BREEDING AND SELECTING LEUCAENA FOR ACID TROPICAL SOILS

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ABSTRACT - In tropical South America 55% of the area (820 million ha), including 68% of Brazil (573 million ha) comprise very acid oxisols and ultisols of pH 5 or less. Tropical Africa and Asia also have substantial areas of these acid soils. Special problems are posed by very acid soils for the growth of legumes, including N_2-fixing trees like leucaena. The properties of oxisols, especially of their subsoils, have been considered as a basis for selection and breeding of leucaena lines adapted to these conditions. Perhaps the principles discussed apply also to other N_2-fixing trees.

In view of the low pH, the very low exchangeable calcium and high percentage aluminium saturation in the subsoils of oxisols (ultisols), pertinent aspects of plant nutrition were reviewed. The constraints imposed on plant growth by these Ca and Al levels were given particular attention. Because of the importance of Ca in root growth, its immobility in plants and its neutralizing effect on Al, ways of increasing downward migration of Ca into the subsoil were considered. The effect of Ca on nodulation was briefly discussed.

Studies of *Leucaena leucocephala* cultivars, especially Cunningham, in the oxisols of the Colombian llanos and the Brazilian cerrados showed that retarded root and top growth was due primarily to Ca deficiency. The high exchangeable aluminium did not have a toxic effect on the roots, but it probably inhibited Ca absorption and intensified Ca deficiency. The work described has been successful in identifying acid soil tolerance and efficient root absorption of Ca, especially in lines of *L. diversifolia* and *L. shannoni*. Their acid soil tolerance has been transferred to fertile crosses with *L. leucocephala*. Selections from these crosses with good agronomic and forestry potential adapted to the South American oxisols are now possible.

Index terms: N_2 fixation, nodulation, *Rhizobium*.

MELHORAMENTO E SELEÇÃO DE LEUCAENA PARA SOLOS TROPICAIS ÁCIDOS

RESUMO - Na América do Sul tropical, 55% da área (820 milhões de hectares), incluindo 68% do Brasil (573 milhões de hectares), compreende oxissolos e ultissolos muito ácidos, com pH = 5,0 ou menor. A África tropical e a Ásia, também, têm áreas substanciais destes solos. Estes solos muito ácidos representam problemas especiais para o crescimento de leguminosas, inclusive das árvores fixadoras de N_2 como a leucena. As propriedades dos oxissolos, especialmente dos seus subsolos, foram consideradas como base para a seleção e melhoramento de linhagens de leucena adaptadas a estas condições. Talvez os princípios discutidos se apliquem também a outras árvores fixadoras de N_2.

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Em vista do pH baixo, do baixo nível de cálcio e alta percentagem de saturação de alumínio no subsolo dos oxissolos, são revisados alguns aspectos de nutrição de planta. Às limitações impostas sobre o crescimento da planta por estes níveis de Ca e Al foi dada atenção particular. Por causa da importância do Ca no crescimento das raízes, sua imobilidade na planta e seu efeito em neutralizar o Al, foram discutidos os modos de aumentar a migração do cálcio da superfície para o subsolo, bem como os efeitos do cálcio sobre a nodulação.

Estudos feitos com cultivares de *Leucaena leucocephala,* especialmente a cultivar Cunningham, nos oxissolos dos "llanos" colombianos e nos cerrados brasileiros, mostraram que o crescimento deficiente de raiz e parte aérea foi devido, principalmente, à deficiência de Ca. O alto nível de alumínio trocável não teve efeitos tóxicos sobre as raízes mas, provavelmente, inibiu a absorção de Ca e intensificou a deficiência deste elemento.

O trabalho mostrou ser possível identificar a tolerância a solos ácidos e a eficiência em absorção de Ca, principalmente nas linhagens de *L. diversifolia* e *L. shannoni,* cuja tolerância a solos ácidos foi transferida a cruzamentos férteis com *L. leucocephala.* A partir dos cruzamentos com bom potencial agronômico e florestal, é possível, agora, selecionar estas linhagens para adaptação aos oxissolos sul-americanos.

