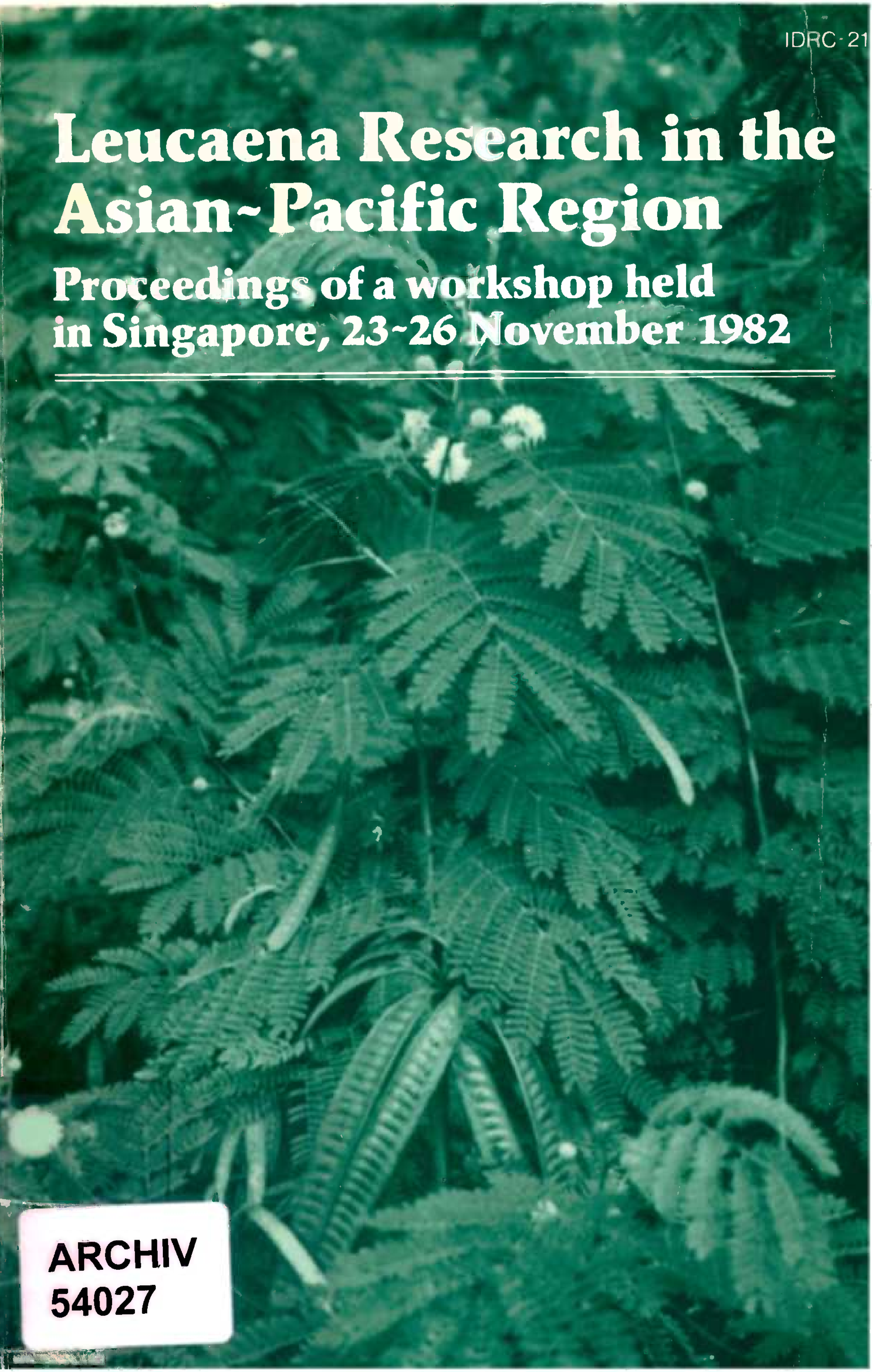
IDRC-211e

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 IDRC-211e

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Leucaena Research in the Asian–Pacific Region

