Rhizobia that nodulate tree legumes: specificity of the host for nodulation and effectiveness[1]

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Received August 26, 1991
Revision received December 18, 1991
Accepted January 2, 1992

TURK, D., and KEYSER, H. H. 1992. Rhizobia that nodulate tree legumes: specificity of the host for nodulation and effectiveness. Can. J. Microbiol. 38: 451-460.

Rhizobial specificity, defined in terms of nodulation and nitrogen-fixing effectiveness characteristics of a group of rhizobia on a host legume, serves as a basis for predicting the need to inoculate, selecting species for most probable number plant-infection assays, and preparing rhizobial inoculants suitable for a range of legume species. A series of inoculation experiments were performed under growth room and greenhouse conditions to delineate rhizobial specificity of a variety of tree legumes. *Gliricidia sepium, Calliandra calothyrsus,* and *Leucaena leucocephala* nodulated effectively with rhizobia isolated from each of the three genera. With a few exceptions, *Sesbania grandif7ora* and *Robinia pseudoacacia* nodulated effectively only with rhizobial strains isolated from each genus respectively. A range of specificity was found among species that nodulate with *Bradyrhizobium.* Whereas *Acacia mearnsii* nodulated with most strains but fixed N_2 effectively with relatively few, *Acacia mangium* and *Lysiloma latisiliqua* were specific for both nodulation and effectiveness.

Key words: nitrogen fixation, effectiveness, nodulation, rhizobia, tree legumes.

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Definie en termes de nodulation et d'efficacite de fixation de 1'azote, la specificite rhizobienne qui caracterise un groupe de rhizobiums de legumineuses-hotes a servi de base pour les aspects suivants : predire la necessite d'inoculer, selectionner des especes pour des essais du nombre le plus probable de plantes inoculees et preparer des inoculants rhizobiens qui conviennent a une gamme d'especes de la famille des legumineuses. Une serie d'experiences d'inoculation ont ete poursuivies en chambre de croissance et dans des conditions de champ pour determiner la specificite rhizobienne dune vari6te d'arbres de cette famille. Les *Gliricidia sepium, Calliandra calaothyrsus* et *Leucaena leucocephala* ont effectivement produit des nodosites avec des souches rhizobiennes isol6es de chacun des trois genres. A peu d'exceptions pres, les *Sesbania grandiflora* et *Robinia pseudoacacia* ont forme des nodosites avec les souches rhizobiennes isolees de chacun des genres respectifs. Une variation etendue de specificite a ete decelee parmi les especes qui ont forme des nodosites avec le *Bradyrhizobium.* D'autre part, tandis que *1'Acacia mearnsii* a forme des nodosites avec la plupart des souches alors que peu d'entre elles ont ete efficace pour la fixation de N_2, *1'Acacia mangium* et le *Lysiloma latisiliqua* ont ete specifiques a la fois pour la nodulation et pour 1'efficacite.

Mots cles : fixation de 1'azote, efficacite, nodulation, rhizobiums, arbres de la famille des legumineuses.

[Traduit par la redaction]

Introduction

Nitrogen-fixing leguminous trees (NFLTs) are being planted on a wide scale in the tropics to provide fuel wood, construction materials, fodder, and nitrogen-rich biomass for improving soil fertility. In most cases, exotic species are being planted, as they have been shown to outperform indigenous taxa in terms of vigor (Hughes and Styles 1987). Although biological nitrogen fixation is an important attribute of NFLTs, little has been done to delineate their rhizobial specificity in terms of nodulation and nitrogen fixing effectiveness. Such knowledge is required to develop effective inoculants for NFLTs, since they are introduced into soils where they have not previously been cultivated and which may lack compatible rhizobia.

According to Allen and Allen (1981), the first description of rhizobia from a leguminous tree was recorded in 1887 on isolates from *Robinia pseudoacacia.* Several tree species were subsequently investigated for nodulation (see Fred *et al.* 1932). However, legume cross-inoculation groups as initially developed included few perennial shrubs or trees. A landmark contribution in this area was that of Allen and Allen (1936). In a survey of the leguminous flora of Hawaii they recorded nodulation of plants ranging in age from 1 to 20 years and recovered isolates from the nodules. Reciprocal cross-inoculation tests were performed with these isolates and *Vigna unguiculata.* Based on these tests, they identified 72 trees and shrubs that were new additions to the cowpea cross-inoculation group. Further cultural and symbiotic characterization of 54 strains from 28 of these species showed that in general they were typical of "cowpea" bacteria (*Bradyrhizobium* sp. in current usage) in their slow growth and alkaline media reactions (Allen and Allen 1939). The strains exhibited marked variation in infectiveness and effectiveness of nodulation on 20 host members of the cowpea cross-inoculation group (Allen and Allen 1939).

The work of Trinick (1965, 1968, 1980) established that true fast-growing rhizobia also nodulate tree legumes. His work in Papua New Guinea showed that *Leucaena leucocephala* did not nodulate with slow-growing rhizobia from 41 tropical legumes, and that the homologous isolates

[1]Journal series No. 3638 of the Hawaii Institute of Tropical Agriculture and Human Resources.

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Printed in Canada / Imprim€ au Canada

TABLE 1. Conflicting reports on rhizobial affinities of tree legumes

Genus or species	Effective nodulation with: *Bradyrhizobium*	*Rhizobium*
Albizia lebbeck	Allen and Allen 1939[a]	Duhoux and Dommergues 1985
Calliandra	Peoples *et al.* 1989	P. Somasegaran, personal communication
Desmanthus	Date 1977, 1982; Date and Halliday 1982; Trinick 1982	Date 1991[a]; Davis 1982[a]
Gliricidia	Date 1977; Dreyfus *et al.* 1987; Peoples *et al.* 1989; Trinick 1982	Akkasaeng *et al.* 1986[a]; Somasegaran *et al.* 1989[a]
Samanea (Albizia)	Allen and Allen 1939[a]	Gibson *et al.* 1982

[a]Supporting data presented in report.

were fast growers, which utilized a wide array of sugars (characteristic of *Rhizobium)* and had some serological relatedness with *Rhizobium meliloti* (Trinick 1965). Subsequent work found that rhizobia isolated from *Mimosa pudica, Mimosa invisa, Acacia farnesiana,* and *Sesbania grandif7ora* were also fast growers, with some strains related to the fast-growing *Rhizobium meliloti* (Trinick 1968, 1980).