Termos para indexação: fixação de N_2, nodulação, *Rhizobium.*

INTRODUCTION

In the tropics when selecting N_2-fixing trees for wood, forage, fuel, shelter etc. it is essential to remember that a high percentage of the soils are very acid oxisols and ultisols with a pH of 5 or less. On an area basis, Sanchez & Salinas (1981) calculated that 43% of the tropics have acid oxisols and ultisols with the highest concentrations in tropical America (55%), tropical Africa (40%), and tropical Asia (37%). In Brazil, Cochrane (1979) estimated that 68% (573 million ha) of the country comprises oxisols and ultisols.

The very acid soils present special problems for the growing of legumes, including N_2-fixing trees. Some of the problems will be outlined later in this paper. In addition, vigorous growth of N_2-fixing legumes requires an active *Rhizobium* symbiosis. Also, more attention than non-legumes, like *Eucalyptus* and grasses must be given to the supply of essential mineral nutrients, including P, S, Ca^{++}, Mg^{++}, K^+ and the micronutrients Mo, Zn, Cu and B. Advantages such as persistence, soil enrichment and forage, provided by N_2-fixing trees outweigh any extra attention needed in the oxisol and ultisol regions.

There is a challenge to collect and evaluate more of the promising native N_2-fixing trees from the tropics, especially from strongly acid soils. Those that are well-known have been reviewed by the National Academy of Sciences (1979). For example, neutral to alkaline soils in tropical Central America are the native habitat for several important species, including *Leucaena leucocephala* and *Calliandra calothyrsus.* Tropical native N_2-fixing trees from Asia include *Albizia falcataria* and *Sesbania grandiflora,* from Africa a number of *Acacia's* and *Tamarindus indica* and from Australia also a number of *Acacia's,* such as *A. mangium.* Brazil has a rich flora of N_2-fixing trees which need exploiting.

This paper outlines the progress made in breeding lines of the tree legume *L. leucocephala* adapted to the oxisols and ultisols of South America with present emphasis on the Brazilian cerrados. Since *L. leucocephala* and related Leucaena species are native to the neutral and alkaline soils of Central American countries, principally Mexico, there are special problems to be solved. In particular, the factors affecting the uptake by *L. leucocephala* of essential nutrients in the very acid oxisols and ultisols needed particular attention. The experience gained with leucena could be useful in selecting and adapting other tree legumes to the vast areas of tropical acid soils.

The problems associated with acid oxisols and ultisols

The soil problems related to growing trees, crops and pastures in oxisols and ultisols are dealt with in the recent review of Sanchez & Salinas (1981). Oxisols and ultisols are highly leached tropical soils deficient in most essential nutrients including P, S, Ca^{++}, Mg^{++}, K^+ and the minor elements, especially Zn. In the virgin soils the low pH of 5 or less in accompanied by 70% or more Al saturation of the clay complex, a low effective cation exchange capacity, and Al concentrations in the soil solution of up to 4-5 ppm. The exchangeable cations determined by soil extraction with neutral salt solutions are dominated by Al so that the percentage saturation of the especially important Ca is less than 20%.

The profile characteristics of three typical oxisols (Salinas 1980, Salinas & Delgadillo 1980) concerning Ca and Al, which significantly affect root and plant growth, and also pH are given in Table 1. In oxisols near Brasilia occasionally much lower calcium levels are found (Ritchey et al. 1982). Of the above oxisols that of Carimagua shows with increasing depth increase pH and Ca concentrations and therefore lower percentage Al saturation. In the ultisols and oxisols from Brasilia and Manaus pH, Ca, Al and percentage Al saturation remain relatively stable with depth, except in the deepest horizon where Al and percentage Al saturation decreases significantly, particularly in the Manaus oxisol. The surface of Manaus oxisol after recent forest burn had high Ca and very low percentage Al saturation.

Plant growth and nutrition with special reference to Ca^{++} and Al^{++} in acid soils

The major nutrients, except Ca, are readily mobile in plants and translocated in an upward and downward direction to tops and roots (Mengel & Kirkby 1979). Translocation in the xylem is upward

TABLE 1. pH, Ca, Al and Al saturation with depth in three representative oxisols of tropical South America.

