

Leucaena Research in the Asian-Pacific Region

**Proceedings of a workshop held
in Singapore, 23-26 November 1982**

Leucaena Research in the Asian-Pacific Region

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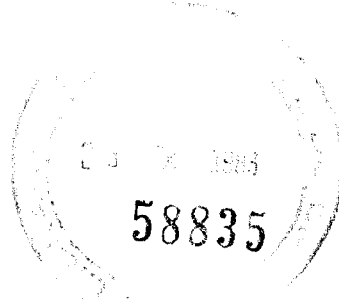
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**Proceedings of a Workshop Held in Singapore, 23–26
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Abstract

Because of *Leucaena leucocephala*'s multiple uses as forage, fuelwood, poles, green manure, etc., this fast-growing, nitrogen-fixing tree has been the subject of much research in the last decade. The results have clarified the capabilities of the plant as well as its limitations. One main constraint to cultivation in vast areas of Latin America and Southeast Asia has been *leucaena*'s inability to survive on acidic, aluminum-saturated soils. At low pH, the aluminum complexes with calcium, which is essential for good growth. Trials have shown that some varieties of *L. diversifolia* can make use of the calcium from the complexes and that crosses between these varieties and *L. leucocephala* perform well on acidic soils. The main constraint to use of *leucaena* as a forage derives from the plant's content of mimosine, a toxic, nonprotein amino acid. Although *leucaena* has proved to be a highly nutritious animal feed, the mimosine and its breakdown product, DHP (3-hydroxy-4[1H]pyridone), have caused toxicity among animals fed high levels of leaf meal. Scientists now have evidence that the mimosine is converted into DHP when brought into contact with an enzyme contained in some of the plant's cells harbouring mimosine. This finding needs follow up; it suggests that simple processing, such as chopping fresh leaves, will convert all the mimosine into the less-toxic DHP. Elsewhere, researchers have found that DHP can be metabolized by anaerobic microorganisms that have been found in the guts of ruminants in countries like Indonesia. They have successfully transferred the microorganisms to animals in Australia where toxicity from DHP has deterred graziers from using *leucaena* as forage. Other research has defined optimal approaches to breeding and genetic improvement of *leucaena*; characteristics of rhizobia that effectively provide the plant with nitrogen-fixing ability; biomass production under widely different soil conditions; effects on fish, poultry, cattle, goats, and sheep fed *leucaena* leaf meal; management and cultural practices for both large-scale and smallholder operations; etc. The results are the subject of this publication, which comprises 30 papers from researchers in the Asian-Pacific Region.

Résumé

Leucaena leucocephala a fait l'objet de nombreuses recherches au cours de la dernière décennie, cet arbre légumineux fixateur d'azote et de croissance rapide ayant de nombreux usages comme fourrage, combustible, poteau, engrais vert, etc. Ces études ont permis d'en délimiter les fonctions. L'un des facteurs limitants de sa culture dans de vastes régions de l'Amérique latine et de l'Asie du Sud-Est est l'incapacité de *Leucaena* de survivre dans des sols acides, saturés d'aluminium. Dans le cas d'un faible pH l'aluminium complexe le calcium, essentiel à une croissance régulière. Des essais ont démontré que certaines variétés de *L. diversifolia* peuvent utiliser le calcium présent dans les complexes et que les croisements entre ces variétés et *L. leucocephala* prospèrent dans des sols acides. Le principal obstacle à l'utilisation de *Leucaena* comme fourrage est sa teneur en mimosine, acide aminé non protéique toxique. Bien que ce fourrage soit hautement nutritif, la mimosine et DHP (3-hydroxy-4[1H]pyridone) ont provoqué des cas de toxicité chez les animaux consommant de grandes quantités de farine de feuilles. Les scientifiques ont découvert que la mimosine se décompose en DHP lorsqu'elle entre en contact avec une enzyme contenue dans certaines cellules où elle est présente. Cette découverte a permis de déterminer des moyens simples de neutraliser cette substance toxique, tel que le hachage des feuilles vertes qui décompose la mimosine en DHP moins toxique. Ailleurs, des chercheurs ont trouvé que le DHP peut être métabolisé par des microorganismes anaérobiques présents dans l'intestin des ruminants dans certains pays comme l'Indonésie. Ils ont réussi à transférer ces microorganismes à des animaux en Australie où les pasteurs refusent l'emploi du fourrage de *Leucaena* à cause de la toxicité de DHP. D'autres recherches préconisent une approche optimale: de la sélection et de l'amélioration génétique de *Leucaena*; des caractères des rhizobiums qui assurent la fonction de la fixation d'azote chez la plante-hôte; de la production de bio-masse dans diverses conditions de sols très variés; des effets des rations de farine de feuilles sur les poissons, les volailles, le bétail, les chèvres et les moutons; de la gestion et des pratiques culturelles des