Proceedings of a Workshop Held in Singapore, 23–26 November 1982

Organized by the Nitrogen Fixing Tree Association and the International Development Research Centre

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 culturales 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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Selection and Breeding of Leucaena for Very Acid Soils

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For satisfactory growth of Leucaena *in oxisols, and presumably ultisols, efficient root absorption of Ca, allowing deep-root penetration into subsoils, is more important than Al tolerance of roots. The acid-soil-tolerant lines are able to absorb sufficient Ca for prolific root growth in spite of the presence of high exchangeable Al. Increase in the subsoil Ca supply from surface applications of lime, dolomite, and* $CaSO_4$ *is slow and not to the depths required for extensive exploitation of subsoils for water and minerals by leucaena roots. Among the Ca sources,* $CaSO_4$ *provides both Ca and S and a better downward movement of Ca.*

The techniques that have been developed have enabled the selection of acid-soil-tolerant leucaena lines. This has enabled crosses to be made in which the acid-tolerance character has been transferred. Chromosome studies are needed, as it seems that, for best field results, it is necessary to have the acid-tolerance character in an L. leucocephala *background. The adaption of leucaena to the oxisols and the ultisols of South America can now be achieved.*

Pour obtenir un développement satisfaisant du Leucaena *dans les oxysols, et sans doute aussi les ultisols, l'absorption satisfaisante du Ca grâce à la pénétration profonde du sous-sol par les racines revêt plus d'importance que la tolérance des racines à Al. Les lignées tolérantes aux sols acides peuvent absorber suffisamment de Ca pour développer un système radiculaire abondant, malgré la présence de beaucoup de Al échangeable. L'amélioration des réserves de calcium du sous-sol par des applications en surface de chaux, de dolomite, et de* $CaSO_4$ *est lente et n'atteint pas les profondeurs nécessaires à une utilisation extensive de l'humidité et des minéraux du sous-sol par les racines du* Leucaena. *Parmi les sources de Ca,* $CaSO_4$ *apporte à la fois Ca et S et facilite la migration du Ca vers le sous-sol.*

Les techniques explorées jusqu'ici ont permis la sélection de lignées de Leucaena *tolérantes aux sols acides ce qui, à son tour, a permis d'effectuer des croisements dans lesquels ce caractère a pu être introduit. Des études de chromosomes s'imposent : il semble en effet que pour obtenir les meilleurs résultats pratiques, le caractère de tolérance à l'acidité doive se retrouver dans les antécédents de* L. leucocephala. *On peut réussir maintenant à adapter le* Leucaena *aux oxysols et ultisols sud-américains.*

Para el crecimiento satisfactorio de la Leucaena *en oxisoles, y presumiblemente en ultisoles, la eficiente absorción radical de Ca, que permita une penetración profunda de la raíz en los subsuelos, es más importante que la tolerancia de la raíz a Al. Las líneas tolerantes a los suelos ácidos pueden absorber suficiente Ca para un crecimiento prolífico de la raíz a pesar de la presencia de Al altamente intercambiable. El aumento en el Ca del subsuelo a partir de aplicaciones superficiales de cal, dolomita y* $CaSO_4$ *es lento y no a la profundidad requerida para una explotación extensa de los subsuelos por agua y minerales por las raíces de* Leucaena. *Entre las fuentes de Ca,* $CaSO_4$ *proporciona tanto Ca como S y un mejor descenso de Ca. Las técnicas desarrolladas permiten la selección de líneas de* Leucaena *tolerantes a los suelos ácidos. Esto ha permitido hacer cruces en los que se ha transferido la tolerancia al ácido. Se necesitan estudios de cromosomas puesto que parece que, para mejores resultados, es necesario tener la tolerancia al ácido en el antecedente de una* L. leucocephala. *La adaptación de la* Leucaena *a los oxisoles y los ultisoles de Suramérica puede ahora lograrse.*