Horizon (depth in cm)	pH			Exchangeable cations-meq/100 g soil						% Al Saturation		
				Ca			Al					
	A*	B*	C*	A	B	C	A	B	C	A	B	C
0- 20	4.1	4.9	4.6	0.36	0.4	1.7	3.6	1.9	1.1	87	79	33
20- 40	4.0	4.8	4.4	0.19	0.2	0.2	2.7	2.0	1.1	90	89	79
40- 80	4.4	4.9	4.3	0.25	0.2	0.2	1.4	1.6	1.2	78	88	82
80-140	4.9	5.0	4.6	0.31	0.2	0.2	0.5	1.5	1.0	52	88	81
140-200	5.0	4.6	4.9	0.36	0.2	0.2	1.1	0.7	0.2	67	76	39

* A = Oxisol Carimagua, Colombian llanos orientales; B = Oxisol Brasilia, cerrado; C = Oxisol Manaus, Brazilian Amazon. Data based on that of Salinas (1980) and Salinas & Delgadillo (1980).

with the transpiration stream of water, the main minerals transported being N (as NO_3), Ca and K, with P, S and Mg transported to a minor extent. Translocation in the phloem is bi-directional and involves mainly P, Mg and K and to a minor extent N (as NH_4) and S. Ca is only in minute quantities in phloem sap and is scarcely moved in a downward direction in the phloem (Loneragan & Snowball 1969).

Foy (1974a) reviewed the effects of soil Ca availability on plant growth. Ca is mono-directional and translocated only upwards in the xylem from roots to plant tops, and becomes irreversibly deposited in the old leaves. The Ca is found in the cell walls and as precipitated salts of organic acids, phosphate, carbonate etc. in cell vacuoles and organelles. Often adequate Ca is absorbed early in the growing season, but absorption is reduced markedly as the plant matures. Thus the older tissues of the above ground growth are usually well supplied with immobile Ca while the growing points of tops and roots can die of Ca deficiency, if the soil is too low in available Ca^{++}. Insufficient translocation of Ca from roots to the growing points of shoots results in a reduced growth rate, yellowing of young apical leaves followed by necrotic spots, and finally browing and death of shoot tips. The meristematic tissues at the tips of roots and shoots are those most affected by Ca deficiency. Dekock et al. (1975) suggested that the browning of root and shoot tips, when Ca is limiting, is due to black melanins derived from oxidation of polyphenols to quinones and their reaction with amino acids and proteins. In soybeans, Ca deficiency occurs in soils with Ca saturations of 20% or less, Al saturations of 68% or more, and when Ca in plant tops is less than 0.58% of the dry matter. The Ca level in plant tops of most species at which Ca deficiency appears is 0.3 - 0.4%.

Ca is especially important in root growth, but healthy roots have very low Ca contents. Ca maintains the integrity of root membranes, and within roots and plants detoxifies cation excesses. The soil Ca level essential for root and top growth is quite low, except in acid soils with pH of about 5 or less and with high exchangeable Al (eg. oxisols). Excess Al increases the soil calcium requirement to neutralize the inhibiting effect of Al on root absorption of Ca by crops such as maize, soybeans and N_2-fixing trees like leucaena. Thus for the growth of crops and N_2-fixing trees in acid oxisols with low pH, low exchangeable Ca and high exchangeable Al (Table 1) there are special constraints due to Ca-Al interactions.

The effects of Al on plant growth are discussed in detail by Foy (1974b). In neutral and alkaline soils, Al solubility is too low to interfere with plant growth. Low Al levels benefit growth and most plants contain about 150-200 ppm Al in the dry matter. In acid soils with pH of 4.2-5.0, Al availability increases markedly and over half the cation exchange sites are occupied (Evans & Kamprath 1970). More than 1/meq exchangeable Al/100 g in these acid soils produces enough Al in the soil solution (commonly 2-3 ppm) to cause inhibition of root growth due to Al toxicity or Al-induced Ca deficiency. The exchangeable Al interacts with Ca and P in the soil and plant. In the plant Al reduces uptake and translocation of Ca and P to the tops (Andrew et al. 1973). Where roots are thickened and deformed by excess Al in species like cotton and sorghum, Al accumulates in or on the roots, but is usually not translocated to the tops. Rasmussen (1968) and McCormick & Borden (1974) found that Al was localized on the root epidermal cells when the root surface remained intact. If Al penetrated into the cortex and conducting tissue of roots, Al and P precipitated as Al phosphate on cell walls and in the free space between cells. There was no relation between the distribution of Ca and Al in the root. This suggests that movement of Al upwards in the plant is by chelates in the phloem.