opérations des petites et des grandes exploitations, etc. Tous ces résultats sont détaillés dans la présente brochure qui contient trente communications exposées par des chercheurs de la région du Pacifique asiatique.

Resumen

Debido a los múltiples usos de la *Leucaena leucocephala* como forraje, combustible, madera, abono, etc., este árbol, de rápido crecimiento y habilidad para fijar el nitrógeno, ha sido objeto de abundante investigación en la última década. Los resultados han aclarado las capacidades de la planta, así como sus limitaciones. Uno de los problemas para su cultivo en vastas áreas de Latinoamérica y el Sudeste Asiático ha sido su incapacidad para sobrevivir en suelos ácidos, saturados de aluminio. A niveles bajos de pH, el aluminio forma complejos con el calcio que es esencial para un buen crecimiento. Las pruebas han mostrado que algunas variedades de *L. diversifolia* pueden usar el calcio de los complejos y que los cruces entre estas variedades y la *L. leucocephala* se desempeñan bien en suelos ácidos. El principal inconveniente para usar la leucaena como forraje proviene de su contenido de mimosina, un aminoácido tóxico no proteínico. Aunque la leucaena ha probado ser un alimento animal altamente nutritivo, la mimosina y su producto de descomposición, el DHP (3-hydroxy-4[1H]pyridone), han causado toxicidad entre los animales alimentados con altos niveles de harina de follaje. Los científicos tienen ahora evidencia de que la mimosina se convierte en DHP cuando entra en contacto con una enzima que se encuentra en algunas células de la planta que contienen mimosina. Este hallazgo necesita seguimiento, pero sugiere que un simple procesamiento, como picar las hojas frescas, convierte toda la mimosina en el menos tóxico DHP. En otras partes, los investigadores han encontrado que el DHP puede ser metabolizado por microorganismos anaeróbicos que han sido hallados en el intestino de rumiantes en países como Indonesia. Ellos han traspasado con éxito los microorganismos a animales en Australia donde la toxicidad del DHP ha impedido que los ganaderos empleen la leucaena como forraje. Otras investigaciones han definido los enfoques óptimos para el fitomejoramiento de la leucaena, las características de la rizobia que efectivamente dotará a la planta de la habilidad de fijar nitrógeno, la producción de biomasa bajo condiciones edáficas ampliamente distintas, los efectos sobre los peces, las aves, el ganado, las cabras y las ovejas alimentadas con harina de hoja de leucaena, las prácticas culturales y de manejo para las actividades a gran escala o del pequeño agricultor, etc. Los resultados son el tema de esta publicación que abarca 30 trabajos de investigadores en la región Asiopacífica.

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Nodulation, Nitrogen Fixation, and Rhizobium Strain Affinities in the Genus *Leucaena*

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Leucaena spp. belong in the subfamily Mimosoideae of the Leguminosae. All 10 species have been reported to nodulate readily, but some doubt remains in the case of *L. retusa*, which has failed to nodulate in Hawaii soils. The infection process has not been studied in *leucaena*. Nodule shape is variable, being astragaloid, desmodioid, or muconoid in form. Attachment to the root is narrow and fragile. Annual nitrogen accumulation rates of 600 kg/ha and as high as 1 t/ha are on record, but annual nitrogen fixation has not been determined with precision. One approximation, based on the acetylene-reduction technique, puts annual fixation at 110 ± 30 kg. The specific designation for the *leucaena* rhizobia is *Rhizobium loti*. These rhizobia are typically fast growing, gummy, and translucent on yeast-mannitol agar (YMA). They are neutral, or slightly acidic, in the pH reaction on YMA. *Leucaena* rhizobia will nodulate other tropical legumes usually nodulated by fast-growing rhizobia and will also nodulate some species normally nodulated by slow growers. Slow-growing strains of *Rhizobium* from other tropical genera do not nodulate *leucaena*.