Dreyfus and Dommergues (1981) found differential affinity among species of *Acacia* for symbiotic effectiveness with *Rhizobium* and *Bradyrhizobium,* with *Acacia seyal* and *Acacia sieberana* nodulating effectively with rhizobia from both genera. Effective nodulation with rhizobia from both genera has since been reported for *Acacia longifolia* (Barnet *et al.* 1985) and Prosopis *glandulosa* (Jenkins *et al.* 1987).

Despite these exceptions, tree legumes in general nodulate effectively almost exclusively with either *Rhizobium* or *Bradyrhizobium* (Dreyfus *et al.* 1987). Tree species that nodulate effectively with fast-growing rhizobia are recognized as being specific for nodulation and effectiveness (Duhoux and Dommergues 1985; Gibson *et al.* 1982; Trinick 1982), whereas species that nodulate with *Bradyrhizobium* are less specific (more promiscuous) for both characteristics (Graham and Hubbell 1975; Date 1977, 1982).

There is considerable confusion surrounding the rhizobial affinities of many tree species (Table 1). Apparent misidentification of the genus of rhizobia that effectively nodulate some trees (e.g., *Calliandra* and *Gliricidia) is* misleading given the view that species that nodulate effectively with *Rhizobium* are more likely to respond to inoculation than species that nodulate effectively with *Bradyrhizobium* (Dommergues 1987; Dreyfus and Dommergues 1981; Peoples *et al.* 1989).

Effectiveness groups of legumes are subsets of cross-inoculation groups that respond similarly to a set of rhizobial strains (Burton 1979) and, therefore, have similar rhizobial specificity for nodulation and effectiveness. Effectiveness groups have served primarily as a guideline for inoculant preparation but can also serve as the basis for predicting the need to inoculate and for forming species substitutions in most probable number (MPN) assays. Despite recognition that trees nodulating with *Rhizobium* have specific rhizobial requirements relative to species nodulating with *Bradyrhizobium* (Trinick 1965, 1968), trees that nodulate with *Rhizobium* have not been examined extensively for specificity for effectiveness with respect to each other. Similarly, specificity for effectiveness has not been systematically evaluated in tree species that nodulate with *Bradyrhizobium.* Burton (NifTAL and FAO 1984) placed only four trees in effectiveness groups: *Leucaena leucocephala* and *Leucaena retusa* were grouped with *Coronilla, Onobrychis,* and *Petalostemon spp.; Robinia pseudoacacia* was grouped with *Robinia hispida.*

To delineate effectiveness groups of 12 fast-growing tree legumes in terms of nodulation and effectiveness, two inoculation experiments were performed in pouches. The first, pouch experiment A, used strains of both *Rhizobium* and *Bradyrhizobium.* The second, pouch experiment B, used only strains of *Bradyrhizobium* with trees known to nodulate effectively with bradyrhizobial strains. A series of pot experiments were also conducted to confirm the results of the pouch studies for three species of *Acacia* and to evaluate the effectiveness of a group of homologous strains (strains isolated from the same legume species on which they are being tested) inoculated on *Acacia auriculiformis* and *Acacia mearnsii.*

Materials and methods

Tree species and rhizobial strains

Tree species (Table 2) were selected for their importance in reforestation and agroforestry in the tropics. Seeds from each species were obtained from the Nitrogen Fixing Tree Association (Waimanalo, Hawaii) or the NifTAL Project.

Rhizobial strains were from the NifTAL Project collection. As much as possible, in pouch experiment A at least three strains isolated from each tree species (or genus) were used (Table 3). The designation of strains as *Rhizobium* or *Bradyrhizobium* (Table 3) was determined by rate of growth on yeast mannitol medium and pH reaction on the same medium with bromthymol blue indicator, as described by Vincent (1970). When these determinations were not conclusive, assignment was based on growth on minimal media supplied individually with lactose and sucrose, and on color reaction with the isopropyl-(3-D-thiogalactopyranoside plus 5-bromo-4-chloro-3-indolyl-(3-n-galactopyranoside (IPTG-X-Gal) assay (Sambrook *et al.* 1989), which indicates the presence of the enzyme ß-galactosidase. Disaccharide utilization and positive (blue) color reaction are indicative of *Rhizobium.* No strains suspected of being *Azorhizobium* or *Sinorhizobium* were used in these experiments.

In pouch experiment B, 34 bradyrhizobial strains were used comprising strains isolated from *Acacia auriculiformis* (TAL 1446, TAL 1521), *Acacia koa* (TAL 881), *Acacia mangium* (TAL 1867), *Acacia mearnsii* (TAL 63, TAL 111, TAL 1388), *Albizia caribaea* (TAL 1852), *Albizia lebbeck* (TAL 1536, TAL 1597), *Albizia saman* (TAL 833, TAL 1280), *Albizia stipulata* (TAL 1122), *Enterolobium cyclocarpum* (TAL 47, 60, 1530), *Erythrina indica* (TAL 69, 749),

TABLE 2. Plant combinations and time from inoculation to harvest for each combination

Experiment	Plant combinations	Days to harvest
Pouch A	*Acacia auriculiformis – Acacia mangium*	62
Pouch A	*Acacia mearnsii*	49
Pouch A	*Calliandra calothyrsus – Paraserianthes falcataria*	47
Pouch A	*Gliricidia sepium – Lysiloma latisiliqua*	39
Pouch A	*Robinia pseudoacacia*	49
Pouch A	*Sesbania sesban – Tephrosia candida*	32
Pouch B	*Acacia auriculiformis – Albizia lebbeck*	88
Pouch B	*Acacia mangium – Acacia mearnsii*	74
Pouch B	*Albizia saman – Enterolobium cyclocarpum*	92
Pouch B	*Lysiloma latisiliqua – Paraserianthes falcataria*	87
Pouch B	*Macroptilium atropurpureum – Vigna unguiculata*	38
Pot A	*Acacia auriculiformis*	101
Pot B	*Acacia mangium*	73
Pot C	*Acacia mearnsii*	59

Faidherbia albida (TAL 1457), *Flemingia macrophylla* (TAL 1883), *Paraserianthes falcataria* (TAL 45), NifTAL's recommended strains for cowpea (TAL 173, TAL 209, TAL 658), peanut (TAL 1000, TAL 1371), lima bean (TAL 22, TAL 209, TAL 658), as well as strains from soybean (TAL 102), *Calopogonium mucunoides* (TAL 651), *Canavalia ensiformis* (TAL 201), *Crotalaria spp.* (TAL 850, TAL 1380), *Desmodium uncinatum* (TAL 569), and *Phaseolus acutifolius* (TAL 644). The same set of strains was used on each of the six tree species and *Macroptilium atropurpureum* as indicated in Table 2. Strains used in the pot experiments were a subset from pouch experiment B, including the best strains identified from each of the three *Acacia* species. In addition, 4 and 18 strains from the NifTAL collection that were isolated from *Acacia auriculiformis* and *Acacia mearnsii,* respectively, were used to inoculate their respective hosts (Fig. 3).