The differences between the symptoms of Al toxicity and Ca deficiency in plant roots are well illustrated in cotton, which is one of the most sensitive crops. Al toxicity from excess Al is manifested

by swollen, stubby and deformed roots (Rios & Pearson 1964). By contrast, Ca deficiency in this plant is indicated by thin straight primary roots with brown tips, and severe Ca deficiency causes death of the roots (Howard & Adams 1965). In their studies of cotton growth in Alabama Coastal Plain soils of southeastern U.S.A. Adams & Moore (1983) found no consistent relationship between soil chemical properties and intensity of Al toxicity in cotton roots. They concluded that lack of cotton root growth into the acid subsoils may be due to Ca deficiency or Al toxicity.

Ca migration downwards in oxisol profiles

All the major nutrients applied to the soil can be absorbed by surface roots and some like N, K and S move readily downwards in the profile. As already discussed, their translocation in the plant is bidirectional, except in Ca which is monodirectional. Because of the importance of Ca in root growth and plant nutrition, and its neutralizing effect on Al toxocity, it is necessary to consider augmenting the low exchangeable Ca levels in oxisol subsoils. This would stimulate root and top growth of N_2-fixing trees, like leucena, in acid oxisols and also in ultisols.

In oxisols (and ultisols) fertility improvement usually includes heavy applications of calcitic or dolomitic lime. The resultant increase in exchangeable Ca and decrease in exchangeable Al occur mainly at the depth of lime incorporation, as there is no significant downward movement of Ca and Mg for probably 6-10 years. Gonzalez (1976) found that both a shallow (0-15 cm) and a deep (15-30 cm) incorporation of 1, 2, 4 and 8 tons of calcitic lime (72.4% $CaCO_3$, 2.4% $Mg CO_3$) into a typical Brazilian LVE oxisol had no effect on pH and exchangeable Ca and Al contents at lower depth (30-45 cm). This was after 3 successive maize crops. Prolific root development in the maize occurred only at the depth of lime incorporation.

In oxisols, promotion of deep rooting in leucena, and probably most N_2-fixing trees, would follow if Ca migration deep into the profile could be stimulated from surface applications. It is known that Ca when combined with other common anions has increased mobility in soils in the order chloride = nitrate $<$ sulfate $>$ HCO_3-CO_3 (Bohn et al. 1979). Ritchey et al. (1980) showed that heavy field applications of simple superphosphate eg 700, 1400 kg/ha to the Brasilia oxisol gave after 4 years an appreciable increase in subsoil Ca, a small pH increase and a decrease in Al saturarion to a depth of 45-60 cm. These beneficial effects are due to about 60% gympsum ($CaSO_4$, $2H_2O$) in simple superphosphate. These effects could also be obtained by supplementing ordinary applications (about 200 kg/ha) of simple superphosphate with relatively heavy amounts (500 - 800 kg/ha) of the low cost gypsum. Supplementing rock phosphate applications (200 - 400 kg/ha) with sypsum could also be considered, as they would provide the essential P, S as well as more mobile Ca. Gypsum could also replace much of the dolomitic limestone used, except that needed as a cheap source of Mg. Transport and application of gypsum causes difficulties because of its physical nature. These may be overcome by mixing and processing the gypsum with dolomite or rock phosphate.

Deep, and usually expensive mechanical incorporation of lime of gypsum into oxisol subsoils would benefit considerably the root growth of N_2-fixing trees, including leucena, and also of crops. This method could be considered with N_2-fixing forest trees which are transplanted into holes. These holes could be deepened and supplied with $CaSO_4$ or lime, as well as rock phosphate or simple super-phosphate. However attention is needed to practical fertilizer mixtures which increase chemically, the downward migration of Ca into oxisol subsoils from surface applications.