Leucaena usually nodulates spontaneously with indigenous soil rhizobia without specific inoculation, but the documented cases of nodulation failure are sufficient to prompt adoption of legume inoculation when *leucaena* is introduced to locations where it has not been grown before. Although *leucaena* performs very poorly in acid-soil sites, strains of *Rhizobium* for *leucaena* are available that exhibit tolerance to low pH. Acid tolerance in a strain is not dependent on an alkali-producing capability. One particular strain, TAL 1145, has proved to be competitive for nodule formation, persistent in the soil microflora, and fully effective as a nitrogen-fixing partner for *leucaena* in acid soils.

Leucaena spp. appartient à la famille des

mimosacées, sous-famille des légumineuses. Il a été constaté que toutes les 10 espèces forment aisément des nodosités mais il subsiste quelque doute au sujet de *L. retusa* qui n'en produit pas dans les sols d'Hawaii. Le processus d'infection n'a pas été étudié chez *Leucaena*. La forme des nodosités varie et peut être astragaloïde, desmodioïde ou muconoïde. Le point d'attache à la racine est étroit et fragile. Les quantités d'azote atmosphérique accumulées annuellement atteignent 600 kg/ha et l'on en a rapportées de 1 t/ha, mais la fixation annuelle n'a pas été établie avec précision. Une approximation fondée sur la technique de réduction à l'acétylène évalue la fixation annuelle à 110 ± 30 kg. La désignation spécifique du rhizobium de *Leucaena* est *Rhizobium loti*. Ces bactéries ont un développement typiquement rapide, et apparaissent gommeuses et translucides sur agar de levure-mannitol (YMA). Elles sont neutres ou légèrement acidulées dans la réaction du pH sur YMA. Le rhizobium de *Leucaena* formera des nodosités sur d'autres légumineuses tropicales généralement réceptives aux nodosités produites par des rhizobiums à croissance rapide, et il en produira également sur certaines espèces normalement réceptives à des rhizobiums à plus lent développement. Les souches de *Rhizobium* à développement lent provenant d'autres espèces tropicales n'inoculeront pas le *Leucaena*.

En général, *Leucaena* forme spontanément des nodosités avec les rhizobiums indigènes, sans inoculation particulière. Mais les cas vérifiés de nodulation manquée sont assez fréquents pour faire adopter l'inoculation de la légumineuse lorsqu'on introduit *Leucaena* dans des endroits où il n'a jamais été cultivé. Bien que celui-ci se comporte très médiocrement dans les régions à sols acides, il existe des souches de *Rhizobium* tolérantes à l'égard d'un faible pH. La tolérance d'une souche à l'acidité ne dépend pas d'une aptitude à produire des alcalis. Une souche particulière, TAL 1145, s'est révélée compétitive pour la formation de nodosités, persistante dans la microflore du sol et tout à fait efficace comme partenaire fixateur de l'azote pour *Leucaena*, en sols acides.

La especie *Leucaena* pertenece a la subfamilia Mimosoideae de las leguminosas. Se ha informado que todas las 10 especies nodulan prontamente, pero hay todavía dudas en el caso de la *L. retusa*, que no ha nodulado en los suelos de Hawaii. El proceso infeccioso no ha sido estudiado en la *leucaena*. La forma del nódulo es variable, siendo astragaloide, desmodioide o muconoide. El agarre a la raíz es delgado y frágil. Se han registrado casos de acumulación anual de nitrógeno de 600 kg/ha y hasta 1 t/ha, pero la fijación anual de nitrógeno no ha sido determinada con precisión. Una aproximación, con base en la técnica de reducción por acetileno, señala la fijación anual en 110 ± 30 kg. La designación específica para la *leucaena* rizobia es *Rhizobium loti*. Estas son rizobias de rápido cre-