Growth systems and experimental design

Pouch experiments

In the pouch experiments, tree seedlings were grown in growth pouches (Northrup King Co.), in most cases with one seedling of each of two species grown per pouch. Pouches were prepared by adding 50 ml, of a nitrogen-free plant nutrient solution (Singleton 1983), except that micronutrients were supplied by adding a commercial micronutrient mix (0.25 mL L^{1}; Hawaiian Horticultural). An additional 50 mL of this solution was added just prior to inoculation. Pouches were arranged in racks on a table in a growth room that received more than 300 ìE M^{-2} S^{-1} of photosynthetically active radiation at plant height from 1000-W high pressure sodium lamps for 16 h daily.

Four replicates of each treatment were included in pouch experiment A, with six uninoculated controls per species. Six uninoculated controls were included per species in pouch experiment B. For pouch experiment B, pouches were independently randomized in racks (Somasegaran and Hoben 1985) in three replicate blocks. Every 2 days racks were rotated around the table to ensure uniformity of light for all racks.

Pot experiments

In the pot experiments, plants were grown in 1-L plastic pots containing moistened horticultural vermiculite (Grace and Co.). Two, three, and four plants were grown per pot for *Acacia mangium, Acacia mearnsii,* and *Acacia auriculiformis,* respectively. Pots were placed in a greenhouse at Hamakuapoko, Maui, Hawaii, under natural light. A microtube irrigation system supplied nutrient solution daily. The nutrient solution was the same as that used for the pouches, except for the first 6 weeks with *Acacia mangium,* when double-strength solution was used. In addition, pots with *Acacia mangium* and *Acacia mearnsii* received 3 mM starter N in the nutrient solution in the form of $CaNO_3$ for the first 3 weeks after planting. Pots were covered with sterile gravel following inoculation to help prevent rhizobial contamination.

Each of the three pot experiments was set up in a randomized complete block design with three blocks for *Acacia mangium* and four for *Acacia auriculiformis* and *Acacia mearnsii.*

Seedling preparation and inoculation

Seeds for all experiments were appropriately scarified and surface sterilized. Seeds for the pouch experiments were germinated on water agar plates except for *Albizia lebbeck,* which was germinated in horticultural vermiculite.

All seedlings for the pot experiments were germinated in horticultural vermiculite and transplanted into pots 10-15 days later.

Rhizobial strains were grown in yeast-extract mannitol (YEM) broth (Vincent 1970) for 8 and 11 days, respectively, for pouch experiments A and B and for 8 days for the pot experiments. Plants were inoculated 8-16 days after planting in pouches, and within 24 h after planting in pots. Pouches were inoculated by applying 1 mL of undiluted inoculant supplying approximately 10^9 rhizobia mL^{-1} in pouch experiment A and 1 mL of inoculant diluted 10-fold supplying approximately 10^8 rhizobia mL^{-1} in pouch experiment B to the roots of each plant. In pots, 1 mL of rhizobial broth culture containing approximately 10^9 rhizobia mL^{-1} was applied to each plant within 24 h after planting and washed in with approximately 25 ml, of sterile water.

Harvest and analysis

Plants were grown for various times before harvest (Table 2) to achieve maximal treatment differences. At harvest, plants in pouch experiment A were scored for nodulation and effectiveness based on presence of nodules, shoot size, and leaf color (Table 3).

In pouch experiment B, shoot dry weight and nodule numbers were determined at harvest. Specificity for nodulation and effectiveness was assessed by rank correlation of nodule numbers and shoot dry weight, respectively. Single-linkage cluster analysis of nodule numbers and shoot dry weight was performed after standardization of nodule numbers and shoot dry weight for each species from zero to one. For shoot dry weight, uninoculated controls and treatments with values less than those of the uninoculated controls were given a value of zero.

In the pot experiments, strains were evaluated using analysis of variance of $\log_{10}$ transformed data except for shoot dry weight of Acacia auriculiformis, where the data were not transformed because Bartlett's test showed that the variances were not significantly different at $P < 0.05$. Performance of strains used for the same

TABLE 3. Effectiveness of *Rhizobium* and *Bradyrhizobium* strains on 10 tree legumes

Rhizobial strain			Tree species									
TAL No.	Genus	Original host	Ll	Gs	Cc	Sg	Rp	Aa	Am	Ame	Pf	Tc
1887	R	*Leucaena leucocephala*	E	E	*e*	0	I	0	I	I	I	0
583	R	*Leucaena leucocephala*	E	E	*e*	0	I	0	I	I	0	*np*
1145	R	*Leucaena leucocephala*	E	E	*e*	0	*e*	0	0	0	I	I
582	R	*Leucaena leucocephala*	*e*	I	I	0	I	I	I	I	0	0
82	R	*Leucaena leucocephala*	*e*	E	*e*	0	0	0	I	0	0	0
7	R	*Gliricidia sepium*	E	E	E	0	I	I	I	0	I	0
1788	R	*Gliricidia maculata*	E	E	E	I	I	I	0	0	I	I
1806	R	*Gliricidia sepium*	E	E	E	0	I	0	0	0	I	I
1770	R	*Gliricidia sepium*	E	E	E	0	I	0	0	0	I	0
1801	R	*Calliandra calothyrsus*	*e*	E	0	E	I	I	I	0	0	0
33	R	*Calliandra calothyrsus*	E	E	E	0	I	I	0	0	I	I
1455	R	*Calliandra surinamensis*	E	E	E	0	*e*	0	0	0	I	I
1779	R	*Sesbania grandiflora*	0	0	0	E	I	0	I	0	I	0
1886	R	*Sesbania longifolia*	*e*	E	I	E	I	0	I	0	I	0
1137	R	*Sesbania* sp.	*e*	E	E	0	0	0	I	I	I	I
1114	R	*Sesbania* sp.	0	0	0	E	I	0	0	0	0	*np*
1119	R	*Sesbania* sp.	0	0	0	E	0	0	0	0	0	0
183	R	*Robinia pseudoacacia*	0	0	0	0	E	0	0	0	0	*np*
1889	R	*Robinia pseudoacacia*	0	0	0	0	E	0	0	0	0	*np*
1907	R	*Robinia pseudoacacia*	0	0	0	0	E	0	0	0	0	*np*
1869	R	*Acacia mangium*	*e*	I	*e*	I	I	I	I	*e*	0	0
1867	B	*Acacia mangium*	0	0	0	*e*	0	E	E	*e*	E	E
1446	B	*Acacia auriculiformis*	0	0	0	0	0	E	0	E	E	E
1521	B	*Acacia* sp.	0	0	0	0	I	E	0	*e*	E	E
45	B	*Paraserianthes falcataria*	0	I	0	I	I	?	E	E	E	E
363	B	*Albizia lebbeck*	0	0	0	0	0	0	I	I	*e*	E
1536	B	*Albizia lebbeck*	0	I	0	0	I	E	*np*	I	E	*np*
795	B	*Tephrosia glauca*	0	0	0	0	0	0	0	I	E	E
1883	B	*Flemingia macrophylla*	0	0	0	0	0	E	I	0	*e*	E
22	B	*Phaseolus lunatus*	0	0	0	0	0	E	E	*e*	E	*np*
1380	B	*Crotalaria paulina*	0	I	0	0	0	E	I	E	E	E
1000	B	*Arachis hypogaea*	0	0	0	0	0	E	0	*e*	*e*	*np*
1908	B	*Glycine max*	0	I	0	0	I	*e*	I	I	*e*	E
102	B	*Glycine max*	0	0	0	0	I	0	0	I	I	*np*