Leucaena nodulation in oxisols

For N_2-fixing trees, including leucena, adequate root nodulation is essential for productive and vigorous growth. Although Ca does not appear to be necessary for in vitro growth of rhizobium (Norris 1959), it is very important for adequate nodulation of the host plant, especially in acid soils. Nodule initiation in subterranean clover has a higher Ca requirement than nodule development and host-plant growth (Lowther & Loneragan 1968). At a Ca concentration of 0.4 ppm no nodules formed at any pH in subterranean clover (Loneragan & Dowling 1958). At pH 4.5 increasing Ca from 4-280 ppm increased nodule numbers from 0-10. At the high Ca concentration, maximum nodulation was obtained at pH 5, but with a low Ca concentration a pH of 6 was required.

In my CIAT glass house experiments on breeding acid soil tolerant leucena, a typical Colombian Llanos oxisol was used, and the only Ca source a relatively immobile one, was provided by a surface application of Huila rock phosphate. Root growth of the selected lines was prolific to a depth of 12 cm, whereas nodulation was confined to the surface 2-3 cm (Hutton unpublished), in spite of using CIAT selected acid soil tolerant *Rhizobium* (Date & Halliday 1979). It was apparent that the Ca requirement for leucena nodulation was greater than for root growth.

Factors affecting Leucaena growth in oxisols

The growth of commercial *L. leucocephala* cultivars, eg. Cunningham and K 8, is markedly retarded in the oxisols of the Colombian llanos and the cerrados of Brazil. The trees grow to no more than about 1.5 m and become yellow, in spite of adequate fertilizer applications, including minor elements, and inoculation with adapted *Rhizobium*. In addition, tree regrowth after cutting back is poor and a proportion of the trees eventually die from the growing tips downwards. The CIAT leucena research in the Colombian llanos oxisol always showed normal foliar Al^{+++} levels of about 120-150 ppm. It was concluded that poor root absorption of Ca was the major problem, and not toxic effects of subsoil Al (Hutton 1982).

At EMBRAPA-CPAC (Planaltina, DF), the growth of three year old Cunningham leucena trees in typical cerrado LVE oxisol was studied (Hutton, Thomas, De Sousa unpublished) at the end of the last wet season. One group of trees has been transplanted from small jiffy pots and the other group direct seeded. At planting in Feb 1980 both groups received one application of 600 kg/ha simple superphosphate, 100 kg/ha KCl and 20 kg/ha of a minor element mixture. Transplanted trees were cut back when 2 years old, and the heights when 3 years old averaged 1.7 m in the direct seeded and just under 1.0 m in the transplanted plots. Typical Ca deficiency symptoms (Gonzales et al. 1980) were observed in both groups. These included significant yellowing of young leaves and some mature leaves, often with necrosis, and also death of shoot tips in most trees. Ca deficiency was substantiated by analyses of the bulked leaves from each of a number of random groups of trees. The young expanded leaves averaged 0.27% Ca and the mature leaves 0.88% Ca, indicating a lack of Ca for active growth. However the leaf samples had normal levels of N, P, K and Mg showing an adequate soil supply of these nutrients.

In view of the apparent Ca deficiency shown by the above ground growth of these 3 year old trees at EMBRAPA-CPAC, it was necessary to study their root development. A number of trees were excavated at random, and the average number of relatively thick lateral roots per tree was 3 and the average lateral root length was 1 m, although some laterals were 2 m long. The lateral roots were usually confined to the surface 10 cm of soil. Taproots were absent in the trees from jiffy pots, but there were relatively thick taproots in those from direct seeding penetrating 60-80 cm down from the soil surface. Develop-

ment of hair-like secondary roots was not profuse. There was no obvious root damage typical of Al toxicity in any of the tree root systems. It appeared that root development had been relatively rapid initially when adequate nutrients including soluble Ca were available. However profuse secondary root growth and root penetration to depths in the soil profile of 1 m and deeper had stopped. Root growth as well as top growth had become static, and deterioration of top growth had followed. Sufficient available Ca deep in the subsoils of oxisols is essential, if roots of the current leucaena cultivars are to reach the depths necessary for vigorous and commercially acceptable growth. The same could apply to the growth of other N_2-fixing trees in the oxisols. When poor growth of leucaena or other N_2-fixing trees occurs in very acid soils (eg oxisols), a method to overcome it would be of considerable interest. Perhaps mechanical or chemical means of deep subsoil lime or $CaSO_4$ incorporation between the trees could be considered. Recently a method of subsoil lime injection has been described by Anderson & Hendrick (1983), and could probably be adapted for this situation.