cimiento, gomosas y translúcidas en agar manitol-levadura (YMA). Son neutras, o ligeramente ácidas, en la reacción del pH en YMA. La rizobia de la leucaena nodulará otras leguminosas tropicales normalmente noduladas por rizobias de rápido crecimiento y también nodulará algunas especies noduladas lentamente. Las líneas de crecimiento lento de la rizobia de otros géneros tropicales no nodulan la leucaena. La leucaena nodula por lo general espontáneamente con rizobia de suelos nativos sin inoculación específica, pero los casos documentados de fracaso de nodulación son suficientes para promover la adopción de inoculación de la leguminosa cuando la leucaena se introduce en localidades donde no se ha sembrado antes. Aunque la leucaena se desempeña muy pobremente en suelos ácidos, se encuentran líneas de *Rhizobium* para leucaena que exhiben tolerancia al pH bajo. La tolerancia al ácido en una línea no depende de la capacidad de producción alcalina. Una línea particular, TAL 1145, ha demostrado ser competitiva en la formación nodular, persistente en la microflora de los suelos, y totalmente efectiva como socio fijador de nitrógeno para la leucaena en suelos ácidos.

The genus *Leucaena* belongs taxonomically in the subfamily Mimosoideae of the Leguminosae. In common with the majority (84%) of the genera in this subfamily, *Leucaena* includes species that are known to bear root nodules (Allen and Allen 1981). These nodules, formed after infection of the roots by compatible strains of the soil bacterium *Rhizobium*, confer leucaena with the ability to fix atmospheric nitrogen. Autosufficiency in nitrogen supply through the process of biological nitrogen fixation (BNF) is a highly desirable trait in any plant. *Leucaena*'s BNF capability undoubtedly contributes to its rapid growth and to its success as a colonizing species on poor soils.

Nodulation

As recently as 1979, all nodulating legumes were presumed to become infected with *Rhizobium* by a process involving penetration of root hairs (Bauer 1981). Root-hair curling, resulting in a characteristic shepherd's crook appearance, and the development of infection threads have been considered prerequisites for nodulation. But, in reality, infection threads have been observed in only a few species and are only presumed to occur in others. Some tropical legumes, initially thought not to have root hairs, have been studied to determine their mode of infection. *Arachis hypogaea* (Chandler 1978) and *Stylosanthes guianensis*

(Dart, Roughley, and Date, as quoted in Bauer 1981) have been shown to nodulate after penetration of the root at the junction of the root hairs and cortical cells. Root hairs in these species are concentrated around the points of emergence of lateral roots, and, consequently, these are the loci at which nodulation occurs. The leucaena-*Rhizobium* symbiosis remains to be elucidated. One obstacle to progress in this area is that leucaena has a rapidly growing taproot that does not lend itself to the standard methods used for observing infection.

Nodule morphology is variable in leucaena. The first nodules forming on the taproot are usually spherical, but elongated, branched, or even coralloid nodules have also been observed. Although coralloid nodules have been recorded with a span of more than 3.0 cm, lateral dimensions of 0.5–1.0 cm are more common. The attachment of the nodule to the root is narrow, not girdling the root. This fragile attachment could well be a factor contributing to the infrequency with which nodules have been observed on the excavated roots of mature leucaena trees.

The variability in nodule form is underscored by the array of nodule-descriptive codes (Corby 1980) that have been attributed to the nodules of the single species *L. leucocephala*. In each of four publications, different basic nodule shapes were reported. Based on these reports, leucaena nodules can be described under Corby's classification as astragaloid, desmodioid, or muconoid.

Nodules are most commonly described for seedlings because of the difficulties involved in extricating the root systems of mature trees from field soils. Many researchers have reported informally that they have been unable to observe the presence of nodules on leucaena more than a year old. Thorough description of the distribution, quantity, and nitrogen-fixing effectiveness of nodules on root systems of mature leucaena specimens is lacking. This information is essential for definitive calculation of the quantity of atmospheric nitrogen fixed by leucaena.

Nitrogen Fixation

Leucaena leucocephala has been credited with some of the highest figures for annual nitrogen fixation ever recorded. But interpretation of the high levels purported to occur must be tempered by an appreciation of the precision with which nitrogen fixation per se, as opposed to soil-nitrogen uptake, can be de-

terminated. For example, an exceptionally high figure for annual nitrogen fixation, 1 t N/ha (Dijkman 1950), was actually the accumulation of nitrogen in leafy matter of trees in a leucaena plantation harvested bimonthly for a 1-year period. This figure cannot be attributed solely to BNF because soil nitrogen also contributed to the total. The often-quoted annual values of 500 kg N/ha given by Guevarra (1976) for K8 leucaena and 600 kg N/ha for K341 leucaena are also total-nitrogen accumulation in the forage fraction.

