Strategic Research Findings in Leucaena

Tolerance of Leucaena to Acid Soil Conditions

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Abstract

Acid soils of the humid tropics and subtropics pose a major limitation to the growth and production of *Leucaena leucocephala* (Lam.) de Wit, an important multipurpose tree legume well-adapted to neutral and alkaline soils. The limitations imposed by acid soils include the effect of low pH itself (i.e. H+ toxicity); deficiencies of many nutrients, especially calcium, magnesium, potassium, phosphorus or molybdenum; and toxicities of aluminium or manganese. These problems may be overcome through the use of lime or gypsum. However, the latter's high cost, limited availability and (in the case of lime) need for deep incorporation, often limit their use among the resource-poor farmers of many tropical countries. There is high potential therefore for the breeding of leucaena cultivars adapted to acid soil factors, especially through inter-specific crosses (especially *L. leucocephala* x *L. diversifolia*).

LEUCAENA leucocephala (Lam.) de Wit and other Leucaena species have value as multipurpose tree legumes. They are native to the alkaline soils of Central America, especially Mexico, but *L. leucocephala* has been introduced to many tropical countries. However, it has often failed on soils that are strongly acid, and is not generally well-adapted to such conditions (Hutton 1981, 1982; Oakes and Foy 1984; Shelton 1994). Where soils are alkaline or only slightly acid, current *L. leucocephala* cultivars thrive, often with only moderate levels of fertilizer, especially superphosphate.

Overall, acid soils occupy 30% of the global land area, predominantly in two major regions of the world: the humid temperate forests and the humid tropics (von Uexkull and Mutert 1993). Sanchez and Salinas (1981) estimated that tropical oxisols and ultisols, with pH 5 or lower, cover 55% of South America, 40% of Africa and 37% of Asia. Together, these soils comprise approximately 2 billion ha, or some 14% of the total ice-free area of the world (von Uexkull and Mutert 1993). Acid soils may be found elsewhere in these regions too, so that acid soils may account for more than 50% of the agriculturally-important lands of the tropics and subtropics. Yet these areas would be particularly suitable for growing leucaena since there is usually enough water for plant growth, unlike the semi-arid tropics and subtropics.

Acid soils occur naturally where parent materials are acidic and where soils are old or in high rainfall regions (i.e. where the soils have been subjected to considerable leaching). Acidification can also result from agricultural practices such as prolonged use of nitrogen (N) fertilizer, particularly the ammonium (NH_4^+) form, and N_2 -fixation by legumes with Rhizobium and Bradyrhizobium bacteria. For example, in large areas of southern Australia, soil pH has decreased from 6.0 in virgin soils to 5.2 in soils that have been under subterranean clover (Trifolium subterraneum) pastures for 50 years (Williams 1980). When nitrate (NO_3^-) in soils exceeds immediate plant requirements it is leached down the soil profile, taking with it the basic cations, calcium, magnesium and potassium. Also, inputs of nitrogen make soils more productive, resulting in greater removal of basic cations in harvested products. Industrial pollution in highly urbanised areas of Europe and North America, and the consequent 'acid rain' problem, contribute to soil acidity in those regions.

The Acid Soil Infertility Complex and Leucaena's Response

The oxisols and ultisols of the tropics are highly leached and, in terms of plant growth, often

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deficient in calcium, magnesium, potassium, phosphorus or molybdenum and exhibiting aluminium or manganese toxicity. Aluminium toxicity is often the most important factor limiting the growth of plants in acid soils (Foy et al. 1978). The aluminium is highly exchangeable and replaces calcium on the soil's cation-exchange complex, a factor of great importance in the production of leucaena (Hutton and Chen 1993).

Virgin oxisols often have less than 0.2 cmol/kg exch. calcium ions (Ca^{2+}) , especially at depth in the soil profile. Calcium not only helps maintain cell wall and plasma membrane integrity, but is also essential for meristematic growth of roots and shoots. Without it, root development may be poor. leading to plant water stress. Calcium moves in the transpiration stream in the xylem with little, if any, movement in the phloem, and becomes immobilised in the older leaves. High levels of soluble and exchangeable aluminium in the soil interfere with calcium uptake. For instance, application of 2 t/ha dolomite and 1.7 t/ha gypsum to an Oxisol with an initial aluminium saturation of 80% failed to improve the poor growth of L. leucocephala cv. Cunningham (Hutton and de Sousa 1987). Insufficient calcium was absorbed for normal meristematic activity in the tips because aluminium inhibited uptake of calcium. There was only 0.22% calcium in the expanded tip leaves, whereas the mature leaves contained 0.75% calcium. The poor calcium status resulted in the death of the stem tips, and plants began to die 3 years after planting.