Characters needed in acid soil tolerant leucena lines

Foy's reviews (1974a, b) contain a number of important suggestions for the breeding of plants adapted to strongly acid soils with high Al saturation. For example, it is considered that plants with the capacity to absorb adequate Ca from low-Ca high Al soils are needed. Such plants also showed be able to redistribute to their growing points previously absorbed Ca that is immobilized in the old tissues.

Although some scientists (eg. Norris 1959) have shown that *Rhizobium* does not require Ca for multiplication in culture, Foy (1974a) suggested that *Rhizobium* strains able to tolerate the low Ca levels in Al-saturated soils are needed. As Ca is essential for legume nodulation (Loneragan & Dowling 1958), perhaps plants with better root absorption of Ca would nodulate better in strongly acid soils. Perhaps it is possible to find genetic differences in nodulation efficiency at low Ca levels. There is little doubt that the rapid growth of leucena and other N_2-fixing trees in very acid oxisols and ultisols is dependent in large measure on appropriate nodulation and N_2-fixation.

Although Ca deficiency rather than Al toxicity is apparently the major problem in the growth of leucena in oxisols, it would be advantageous to have Al tolerant lines, as these would prevent absorption of excess Al by roots, or detoxify Al after absorption (Foy 1974b).

The physiology and biochemistry of Al tolerance are still being debated and researched. The results from one species are not necessarily applicable to another species. Al tolerant plants may exclude Al from roots and tops, or may take it up more slowly. Some species have low Al concentrations in tops and high concentrations in roots, while others behave in opposite way. It is general concept that tolerance to excess Al, is confered by extracting P more effectively from Al · Fe compounds, and by utilizing Ca and P more efficiently in the presence of excess Al.

The search for acid soil tolerance in leucena

Although *L. leucocephala* and its related species are native to the neutral and alkaline soils of Southern Mexico and Central America (Brewbaker & Hutton 1979), it was shown by Hutton & Andrew (1978) that some scope exists within *L. leucocephala* for breeding lines with greater tolerance to acid soils. In their investigation, the bred line 3 (Subsequently Cunningham), among the 4 leucena lines studied, maintained the highest Ca concentration and the lowest Al content.

In my initial leucena research at CIAT, Al tolerance was studied in populations from an F_1 (*L. leucocephala* cv. Cunningham x *L. pulverulenta*) back-crossed twice to Cunningham. The back-cross was developed previously in Australia (Hutton unpublished). The subsequent F_3 and F_4 populations provided greater variability for selection for selection than crosses between *L. leucocephala* varieties. About 45,000 seedlings of the populations were evaluated in sand culture supplied with complete nutrients, but low P (0.5 ppm) and Ca^{++} (1 ppm) and 5 ppm Al, at a pH maintained at 4.2. Of the seedlings tested, 12.6% were selected for high Al tolerance on the basis of growth. When the selected lines were grown in Carimagua oxisol (pH 4.2, 85% Al saturation) in the glasshouse, and in the field at Carimagua, their growth was slow. Within 3 months in the glasshouse most lines lacked vigor and had some leaf yellowing. At the end of the first season's growth at Carimagua, none of the Al tolerant selections were more that 1 m high, and most had yellowish leaves. Leaf samples showed normal Al levels (about 130 ppm) but tended to have a low Ca content (about 0.4%).