NOTE: Effectiveness code: 0, no nodules on at least three of four plants; I, ineffective: plants nodulated but were not greener than uninoculated plants; *e*, moderately effective: at least two plants were greener than uninoculated plants; E, effective: at least two plants were greener and larger than *e* plants; ?, inconsistent response; *np*, not performed. Legume species: Ll, *Leucaena leucocephala*; Gs, *Gliricidia sepium*; Cc, *Calliandra calothyrsus*, Sg, *Sesbania grandiflora*; Rp, *Robinia pseudoacacia*; Aa, *Acacia auriculiformis*; Am, *Acacia mangium*; Ame, *Acacia mearnsii*; Pf, *Paraserianthes falctaria*; Tc, *Tephrosia candida*. Rhizobial genera: R, *Rhizobium*; B, *Bradyrhizobium*.
= *Leucaena-Gliricidia-Calliandra Rhizobium* group;
= *Sesbania Rhizobium* group;
= *Robinia Rhizobium* group;
= *Bradyrhizobium* group.

species in pouch experiment B and in the corresponding pot experiment were compared by Spearman rank correlation.

Correlation and cluster analyses were carried out using the SYSTAT program (Wilkinson 1990); the SAS program (SAS Institute 1986) was used to perform analysis of variance with Tukey's HSD test for the pot experiments.

Results

Pouch experiment A

In pouch experiment A, species nodulated effectively (meaning nodulation that resulted in sufficient nitrogen fixation to detect a growth response by the plant) with either strains of *Rhizobium* or *Bradyrhizobium* (Table 3). Tree species that nodulated effectively with *Rhizobium* formed three fairly distinct groups based on specificity for effectiveness. *Leucaena leucocephala, Gliricidia sepium,* and *Calliandra calothyrsus* consistently formed effective symbioses with rhizobia isolated from members of all three genera, with some effective nodulation with two strains from *Sesbania* species. *Sesbania grandiflora* and *Robinia pseudoacacia* only nodulated effectively with rhizobia isolated from their respective genera except for effective and moderately effective nodulation of *S. grandiflora* with a strain from *Calliandra* (TAL 1801) and a strain from *Acacia mangium* (TAL 1867), respectively, and moderately effective nodulation of *Robinia pseudoacacia* with TAL 1145 and TAL 1455. *Robinia pseudoacacia* nodulated ineffectively with 18 of 34 strains, including strains of both *Rhizobium* and *Bradyrhizobium.*

Acacia auriculiformis, Acacia mangium, Acacia mearnsii, Paraserianthes falcataria, and *Tephrosia candida* nodulated effectively only with *Bradyrhi,zobium* strains (Table 3) but revealed a range of specificity for nodulation and effectiveness. *Paraserianthes falcataria* and *Tephrosia candida* formed effective symbioses with all bradyrhizobial strains applied except TAL 102. At the other extreme, *Acacia*