The precise annual nitrogen fixation by leucaena remains to be determined. Deriving such a value is especially complex in a tree legume. In approximation methods, a non-leguminous crop is usually grown alongside a legume. Then, nitrogen accumulation in the nonlegume is assumed to be the soil contribution. This is subtracted from the total accumulation in the legume as an estimate of the nitrogen gain through fixation. But, with leucaena and other nitrogen-fixing trees, this approach is confounded by lack of an appropriate perennial nonleguminous crop for comparison.

The more precise methods, in which isotopic nitrogen is incorporated in the soil for measurements of the legume's soil-nitrogen uptake, are difficult to accomplish because of the depth and total volume of soil explored by tree roots.

In a recent study with *L. leucocephala* in Tanzania (Hogberg and Kvarnstrom 1982), rough calculations, based on acetylene-reduction rates in nodule tissue, gave a figure for annual nitrogen fixation of 110 ± 30 kg/ha. The calculation was based on experimentally derived values for nodule biomass and specific-nodule nitrogenase activity but involved crude assumptions for the ratio of acetylene reduced to nitrogen fixed and for the seasonal fluctuation in nodular activity. No correction for diurnal variation in nitrogenase activity was attempted. Each of these factors is researchable. Thus, it should be possible to arrive at a sounder figure for annual nitrogen fixation in leucaena using approaches such as those found satisfactory for other perennials (Halliday and Pate 1976a,b).

Rhizobia Affinities

The rhizobia for the majority of tropical legumes are characteristically slow growing compared with the rhizobia of temperate

legumes. Generation times in culture are in the range of 3–5 h for the fast growers and 8–10 h for the slow growers. Also, the tropical rhizobia generally produce alkali under standard culture conditions, whereas the temperate rhizobia usually produce an acid reaction. So distinct are the cultural characteristics of these two types of rhizobia that a new genus, *Bradyrhizobium*, has been described for the slow-growing rhizobia. The strains of *Rhizobium* isolated from leucaena are fast growing and neutral, or mildly acid-producing, in their pH reaction. Thus, they are atypical of the rhizobia usually isolated from tropical legumes. Under the old classification, they were placed in the "cowpea miscellany" *Rhizobium* spp. Now, they have been separated from this miscellany, and their correct designation is *Rhizobium loti*.

Leucaena rhizobia can nodulate legumes of other genera more commonly nodulated by the slow-growing rhizobia and usually result in effective, nitrogen-fixing symbioses. But the converse does not occur. Leucaena is specific in its rhizobial requirements, under the categories advocated by Date and Halliday (1980). Leucaena was not nodulated in a field trial in Australia where 18 of 24 other species of legumes were nodulated by indigenous rhizobia in the soil. In the eastern plains of Colombia, which have an array of native legumes nodulated by indigenous strains of rhizobia, leucaena that is sown without inoculation fails to nodulate.

Trinnick (1980) has described the ability of leucaena rhizobia to cross-inoculate with some of the few other tropical legumes that have fast-growing rhizobia (*Mimosa invisa*, *M. pudica*, *Acacia farnesiana*, and *Sesbania grandiflora*) and even with an unusual isolate from *Lablab purpureus* that was fast growing. In our own lab, leucaena nodulated with a fast-growing rhizobial isolate from *Calliandra calothyrsus*.

The cross-inoculation capabilities of the leucaena rhizobia are evident from a study with a mixed inoculant containing three fast-growing rhizobia originally from leucaena and three slow-growing rhizobia isolated from different tropical legumes (Table 1). As specific antisera had been developed for each strain, and each strain was serologically distinct from each of the others, it was possible to determine which strains occupied nodules on 14 species of leguminous trees after inoculation with the six-strain mixture. The infection range of the

Table 1. Presence (+) or absence (-) of each of six strains of *Rhizobium* in the nodules of field-grown leguminous trees inoculated with all six strains at planting (J. Halliday and P.L. Nakao, unpublished).^a