It was apparent from these results that sources of higher acid soil tolerance would have to be found in leucena. Also that considerable emphasis was needed on breeding for higher efficiency in root absorption of Ca, as already discussed earlier in this paper. A number of ecotypes of *L. collinsii, L. diversifolia, L. esculenta, L. macrophylla, L. pulverulenta, L. shannoni* and *L. trichodes* collected by Prof. James Brewbaker were grown together in the field at CIAT. Populations from seed of these, and *L. leucocephala* controls, were grown under Ca stress conditions in Carimagua oxisol in the glasshouse and in the field at Carimagua. There was significant variation in the populations of these species, except those of *L. collinsii, L. trichodes* and the *L. leucocephala* controls. It was apparent that natural interspecific crossing had taken place previously CIAT field, and that some lines had high acid soil tolerance (Hutton 1981). *L. collinsii, L. esculenta, L. trichodes* and one *L. pulverulenta* were particularly sensitive to the Ca stress in the acid oxisol. However *L. diversifolia, L. shannoni, L. macrophylla* and their natural interspecific hybrids proved to have high tolerance to the acid oxisol and Ca stress. Another *L. pulverulenta* line had a few promising tolerant plants, and the *L. leucocephala* controls had some tolerance, especially one of the "giant" varieties (K420). Leaf samples collected in August and October 1981, when the trees were growing strongly and 14-16 months old, gave the analyses shown in Table 2.

TABLE 2. Foliar analyses of trees of promising acid soil tolerant *Leucaena* species from seed planted June 1980 at Carimagua (Colombian llanos).

Species	Acession	Dry matter analyses[1]				
		% N	% P	% Ca	% Mg	Al ppm
L. leucocephala	78-15 K420[2]	3.12	0.11	0.40	0.22	70
	78-15 K420[3]	2.91	0.10	0.46	0.29	130
L. diversifolia	78-49 K454[2]	3.46	0.11	0.54	0.16	150
	78-49 K454[3]	2.79	0.12	0.60	0.20	140
L. macrophylla (natural species hybrids)	78-65 K468[2]	3.86	0.12	0.72	0.21	70
L. shannoni (natural species hybrids)	78-70 K473[3]	3.50	0.10	0.74	0.39	170

[1] Means for two replicates in close agreement

[2] Samples August 81

[3] Samples October 81.

From Table 2 it is apparent that Ca content in the second season was lowest in *L. leucocephala* K420, intermediate in *L. diversifolia* K454, and highest in the natural interspecific hybrids of *L. macrophylla* and *L. shannoni* (Hutton 1982). This was reflected in the growth of the their roots and tops. The roots of *L. leucocephala* K420 had penetrated the soil 20 cm, while those of the other species had penetrated 1 m or more. In *L. leucocephala* K420 growth was retarded and the trees about 1.2 m high with yellowing leaves, while *L. diversifolia* K454 trees were well branched, 2 m high, green and leafy. The natural *L. macrophylla* and *L. shannoni* hybrids had one relatively thick stem (3-4 cm diam), were up to 3 m high, green but not leafy.

Breeding acid soil tolerant Leucaena from interspecies crosses, especially with *L. diversifolia* and *L. shannoni*

Among the most acid soil tolerant, some *L. diversifolia* and *L. shannoni* lines were the most promising as they crossed by hand with *L. leucocephala*. However crosses with the tolerant *L. macrophylla* were unsuccessful. The intolerant *L. esculenta* was also crossed by hand with *L. leucocephala*. No hand crosses with *L. pulverulenta* were made. This was because the Al tolerant hybrids selected from the populations of my previous Australian *L. pulverulenta* backcross with *L. leucocephala* were not highly acid soil tolerant in the initial phase of the current investigation. However more recently, some *L. pulverulenta* lines have shown potential, as one line tested later in this investigation had a small percentage of plants in the population with high acid soil tolerance.

L. leucocephala has 2n = 104 chromosomes, is mainly self-fertilized and seed production is usually plentiful (Brewbaker & Hutton 1979). The other leucena species of interest have 2n = 52 chromosomes, except *L. pulverulenta* with 2n = 56 chromosomes. They are self-incompatible and outcrossing, and seed production is variable and less than that of *L. leucocephala*. The populations of *L. diversifolia, L. shannoni* and *L. pulverulenta* show considerable plant variation in morphological characters, as well as acid soil tolerance. The same applies to the *L. esculenta* lines studied, except that most plants in the populations are intolerant to acid soil.