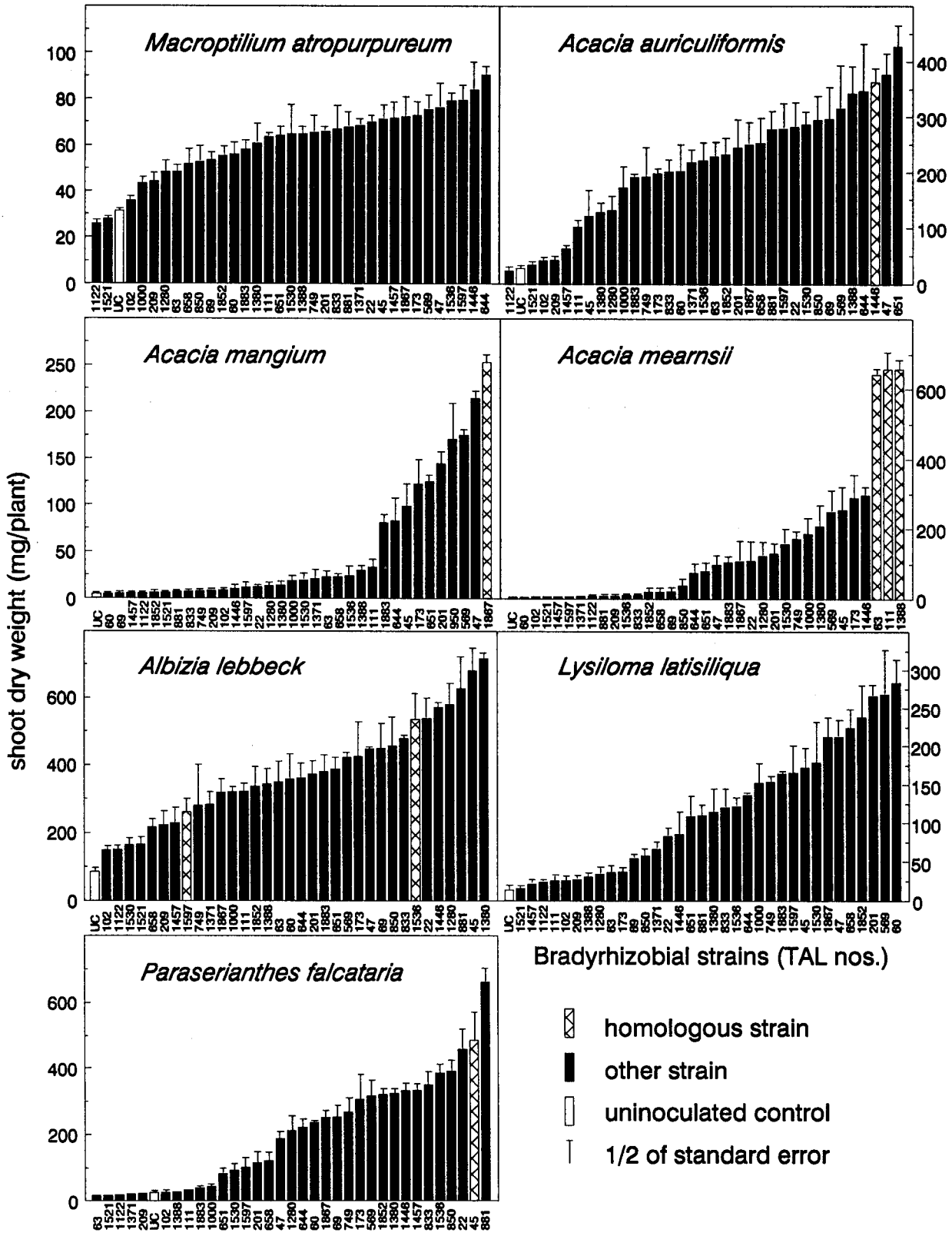

FIG. 1. Shoot dry weight of six trees and *Macroptilium atropurpureum* inoculated with 34 bradyrhizobial strains.

mangium failed to nodulate with 5 of 12 bradyrhizobial strains and nodulated ineffectively with 4 of the remaining strains. All species that nodulated effectively with bradyrhizobial strains nodulated ineffectively with some of the rhizobial strains.

Pouch experiment B

Distinct differences in the performance of different species with 34 strains of *Bradyrhizobium* were observed in pouch experiment B with respect to shoot dry weight (Fig. 1). A greater proportion of strains produced relatively effective

TABLE 4. Comparison of species used in pouch experiment B on the basis of rank correlation (Spearman's rank correlation coefficients) of (A) shoot dry weight and (B) nodule numbers produced by individual rhizobial strains, and (C) rank correlation comparison of shoot dry weight and nodule numbers in pouches versus pots

(A) Shoot dry weight in pouch experiment B

	Ma	Aa	Am	Ame	Al	Ll
Aa	0.402*					
Am	0.374*	0.328*				
Ame	0.189	0.192	0.530**			
Al	0.375*	0.316*	0.230	0.366*		
Ll	0.366*	0.372*	0.314*	0.090	0.164	
Pf	0.528**	0.238	0.019	0.086	0.693**	0.296

(B) Nodule numbers in pouch experiment B

	Ma	Aa	Am	Ame	Al	Ll
Aa	0.212					
Am	0.209	0.269				
Ame	0.304*	0.445**	0.149			
Al	0.094	0.277	0.148	0.320*		
Ll	0.300*	0.289	0.109	0.059	−0.294	
Pf	0.020	−0.159	−0.258	−0.404	0.104	0.094

(C) Shoot dry weight and nodule numbers for strains used in pouch B and pot experiments

Shoot dry weight			Nodule no.	
Aa	Am	Ame	Am	Ame
0.25	0.72***	0.67***	0.72***	0.80***

NOTE: Legume species: Ma, *Macroptilium atropurpureum*; Aa, *Acacia auriculiformis*; Am, *Acacia mangium*; Ame, *Acacia mearnsii*; Al, *Albizia lebbeck*; Ll, *Lysiloma latisiliqua*; Pf, *Paraserianthes falcataria*. *, **, and ***, significant at $P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively, by one-tailed test.

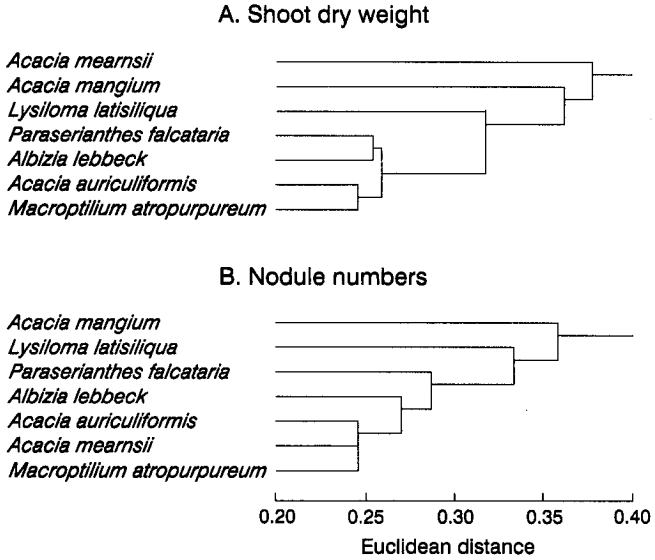

FIG. 2. Single-linkage cluster analysis of (A) shoot dry weight and (B) nodule numbers of legume species from pouch experiment B.

symbioses with *Macroptilium atropurpureum*, *Acacia auriculiformis*, and *Albizia lebbeck* than with *Acacia mangium* and *Acacia mearnsii* (Fig. 1) as evaluated by shoot dry weight. *Lysiloma latisiliqua* and *Paraserianthes falcataria* were intermediate between these groups. The three strains that produced the highest shoot dry weight on *Acacia mearnsii* were homologous, as was the best strain on *Acacia mangium*.

Nodulation (data not shown) by the 34 strains on a per plant basis can be condensed to the following summary: all strains produced more than 10 nodules on *Macroptilium atropurpureum*; only 3 strains produced less than 10 nodules on *Acacia auriculiformis* and *Albizia lebbeck*, but not the same three strains; only 2 strains produced less than 10 nodules on *Acacia mearnsii*; only 1 strain produced less than 5 nodules on Paraserianthes falcataria; and 11 strains produced less than 5 nodules on *Acacia mangium* and *Lysiloma latisiliqua*, but not the same 11 strains. In addition, *Acacia mangium* and *Lysiloma latisiliqua* did not form nodules with 3 and 4 strains, respectively.

Single-linkage cluster analysis, a multivariate procedure for detecting groupings in data (Wilkinson 1990), was used to summarize these relationships (Fig. 2). With respect to nodulation, there were no differences discernable among *Macroptilium atropurpureum*, *Acacia auriculiformis*, and *Acacia mearnsii*. The other species were separated from this group by increasing intervals, with *Acacia mangium* being furthest apart. Similarly, whereas *Acacia mearnsii* had significant rank correlation with *Macroptilium atropurpureum* and *Acacia auriculiformis* with respect to nodule numbers, nodulation of *Acacia mangium* was not significantly correlated with nodulation of any other species (Table 4).