South America has the largest area of very acid soils in the world, consisting of more than 8.0×10^8 ha of oxisols and ultisols with a pH about 4.5 and aluminum saturation of about 85% (Sanchez and Salinas 1981). It includes the Llanos of Colombia and Venezuela, the Amazon of Brazil and Peru, and the Cerrados of Brazil. In these areas, there is considerable interest in obtaining leguminous trees, especially *Leucaena*, for the production of wood and forage. Agroforestry systems based on edible leguminous trees would, in the Amazon, reduce the problems that follow forest clearing as well as supplying necessary protein for cattle. In the Cerrados, these systems would relieve the critical dry-season shortage of feed protein.

After the Amazon forest has been cleared and burned, there is a migration of ash minerals toward the subsoil. Under these conditions, such as near Manaus, Cunningham leucaena grows satisfactorily for some years. However, calcium deficiency can be expected when the roots penetrate into the very acid subsoils.

Cunningham, Peru, and other *L. leucocephala* varieties, including the giants, have given poor growth in the oxisols of the Llanos, as at Carimagua, and in the Cerrados as at Brasilia. Early growth from applied fertilizer, including superphosphate, dolomite, potash, and minor elements, is good, but growth is retarded by the end of the season. Root development is superficial and mainly in the fertilized layer, with deep penetration being prevented by factors associated with the high subsoil acidity. These factors were considered principally to be high exchangeable Al and low Ca levels.

Researchers at the Centro Internacional de Agricultura Tropical (CIAT) originally concentrated on selection of leucaena for Al tolerance in sand culture containing all essential nutrients and Al at 5 ppm. Variable populations were studied from second- and third-generation seed of a Cunningham–*L. pulverulenta* F_1 backcrossed twice to Cunningham. From 45 000 seedlings evaluated, 12.6% were selected for Al tolerance. When the selections were grown in Carimagua oxisol in the glasshouse with restricted fertilizer applications, only one-fourth grew satisfactorily. Foliar analyses proved that Al levels were usually below 200 ppm and that P (0.15%) and Mg (0.2%) levels were normal. However, Ca contents were low (0.14–0.24%) in those with poor, yellowish growth and poor nodulation and medium (0.4%) in the small proportion with good, green growth and good nodulation.

The most promising *Leucaena* hybrids from the glasshouse trials were grown in the field at Carimagua with appropriate fertilizer and with Cunningham as control. None of the selections grew more than 1 m high, and all developed yellowish leaves, with Al at less than 200 ppm. It was apparent that their medium root absorption of Ca was not high enough to give good root and top growth in the Carimagua oxisol, even with adequate fertilization. These results clearly indicated that Al per se was not the major problem and that higher root absorption of Ca against the antagonism of high exchangeable Al was necessary for good leucaena growth in oxisols.

Superior Adaptation

It did not appear possible to obtain high tolerance to oxisols among current leucaena varieties, so it was necessary to investigate a range of different types. A number of Brewbaker introductions from Central America of species other than *L. leucocephala*, as well as *L. leucocephala* controls including Cunningham and some giants, were grown simultaneously in Carimagua oxisol in the glasshouse and in the field at Carimagua.

Important findings resulted from this investigation:

- Natural interspecific crossing had taken place in some species, notably *L. diversifolia*, *L. esculenta*, *L. macrophylla*, *L. pulverulenta*, and *L. shannoni*. These are all self-incompatible and have 2n=52 chromosomes, except *L. pulverulenta* with 2n=56 chromosomes. The natural hybrids were relatively easy to identify and, in the field-grown populations, constituted about 90% in *L. esculenta* and *L. shannoni*, 6–17% in *L. macrophylla* and *L. pulverulenta*, and 1.5–9% in *L. diversifolia*. All the natural hybrids, except those of *L. esculenta*, were fertile. The majority had chromosome numbers about 2n=78, indicating that the main natural crossing was with *L. leucocephala* with 2n=104 chromosomes. *Leucaena trichodes*, also self-incompatible with 2n=52 chromosomes, exhibited no natural interspecific crosses and appeared genetically isolated. *Leucaena collinsii*, with apparently 2n=102 chromosomes, did not show any definite natural crosses, but this possibility needs further investigation.
- High acid-soil tolerance was exhibited by *L. diversifolia*, *L. macrophylla*, *L. shannoni*, and their natural hybrids. The two *L. diversifolia* lines were relatively uniform for high tolerance, whereas *L. macrophylla* and *L. shannoni* were variable for high tolerance. In the field at Carimagua, these tolerant lines remained green and grew 3 m high by the end of the 2nd season. The *L. leucocephala* controls became yellow and were no more than 1 m high, although one giant with high Al tolerance reached 1.5 m high. Foliar analyses showed that Al levels in the *L. leucocephala* controls and the highly tolerant lines were no more than 170 ppm. However, Ca contents in *L. leucocephala* were 0.4% or less, 0.6% in *L. diversifolia*, and 0.72% and 0.74%, respectively, in the *L. macrophylla* and *L. shannoni* hybrids. Root excavations to a depth of 1 m

or more showed superficial rooting in *L. leucocephala* lines. In *L. diversifolia*, a high proportion of the trees remained green and vigorous, and most of these had deep thick roots that continued into the subsoil. In *L. macrophylla*, *L. shannoni*, and their hybrids, relatively small numbers of trees were green and vigorous, and most of these also had deep penetration of thick roots into the subsoil.

- Intolerance to acid oxisols occurred in *L. collinsii*, *L. esculenta*, *L. trichodes*, and *L. pulverulenta* populations with intense yellowing and death of a proportion of the trees in both glasshouse and field. In the two *L. pulverulenta* lines, a low proportion of hybrid trees proved to be tolerant and the progenies of these are now giving promising results in the Carimagua oxisol. Further introductions of *L. diversifolia* proved to have low acid-soil tolerance, so high tolerance is not a feature of all *L. diversifolia* lines.

Natural and Hand-Made Interspecific Hybrids

A number of promising natural interspecific hybrids have been selected in *L. diversifolia*, *L. macrophylla*, and *L. shannoni* and also in *L. pulverulenta* (Hutton 1981). Because they are fertile, further field trials in the acid oxisols at Carimagua and Brasilia have been possible as well as intensive glasshouse evaluation in the Carimagua oxisol.

As a corollary to this work, hand crosses have been made between *L. diversifolia* and *L. shannoni* on the one hand and *L. leucocephala* Cunningham and the giant K420 on the other. Because of flowering problems, no crosses were obtained between *L. macrophylla* and *L. leucocephala*. The acid-intolerant *L. esculenta* was crossed successfully with *L. leucocephala*, but this did not prove possible with the acid-intolerant *L. trichodes*.

The hand-made interspecific hybrids with *L. diversifolia* and *L. shannoni* have proved fertile, as in the natural ones. Their chromosome numbers are mainly intermediate, 2n=78. Flower head and leaflet size are also intermediate between these species and *L. leucocephala*. *L. diversifolia* flower heads often are deep rose, so, in some of the *L. diversifolia* crosses, a proportion of the flower heads were light rose.

In the breeding of acid-soil-tolerant leucaenas for the very extensive oxisol and ultisol areas of South America, it appears necessary to transfer the high-acid tolerance of *L. diversifolia* or *L. shannoni* into an *L. leucocephala* background.

This has a number of advantages, including self-pollination, high seed production, and stability of the populations, as well as high potential for wood and forage production. *L. diversifolia* wood tends to be brittle, a character to be avoided. However, apart from its acid-soil tolerance, *L. diversifolia* could confer its rapid early growth, which would be important in field establishment.

In glasshouse investigations, the high acid-soil tolerance of *L. diversifolia* and *L. shannoni* has been transferred in the F_1 of the crosses with *L. leucocephala*. The F_2 populations of these crosses are now being investigated for tolerance, with particular attention to chromosome levels. Whether it will be necessary to backcross to *L. leucocephala* to raise chromosome numbers to 2n=104 is not known at present.