As expected, the F_1 of *L. leucocephala* crosses with *L. diversifolia* and *L. shannoni* have 2n = 78 chromosomes and show marked plant to plant variation, especially in acid soil tolerance. Unexpectedly, the F_1 of these crosses is fertile and produces ample seed which facilitate the investigation of the F_2 populations for acid soil tolerance (Hutton unpublished). Due to the complication of infertility of the F_1 from *L. leucocephala* hand crosses with *L. pulverulenta* only natural fertile crosses have been studied.

A large screening of acid soil tolerant and intolerant lines of leucaena (selected previously at CIAT, Colombia) was made during the 1982-83 season at EMBRAPA-CPAC (Planaltina, DF) on typical cerrado soil (Hutton & De Sousa unpublished). The seed inoculated and rock phosphate pelleted seed was planted early November 1982 into native LVE oxisol, the whole area being fertilized with 200 kg/ha simple superphosphate, 200 kg/ha dolomite, 100 kg/ha KCl and 20 kg/ha minor element mixture. Of the 108 lines planted, 30 were from hand crosses, 62 from natural crosses and 16 from *L. leucocephala* varieties and leucena species. Initial germination and emergence was good, but in 42% of the lines all plants eventually died, due apparently to Ca deficiency as evidenced by root symptoms. The lines which died out included 47% of those from hand crosses, 34% of those from natural crosses, and 63% of those from *L. leucocephala* varieties and leucena species. Only one out of 4 lines of *L. leucocephala* survived. The species which died included *L. collinsii, L. esculenta, L. macrophylla, L. retusa, L. trichodes,* 3 lines of *L. diversifolia* and one of *L. shannoni*.

Among the lines which had varying numbers of plants persisting by the end of the 1983 dry season, 14% showed relatively high persistence and had a proportion of young trees 55-70 cm high. These promising lines included the "giant" *L. leucocephala* (K420), 2 of the *L. diversifolia*, 4 natural *L. diversifolia* crosses, 2 natural *L. shannoni* crosses and 6 selections from 2 natural *L. pulverulenta* crosses. Some young vigorous trees were excavated at the end of the 1982-1983 wet season and most had deeply penetrating roots up to 1 m long.

In a proportion of the F_2 lines from the hand crosses between *L. leucocephala* (cv. Cunningham) and acid soil tolerant lines of *L. diversifolia* and *L. shannoni* there was good persistence of plants with a number of promising segregates. This indicates that it is possible to transfer the character of acid soil tolerance from *L. diversifolia* and *L. shannoni* to crosses with *L. leucocephala* varieties such as Cunningham. This can be achieved without loss of fertility in the F_1 and F_2, and possibly in subsequent generations.

CONCLUSIONS

In the adaptation and use of N_2-fixing trees in the tropics, the extensive areas of very acid tropical soils present a challenge. Emphasis should be given to the collection of further native N_2-fixing trees from acid soils. Also the leucena work presented in this paper shows that acid soil tolerant lines can be selected or bread from introductions from neutral to alkaline soils.

N_2-fixing trees, unlike eucalyptus, require special attention to mineral nutrition, *Rhizobium* and nodulation. This is the price paid for the fertility and N build up in soil by N_2-fixing trees, and their greater potential productivity and persistence over time. Foliar analysis is an essential tool for monitoring the nutritional growth requirements of N_2-fixing trees.

It is necessary to understand the main chemical facts about acid soils, if N_2-fixing trees are to be successfully adapted to them by selection and breeding. The relevant facts about plant nutrient uptake in acid soils also need consideration. Apart from mineral deficiencies, their low Ca status and high Al^{+++} saturation are the main factors influencing the adaptation and growth of legumes, including N_2-fixing trees.

As shown in the leucena work, the successful growth of N_2-fixing trees in oxisols requires selection (or breeding) of lines with efficient root absorption of Ca from low Ca subsoils. Also replenishment of subsoil Ca needs to be considered by mechanical or chemical means.

Bred leucena lines which will grow satisfactorily in oxisols can now be achieved from fertile crosses between *L. leucocephala* and acid soil tolerant lines of species like *L. diversifolia*. Perhaps the same approach could apply to other promising N_2-fixing trees which may not be well adapted to acid soils.

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