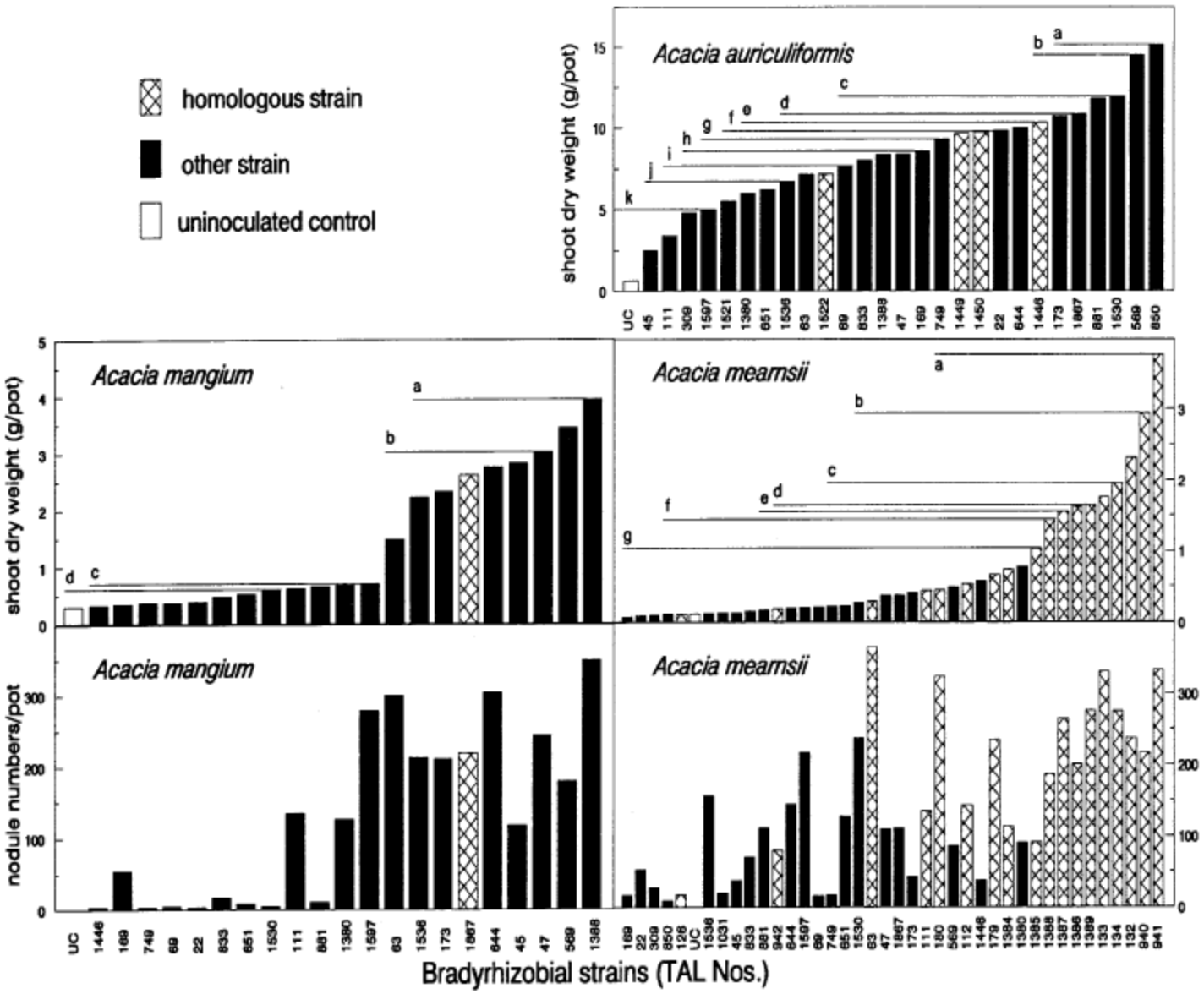

FIG. 3. Shoot dry weight and nodule numbers from pot experiments. Bars under a line are not significantly different ($P < 0.05$) by Tukey's HSD test.

TABLE 5. Rhizobial specificity of tree legumes

	Specificity[a]	
	Nodulation	Effectiveness
Trees nodulating effectively with *Rhizobium*		
Leucaena leucocephala, Gliricidia sepium, Calliandra calothyrsus	S	S
Sesbania grandiflora	S	S
Robinia pseudoacacia	P	S
Trees nodulating effectively with *Bradyrhizobium*		
Acacia auriculiformis, Albizia lebbeck, Paraserianthes falcataria, Tephrosia candida	P	P
Acacia mearnsii	P	S
Acacia mangium	S	S
Lysiloma latisliqua	S	S

[a]S, specific; P, promiscuous.

In terms of shoot dry weight, *Acacia auriculiformis, Macroptilium atropurpureum, Albizia lebbeck,* and *Paraserianthes falcataria* formed a group distinct from the other species (Fig. 2). *Acacia mearnsii* was the only species whose shoot dry weights were not significantly correlated with shoot dry weights of *Macroptilium atropurpureum* by Spearman rank correlation (Table 4).

Pot experiments

Nodulation and shoot dry weight response patterns observed in pouch experiment B were also evident in the pot experiments (Fig. 3). Most strains tested were effective on *Acacia auriculiformis* in pots, with all but 5 of 27 producing significantly higher shoot dry weight than the uninoculated control. As in pouches, a large proportion of strains were relatively ineffective on both *Acacia mangium* and *Acacia mearnsii.* Correlation of shoot dry weight in the pot experiments with the dry weights from the same strains used in pouch experiment B yielded significant Spearman correlation coefficients ($P < 0.05$) for shoot dry weight and nodule numbers of *Acacia mangium* and *Acacia mearnsii* (Table 4).

The 10 strains with the highest dry weights for *Acacia mearnsii* in pots were all homologous strains (Fig. 3). Homologous strains also were among the best strains for *Acacia auriculiformis* and *Acacia mangium* in the pot experiments.