Tree species	<i>Rhizobium</i>					
	Fast growers			Slow growers		
	TAL 1145	TAL 82	CB 81	CB 756	CB 1024	CIAT 71
<i>Acacia auriculiformis</i>	-	-	-	-	-	+
<i>A. mearnsii</i>	+	-	-	+	-	+
<i>Albizia falcataria</i>	-	-	-	+	-	+
<i>A. procera</i>	-	-	-	+	-	+
<i>Calliandra calothyrsus</i>	+	-	-	-	-	-
<i>Enterolobium cyclocarpum</i>	-	-	-	+	-	+
<i>Erythrina poeppigiana</i>	-	-	-	-	-	+
<i>Glyricidia sepium</i>	+	-	-	-	-	-
<i>Leucaena diversifolia</i>	+	-	-	-	-	-
<i>L. leucocephala</i>	+	-	-	-	-	-
<i>Mimosa scabrella</i>	+	-	-	-	-	-
<i>Sesbania grandiflora</i>	+	-	-	-	-	-

^a No stand was produced by *Acacia mangium*, and no nodulation occurred in *Acrocarpus fraxinifolius* and *Dalbergia sissoo*.

leucaena *Rhizobium* TAL 1145 was considerably greater than that of all the other strains, exhibiting a competitive edge even over the other leucaena rhizobia for formation of nodules.

Nodulation and nitrogen fixation have been reported (Halliday and Nakao 1982) for all 10 of the leucaena species recognized by Brewbaker. However, *L. retusa*, a native of Texas, has not nodulated in Hawaii soils. This finding is especially surprising given the readiness with which other leucaena species nodulate on the same sites.

Bacteriologically controlled plant-infection tests have confirmed that *L. retusa* is not nodulated by the strains of rhizobia normally used for inoculating leucaena (Table 2).

Tests with several different provenances have been performed, ruling out any possibility that the absence of nodulation was a freak occurrence limited to one *L. retusa* genotype (Table 3). Attempts to nodulate *L. retusa* with slow-growing rhizobial strains isolated originally from *Dolichos lablab* (CB 756), *D. bifloris* (CB 1024), and *Stylosanthes guianensis* (CIAT 71) have so far been unsuccessful. Nodules from *L. retusa* in its native environment have been collected and are under investigation in Hawaii. As yet, these have not yielded a single authenticated strain of *Rhizobium*.

The concept of co-evolution of rhizobial strains and their homologous hosts implies that leucaena introduced to new areas would be unlikely to encounter its specific rhizobial

Table 2. Numbers of nodules formed on roots of different leucaena species raised under bacteriologically controlled conditions in growth pouches and inoculated with *Rhizobium* strains TAL 1145, TAL 582, TAL 600 (P. Somasegaran and H.M. Kamel, unpublished).

Leucaena species	Number of root nodules/plant		
	TAL 1145	TAL 582	TAL 600
<i>L. diversifolia</i>	17.50	8.00	22.00
<i>L. lanceolata</i>	21.00	19.75	21.00
<i>L. leucocephala</i>	16.75	13.75	15.75
<i>L. pulverulenta</i>	0.50 ^a	1.25 ^b	4.50 ^a
<i>L. retusa</i>	none	none	none
<i>L. shannoni</i>	10.00	none	5.75

^a Two of three replicates nodulated.

^b Only one of three replicates nodulated.

Table 3. Presence (+) or absence (–) of nodules on four *L. retusa* (K501, K502, K504, K506) accessions when inoculated under bacteriologically controlled conditions with strains of *Rhizobium* (J. Halliday and E. Cuautle-Fabian, unpublished).^a

<i>Rhizobium</i> strain	Original host	K501	K502	K504	K506
TAL 1145	<i>L. leucocephala</i>	–	–	–	–
TAL 582	<i>L. leucocephala</i>	–	–	–	–
TAL 583	<i>L. leucocephala</i>	–	–	–	–
TAL 309 ^b	<i>Dolichos africanus</i>	–	–	–	–
TAL 310 ^b	<i>D. bifloris</i>	–	–	–	–
TAL 658 ^b	<i>Stylosanthes</i> sp.	–	–	–	–
Nitrogen control		–	–	–	–
Uninoculated control		–	–	–	–

^a TAL 1145 = CIAT 1967; TAL 582 = CB 81; TAL 583 = NGR8; TAL 309 = CB 756; TAL 310 = CB 1024; TAL 658 = CIAT 710.

^b Strains TAL 309, 310, and 658 were applied as mixed inoculant.

partner. In practice, however, despite being specific in its *Rhizobium*-strain requirement, leucaena usually encounters compatible soil rhizobia with less difficulty than do other rhizobia-specific legumes, such as soybean.