Factors Affecting Breeding

The main factors that breeders must deal with when attempting to produce leucaena adapted to acid oxisols and ultisols are:

- Soil pH 4.2–4.5, Al saturation 80–90%, soil solution Al 4–5 ppm.
- Deficiencies of P, S, Ca, Mg, K, and micronutrients like Zn; Ca saturation less than 20% except in the deepest horizons.
- Surface applications of P remain in the surface, whereas those of Ca and Mg move slowly downward to the subsoil. Ca in $CaSO_4$ has a better downward movement than Ca in lime or dolomite (Ritchey et al. 1980).
- In plants, all important mineral nutrients, except Ca, are translocated to both tops and roots. Ca is translocated only to plant tops, where it is immobilized in old leaves (Mengel and Kirkley 1978).
- Essential characters are associated mainly with the roots. Roots need to prevent excess Al absorption or to detoxify absorbed Al. Roots will not grow without Ca. For the roots to grow and penetrate the acid subsoils, they need to absorb Ca efficiently from the low amounts available, in spite of the presence of high exchangeable Al.

Most of the work on selection of acid-tolerant leucaena lines and hybrids has been done in the Carimagua oxisol in either the glasshouse or the field. In the soil profile at Carimagua, there is a zone from about 0.20–1 m deep that has very unfavourable conditions for root growth because of the high Al saturation and low Ca saturation. Deeper than 1 m, there is an increase in pH and Ca and a decrease in Al saturation, so that conditions are more favourable for root growth.

The glasshouse evaluations for acid-soil tolerance have been done in soil from the unfavourable zone at Carimagua, i.e., with high Al saturation and low Ca saturation. The glasshouse selections, with green, vigorous top growth and long prolific roots that are well nodulated, are the ones with high acid-soil tolerance. Those with poor, yellowish top growth and poor root growth and nodulation lack acid-soil tolerance. Root damage from Al was not apparent in any of the lines, even in the intolerant ones with poor growth. It seems that the roots of intolerant lines, however, are unable to grow because of insufficient absorption of Ca caused by the antagonistic effect between Al and Ca at the root surface. Why the lines with high acid-soil tolerance are able to absorb sufficient Ca for prolific root growth, in spite of the high Al saturation, is the key question that needs answering.

There is a good correlation between the reactions of acid-soil-tolerant and intolerant lines in the glasshouse and in the field at Carimagua. It is apparent that the roots of those leucaena lines with efficient Ca absorption are able to grow through the unfavourable subsoil zone into the deeper, more favourable zones. This results in green, vigorous top growth, with trees up to 3 m high by the end of the 2nd wet season. Glasshouse evaluation can accelerate the selection of acid-soil-tolerant leucaena lines. In 3 months in the glasshouse, a large number of lines can be evaluated for acid-soil tolerance — evaluations that would take up to 2 years in the field.

Other oxisols, such as those in the Cerrados and Amazon of Brazil, have similar characteristics to the Carimagua profile. Although the favourable zone for root growth in these is deeper (1.5–2 m) than that at Carimagua, preliminary results indicate that acid-soil-tolerant leucaena lines, selected at CIAT in the Carimagua oxisol by the methods outlined, will also grow well in the field in the typical Cerrados oxisol at Brasilia. Similar results are expected in the Amazon. Field evaluations of promising selections have not been made as yet in a typical ultisol, as at Pucallpa in Peru. However, the results could be satisfactory, as the subsoil Ca levels are almost double those in the oxisols.

To date, sufficient hand crosses have been made to combine the best characters of Cunningham cultivar and the giant K420, respectively, with the most acid-soil-tolerant selections from *L. diversifolia* and *L. shannoni*. It is now necessary to make acid-tolerant selections within the F_2 populations from these crosses in oxisols, both in the glasshouse and in the field. Current results indicate that high-yielding forage and wood types of leucaena with high acid-soil tolerance are now a definite possibility.