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 N 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., 1991b) 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 Hamakuaoko, Maui, Hawaii using four soils (Table 1), seven legumes, and two treatments.
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 Waiakea 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 Waiakea soils, respectively, to give equivalent soil volumes. The following fertilizers were added (kg 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 soil as NH4N03 were added to the mineral N controls as follows: 50 mg kg soil at the time of inoculation, 150 mg kg soil 3 weeks after inoculation, and 150 mg kg 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-glucose broth.
medium (Sadowsky et al., 1987). The inoculum was applied evenly around the roots of each seedling to give ca. $5 \times 10^6$ 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 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.

**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 NH$_4$SO$_4$. 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 $R = \frac{100(N - Nu)}{Nu}$ where $R =$ response to inoculation, $N_i =$ average shoot N in inoculated pots, and $Nu =$ 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
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.

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

<table>
<thead>
<tr>
<th>Species</th>
<th>Soil</th>
<th>+N</th>
<th>Inoculated</th>
<th>Uninoculated</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>A. mearnsii</em></td>
<td>Pane</td>
<td>24.5</td>
<td>9.1</td>
<td>6.5</td>
</tr>
<tr>
<td><em>S. grandiflora</em></td>
<td>Pane</td>
<td>23.5</td>
<td>17.5</td>
<td>6.7</td>
</tr>
<tr>
<td><em>L. diversifolia</em></td>
<td>Makawao</td>
<td>25.2</td>
<td>12.1</td>
<td>11.0</td>
</tr>
<tr>
<td><em>A. auriculiformis</em></td>
<td>Keahua</td>
<td>14.3</td>
<td>5.4</td>
<td>5.7</td>
</tr>
<tr>
<td><em>R. pseudoacacia</em></td>
<td>Keahua</td>
<td>20.4</td>
<td>8.8</td>
<td>2.4</td>
</tr>
<tr>
<td><em>A. mangium</em></td>
<td>Waiakea</td>
<td>14.5</td>
<td>1.0</td>
<td>1.2</td>
</tr>
<tr>
<td><em>S. grandiflora</em></td>
<td>Waiakea</td>
<td>40.2</td>
<td>21.9</td>
<td>3.6</td>
</tr>
</tbody>
</table>

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

**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

![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, Waiakea.](image)
A hyperbolic model taking the form: \( y = \frac{596.3}{1 + \text{MPN}} \), 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 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...

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

<table>
<thead>
<tr>
<th>Model Description</th>
<th>Coefficients</th>
<th>Residual mean square</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( a )</td>
<td>( b )</td>
</tr>
<tr>
<td><strong>Models with rhizobial density alone</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( y = a + bx )</td>
<td>191.0</td>
<td>-0.001</td>
</tr>
<tr>
<td>( y = ax^{-2} )</td>
<td>550.3</td>
<td>20.84</td>
</tr>
<tr>
<td>( y = a + b(\log x) + c(\log x)^2 )</td>
<td>525.8</td>
<td>-129.7</td>
</tr>
<tr>
<td>( y = a/x )</td>
<td>596.3</td>
<td></td>
</tr>
<tr>
<td><strong>Models with rhizobial density and mineralized N pot(^{-1})</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( y = (a + bm)/x )</td>
<td>1105</td>
<td>-149.1</td>
</tr>
<tr>
<td>( y = (ae^{-rm})/x )</td>
<td>1461</td>
<td>0.322</td>
</tr>
<tr>
<td>( y = (am^{-1}/x )</td>
<td>1106</td>
<td>0.755</td>
</tr>
<tr>
<td>( y = (a + b/m)/x )</td>
<td>155.4</td>
<td>947.9</td>
</tr>
</tbody>
</table>

\( y \) = 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. pseudodacacia* grown in the Makawao and Pane soils, respectively (data not shown), indicated that *R. pseudodacacia* 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. pseudodacacia*, 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 10⁶ 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 < 10⁶ 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. pseudodacacia* in the Pane, Makawao and Waiakea 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. pseudodacacia* 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. pseudodacacia* and *A. mearnsii* that are relatively promiscuous for nodulation but specific for effectiveness in their rhizobial requirements.

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REFERENCES


Response of tree legumes to inoculation