The documented cases of leucaena's failure to nodulate in geographic areas (e.g., in Australia and Colombia) that are remote from its natural distribution, however, make it prudent to inoculate leucaena seed, at planting, with preselected, elite strains of *Rhizobium*.

Symbiosis in Acid Soils

Leucaena leucocephala is acknowledged to have limited tolerance to acid-soil conditions. Alkali production, which is typical of tropical rhizobia that occur in acid soils, is not a trait of leucaena rhizobia and has been proposed as a reason for leucaena's failure in acid soils. Identification of alkali-producing rhizobia has been regarded as a valid approach to overcoming acid sensitivity in the leucaena–*Rhizobium* symbiosis. However, the thesis has been seriously challenged.

After calling into question the agricultural significance of acid–alkali production (Date and Halliday 1979), one of us (J.H.) conducted selection programs in Colombia and in

Hawaii to identify strains of *Rhizobium* with high tolerance to soil-acidity factors. On the premise that acid-tolerant strains need to be capable of multiplying rapidly at low pH if they are to colonize leucaena roots successfully and to cause nodulation in an acid soil, acidified, artificial media were developed to test for this trait (CIAT 1977).

An investigation of the pH-tolerance ranges of leucaena rhizobia isolated from plants growing in soil from three sites of widely differing pH revealed that all of the strains that were recovered from the low-pH (4.5) site possessed the ability to multiply in medium acidified to pH 4.5. None of the isolates from a high-pH (8.5) soil could grow in the acid medium. Data from these investigations (Table 4) emphasize the differing pH-tolerance ranges among rhizobia in naturally occurring populations at sites differing in their soil pH. In these tests, all of the leucaena rhizobia were of the fast-growing type. They were neutral or mildly acid-producing. None of the isolates, including those isolates tolerant to acid pH, produced alkali under the prevailing conditions.

When acid-tolerance screening was combined with the conventional step-wise strain selection (Halliday 1979), strains emerged that were capable of nodulating leucaena abun-

Table 4. Numbers of rhizobial isolates (from nodules on leucaena roots from acid, neutral, and basic soils from the island of Maui, Hawaii) that have the ability to grow on YMA–culture media adjusted to pH 4.5, 7.0, or 8.5 (J. Halliday and K. McGlashin, unpublished).

Collection site	Soil pH	Total isolates	Number of isolates able to grow		
			pH 4.5	pH 7.0	pH 8.5
Kuiaha	4.5	35	35	35	35
Hamakuapoko	7.0	102	79	102	93
Kahului	9.0	39	0	39	39

dantly and effectively in a highly acid (pH 4.1) soil (Llanos Orientales, Colombia). Subsequent testing of the best strain from the Colombian trials (CIAT 1967, now TAL 1145) at an acid-soil site in Hawaii revealed this strain to be more competitive for nodulation than the previously recommended strains CB 81 and NGR8 when inoculated as a mixed inoculant containing equal numbers of each strain.

The strain TAL 1145 also proved highly competitive in comparisons with indigenous rhizobia at the Hawaii site, forming virtually 100% of the nodules tested. At a separate site on the island of Oahu, TAL 1145 was the most competitive of a wide array of introduced strains (H. Maowad, personal communication).

Serological approaches to the study of competition and persistence of leucaena rhizobia are facilitated by the extreme degree of antigenic heterogeneity encountered among the strains in indigenous populations (H. Maowad, personal communication).

As TAL 1145 was neutral in its pH reaction on YMA and as there was no correlation

between ability to multiply in acidified media and production of alkali by any of the strains, the relevance of alkali production to success of a strain as an inoculant for acid soils is again disputed.

Nevertheless, successful nodulation was not a sufficient condition to render the leucaena productive in the acid-soil locations. Despite the presence of effective nodulation, plants in plots at pH 4.5 remained stunted and yellow, yielding only 6% of the level of dry-matter production measured for neighbouring plots that had been limed to pH 6.0. Thus, it appears that leucaena rhizobia are able to multiply at a pH in which leucaena itself cannot grow. Leucaena's intolerance of soil acidity can be attributed to the leucaena-plant genome rather than to its rhizobial partner, which has been shown to be acid competent.

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