Discussion

Effective nodulation of *Gliricidia septum, Calliandra calothyrsus,* and *Leucaena leucocephala,* by rhizobia from each of the three species but failure to nodulate effectively with most rhizobia isolated from other legumes indicates that *Gliricidia septum, Calliandra cadothyrsus,* and *Leucaena leucocephala* belong to a common effectiveness group. Previously, *Leucaena leucocephala* had been reported to nodulate with a strain isolated from *Calliandra calothyrsus* (Halliday and Somasegaran 1983) but without information on effectiveness. Other researchers found *Gliricidia septum* (Somasegaran *et al.* 1989) and *Gliricidia maculata* (Akkasaeng *et al.* 1986) to nodulate effectively with fast-growing rhizobial strains. Our data conflict with unsubstantiated reports that *Gliricidia septum* (Date 1977; Dreyfus *et al.* 1987; Peoples *et al.* 1989; Trinick 1982) and *Calliandra* (Peoples *et al.* 1989) nodulate with "cowpea,"

The results from pouch experiment A support evidence that *Sesbania grandiflora* has highly specific rhizobial requirements (Abdel Magid *et al.* 1988; Johnson and Allen 1952; Ndoye *et al.* 1990; Trinick 1982). Pouch experiment A data also substantiate reports of specificity for effectiveness of *Robinia pseudoacacia* (Allen and Allen 1981; NifTAL and FAO 1984). Allen and Allen (1981) concluded that *Robinia pseudoacacia is* specific for both effectiveness and nodulation, but data in pouch experiment A show *Robinia pseudoacacia* to nodulate ineffectively with a wide variety of legumes. Other researchers (Burton 1977; Crow *et al.* 1981; Wilson 1944) have also found ineffective nodulation of *Robinia pseudoacacia* with rhizobia isolated from a variety of hosts.

Based on the pattern of effective nodulation in pouch experiment A and on cluster analyses and rank correlation of shoot dry weight and nodule numbers in pouch experiment B, *Acacia auriculiformis, Albizia lebbeck, Paraserianthes falcataria,* and *Tephrosia candida* appear to be promiscuous for both nodulation and effectiveness, while *Acacia mearnsii is* promiscuous for nodulation but specific for effectiveness, and *Acacia mangium* and *Lysiloma lysiliqua* are specific for both nodulation and effectiveness. These data support the findings of Allen and Allen (1939) for *Albizia lebbeck* and of Galiana *et al.* (1990) for *Acacia mangium,* but are in disagreement with an unsubstantiated report that *Albizia lebbeck is* specific in its rhizobial requirements (Duhoux and Dommergues 1985).

Based on all of the experiments conducted, the trees studied can be categorized based on effective nodulation with *Rhizobium* or *Bradyrhizobium* and specificity for nodulation and effectiveness (Table 5). This classification scheme is similar to those proposed by Graham and Hubbell (1975), Date (1977), and Date and Halliday (1980).

The results suggest that species-specific inoculants should be used with trees such as *Sesbania grandiflora, Robinia pseudoacacia,* and *Acacia mearnsii* that are specific for effectiveness. A common inoculant could be developed for *Leucaena leucocephala, Gliricidia septum,* and *Calliandra calothyrsus,* an expansion of the host range suggested by Somasegaran *et al.* (1989). Similarly, a single inoculant could be developed for use with several tree species that are promiscuous for both nodulation and effectiveness. However, because no strains in pouch experiment B were distinctly superior for all trees in this category, it would seem

prudent to develop an individual inoculant for each species. Another approach would be to develop a group inoculant consisting of a mixture of highly effective strains for each species. The data suggest that MPN assays conducted with *Leucaena leucocephala, Gliricidia sepium,* or *Calliandra calothyrsus* should provide a reasonable estimate of the rhizobial population present capable of effectively nodulating the other two species. Similarly, *Macroptilium atropurpureum* will likely provide a reasonable rhizobial density estimate for tree species that nodulate with *Bradyrhizobium* and are promiscuous for nodulation, such as *Acacia auriculiformis* and *Acacia mearnsii.* An assessment of effectiveness measured along with an MPN assay, such as described by Brockwell *et al.* (1988) for *Trifolium subterraneum* and *Medicago sativa,* would be useful in determining the "effective population" of rhizobia that nodulate particular tree species.

Acknowledgements

The authors thank Patty Nakao, Kathy MacGlashan, and Joe Rourke for excellent technical assistance. This research was supported in part by USAID cooperative agreement DAN-4177-A-00-6035-00 to the NifTAL Project.

Abdel Magid, H.M., Singleton, P.W., and Tavares, J.W. 1988. *Sesbania-Rhizobium* specificity and nitrogen fixation. Desert Plants, 9(2): 45-47.

Akkasaeng, R., Whiteman, P.C., and Date, R.A. 1986. *Rhizobium* requirements for two accessions of *Gliricidia maculata.* Tropical Grasslands, 20(1): 26-29.

Allen, O.N., and Allen, E.K. 1936. Root nodule bacteria of some leguminous plants: I. Cross-inoculation studies with *Yigna sinensis* L. Soil Sci. 42(1): 61-77.

Allen, O.N., and Allen, E.K. 1939. Root nodule bacteria of some tropical leguminous plants: II. Cross-inoculation tests within the cowpea group. Soil Sci. 47(1): 63-76.

Allen, O.N., and Allen, E.K. 1981. The Leguminosae: a source book of characteristics, uses, and nodulation. University of Wisconsin Press, Madison, Wis.

Barnet, Y.M., Catt, P.C., and Hearne, D.H. 1985. Biological nitrogen fixation and root-nodule bacteria *(Rhizobium* sp. and *Bradyrhizobium sp.)* in two rehabilitating sand dune areas planted with *Acacia spp.* Aust. J. Bot. 33: 595-610.

Brockwell, J., Holliday, R.A., and Pilka, A. 1988. Evaluation of the symbiotic nitrogen-fixing potential of soils by direct microbiological means. Plant Soil, 108: 163-170.

Burton, J. C. 1977. Associations of some uncommon leguminous plants with various rhizobia. Coll. Trop. Agric. Misc. Publ. (LJniv. Hawaii), 145: 381-384.

Burton, J. C. 1979. *Rhizobium* species. *In* Microbial technology. 2nd ed. Vol. I. Academic Press, New York. pp. 29-58.

Crow, V.L., Jarvis, B.D.W., and Greenwood, R.M. 1981. Deoxy-ribonucleic acid homologies among acid-producing strains of *Rhizobium.* Int. J. Syst. Bacteriol. 31: 152-172.

Date, R.A. 1977. Inoculation of tropical pasture legumes. Coll. Trop. Agric. Misc. Publ. (Univ. Hawaii), 145: 293-3311.

Date, R.A. 1982. Assessment of the rhizobial status of the soil. *In* Nitrogen fixation in legumes. *Edited by* J.M. Vincent. Academic Press, Sydney, Australia. pp. 85-94.

