomy, Madison.
- Singleton P. W., Woomer P. L., Thies J. E., Nakao P. L. and Bohlool B. B. (1991) Ni1TAL Project, University of Hawaii, Paia.
- Soil Conservation Service (1972) *Soil Survey of Islands of Kauai, Oahu, Maui, Molokai, and Lanai, State of Hawaii*. Soil Conservation Service, USDA, Washington.
- Thies J. E., Singleton P. W. and Bohlool B. B. (1991a) Influence of the size of indigenous rhizobial populations on establishment and symbiotic performance of introduced rhizobia on field-grown legumes. *Applied and Environmental Microbiology* 57, 19-28.
- Thies J. E., Singleton P. W. and Bohlool B. B. (1991b) Modeling symbiotic performance of introduced rhizobia in the field by use of indices of indigenous population size and nitrogen status of the soil. *Applied and Environmental Microbiology* 57, 29-37.
- Thompson J. A. and Vincent J. M. (1967) Methods of detection and estimation of rhizobia in soil. *Plant and Soil* 26, 72-84.
- Turk D. and Keyser H. H. (1992) Rhizobia that nodulate tree legumes: specificity of the host for nodulation and effectiveness. *Canadian Journal of Microbiology*.
- Turk D. and Keyser H. H. (1993) Accuracy of most-probable-number estimates of rhizobia for tree legumes. *Soil Biology & Biochemistry* 24, 69-74.
- Vincent J. M. (1970) *A Manual for the Practical Study of Root-Nodule Bacteria*. Blackwell, Oxford.
- Wilkinson L. (1990) *SYSTAT: The System for Statistics*. Evanston, SYSTAT, Evanston.
- Woomer P., Singleton P. W. and Bohlool B. B. (1988) Ecological indicators of native rhizobia in tropical soils. *Applied and Environmental Microbiology* 54, 1112-1116.
- Woomer P., Bennett J. and Yost R. (1990) Overcoming the inflexibility of most probable number procedures. *Agronomy Journal* 82, 349-353.