Date, R.A. 1991. Nitrogen fixation in *Desmanthus:* strain specificity of *Rhizobium* and responses to inoculation in acidic and alkaline soil. Tropical Grasslands, 25: 47-55.

Date, R.A., and Halliday, J. 1980. Relationships between *Rhizobium* and tropical forage legumes. *In* Advances in legume science. *Edited by* R.J. Summerfield and A. H. Bunting. Royal Botanic Gardens, Kew. pp. 597-601.

Davis, P.E. 1982. *Desmanthus virgatus* as a test plant for dilution-frequency infection tests using *Leucaena leucocephala* rhizobia. Soil Biol. Biochem. 14: 313-314.

Dommergues, Y.R. 1987. The role of biological nitrogen fixation in agroforestry. *In* Agroforestry, a decade of development. *Edited by* H.A. Steppler and P.K.R. Nair. International Council for Research in Agroforestry (ICRAF), Nairobi, Kenya. pp. 245-271.

Dreyfus, B.L., and Dommergues, Y.R. 1981. Nodulation of *Acacia* species by fast- and slow-growing tropical strains of *Rhizobium.* Appl. Environ. Microbiol. 41(1): 97-99.

Dreyfus, B.L., Diem, H.G., Freire, J., *et al.* 1987. Nitrogen fixation in tropical agriculture and forestry. *In* Microbial technology in the developing world. *Edited by* E.J. DaSilva, Y.R. Dommergues, E.J. Nyns, and C. Ratledge. Oxford University Press, Oxford. pp. 9-50.

Duhoux, E., and Dommergues, Y. R. 1985. The use of nitrogen fixing trees in forest and soil restoration in the tropics. *In* Biological nitrogen fixation in Africa: proceedings of the first conference of the African association for biological nitrogen fixation. *Edited by* H. Ssali and S.O. Keya. Matianum Press Consultants, Nairobi. pp. 384-400.

Fred, E.B., Baldwin, LL., and McCoy, E. 1932. Root nodule bacteria and leguminous plants. Univ. Wisconsin Stud. Sci. 5.

Galiana, A., Chaumont, J., Diem, H.G., and Dommergues, Y.R. 1990. Nitrogen-fixing potential of *Acacia mangium* and *Acacia auriculiformis* seedlings inoculated with *Bradyrhizobium* and *Rhizobium spp.* Biol. Fertil. Soils, 9: 261-267.

Gibson, A.H., Dreyfus, B.L., and Dommergues, Y.R. 1982. Nitrogen fixation by legumes in the tropics. *In* Microbiology of tropical soils and plant productivity. *Edited by* Y.R. Dommergues and H.G. Diem. Martinus Nijhoff/Dr. W. Junk Publishers, The Hague. pp. 37-73.

Graham, P.H., and Hubbell, D. H. 1975. Legume *Rhizobium* relationships in tropical agriculture. *In* Tropical forages in livestock production systems. *Edited by* E.C. Doll and G.O. Mott. ASA Spec. Publ. 24: 9-21.

Halliday, J., and Somasegaran, P. 1983. Nodulation, nitrogen fixation, and *Rhizobium* strain affinities in the genus *Leucaena. In* Leucaena research in the Asian Pacific Region. Proceedings of a workshop held in Singapore, 23-26 November, 1982, International Development Research Centre, Ottawa, Ont. pp. 27-32.

Hughes, C.E., and Styles, B.T. 1987. The benefits and potential risks of woody legume introductions. Int. Tree Crops J. 4: 209-248.

Jenkins, M.B., Virginia, R.A., and Jarrell, W.M. 1987. Rhizobial ecology of the woody legume mesquite *(Prosopis glandulosa) in* the Sonoran desert. Appl. Environ. Microbiol. 53(1): 36-40.

Johnson, M.D., and Allen, O.N. 1952. Nodulation studies with special reference to strains isolated from *Sesbania* species. Antonie van Leeuwenhoek J. Microbiol. Serol. 18(1): 1-12.

Ndoye, L, Tomekpe, K., Dreyfus, B., and Dommergues, Y.R. 1990. *Sesbania* and *Rhizobium* symbiosis: nodulation and nitrogen fixation. *In* Perennial *Sesbania* species in agroforestry systems. *Edited by* B. Macklin and D.O. Evans. Nitrogen Fixing Tree Association, Waimanalo, Hawaii.

NifTAL and FAO. 1984. Legume inoculants and their use. Food and Agriculture Organization of the United Nations, Rome.

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.

Sambrook, J., Fritsch, E.F., and Maniatis, T. 1989. Molecular cloning: a laboratory manual. 2nd ed. Vol. 1. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.

SAS Institute. 1986. SAS users' guide: statistics. SAS Institute Inc., Cary, N.C.

Singleton, P.W. 1983. A split-root growth system for evaluating the effect of salinity on components of the soybeanlRhizobium japonicum symbiosis. Crop Sci. 23: 259-262.

Somasegaran, P., and Hoben, H.J. 1985. Methods in legume-*Rhizobium technology*. NifTAL Project, University of Hawaii, Paia.

Somasegaran, P., Keyser, H.H., and Roskoski, J.P. 1989. Rhizobial requirement and inoculation response of Gliricidia *sepium* (Jacq.) Walp. The 12th North American Symbiotic Nitrogen Fixation Conference Proceedings. Ames, Iowa. p. 77.

Trinick, M.J. 1965. *Medicago sativa* nodulation with Leucaena *leucocephala* root-nodule bacteria. Aust. J. Sci. 27: 263-264.

Trinick, M.J. 1968. Nodulation of tropical legumes. I. Specificity in the *Rhizobium* symbiosis of *Leucaena leucocephala.* Exp. Agric. 4: 243-253.

Trinick, M.J. 1980. Relationships amongst the fast-growing rhizobia of *Lablab* purpureus, *Leucaena leucocephala, Mimosa spp., Acacia ,f'arnesiana, and Sesbania grandiflora and* their affinities with other rhizobial groups. J. Appl. Bacteriol. 49: 39-53.

Trinick, M.J. 1982. *Host-Rhizobium associations.* In Nitrogen fixation in legumes. *Edited by* J.M. Vincent. Academic Press, Sydney. pp. 111-122.

Vincent, J.M. 1970. A manual for the practical study of rootnodule bacteria. IBP Handbook No. 15. Burgess and Son (Abingdon) Ltd., Berkshire.

Wilkinson, L. 1990. SYSTAT: the system for statistics. SYSTAT, Inc., Evanston, Ill.

Wilson, J.K. 1944. Over five hundred reasons for abandoning the cross-inoculation groups of the legumes. Soil Sci. 58: 61-69.