Legumes often fix less N_2 when they are grown on acid soils. Many factors of the acid soil infertility complex reduce not only *Rhizobium* and *Bradyrhizobium* populations, but also depress infection of roots by these bacteria. The acidtolerant *Rhizobium* strains, such as those selected at Centro International de Agricultura Tropical (CIAT), are essential when growing acid-tolerant leucaena cultivars on oxisols and ultisols. No nodulation occurred, however, when *L. leucocephala* cv. Cunningham was grown in a Cerrado Oxisol. Brandon (1992) found that strain CB3060 (TAL1145) was particularly effective in N₂-fixation in leucaena.

Acid soils also depress infection by vesicular arbuscular mycorrhiza (VAM) which are an important factor in leucaena's growth in infertile soils. For example, leucaena production was reduced in soils that had been sterilised to reduce the VAM population, compared to its growth when adequately infected with VAM (Brandon 1992). This poor growth was particularly evident where no or little P fertilizer had been applied to sterilised soil.

Overcoming Limitations to Leucaena Production on Acid Soils

Commonly, lime or gypsum is applied to overcome yield limitations on acid soils. Liming raises soil pH, reduces phytotoxic aluminium and manganese, and increases plant-available calcium and magnesium in the soil. However, calcium applied as lime moves very slowly down the profile, and the lime needs to be incorporated as deeply as possible. When applied as gypsum, calcium moves more easily down the profile since gypsum does not affect the soil pH of the surface layer (McCray and Sumner 1990). Gypsum is less efficient than lime in reducing phytotoxic aluminium, and may increase the leaching of other exchangeable cations, especially magnesium and potassium, out of the rooting zone. Nevertheless, the increase in plant-available calcium in the subsoil may increase root proliferation at depth.

It is doubtful whether lime or gypsum treatments are appropriate for ameliorating virgin soils to produce leucaena. High application rates of lime or gypsum are required, with deep tillage to incorporate the lime. On old cultivated soils, however, where exchangeable calcium in the subsoil is about 0.34 to 0.49 cmol (+) per kg, as at Serdang in Malaysia (Hutton and Chen 1993), satisfactory production from *L. leucocephala* cv. Cunningham is possible with annual applications of lime. Even then, root growth is limited to about 0.5 to 0.8 m, which predisposes the plants to drought. It is also probable that application of other nutrients (e.g. phosphorus and molybdenum) will be necessary.

As an alternative, organic amendments such as compost, farmyard manure, leaf material and suchlike can successfully ameliorate acid soils, whether old or virgin. Ground-up leaves of *Calliandra calothyrsus* applied to a red podzolic soil (epiaquic Haplustult) increased root growth of mung bean, *Vigna radiata* (Bessho and Bell 1992). This resulted from the precipitation of soluble aluminium and the formation of aluminium-organic matter complexes, decreasing the activity of monomeric aluminium in the soil solution. The applied leaf material also increased the effective cation exchange capacity and exchangeable calcium and magnesium in the soil.

Other multipurpose tree legumes may be better adapted than leucaena to acid soils, and not need expensive fertilizer application and soil amelioration. While relatively little information is available, Shelton (1994) has evaluated the environmental adaptation of several multipurpose tree legumes. *Flemingia macrophylla* was assessed as being very well-adapted to acid soils, while *Acacia aneura*, *Acacia villosa*, *Albizia chinensis*, *Albizia lebbek*, *Calliandra calothyrsus* and *Gliricidia sepium* were moderately well-adapted. In contrast, *Chamaecytisus palmensis* and *L. leucocephala* were assessed as being only marginally adapted to acid soils. Furthermore, *L. leucocephala* was rated as being intolerant of low fertility, a problem often encountered on acid soils. This was in marked contrast with *Acacia* and *Albizia* spp., *C. calothyrsus*, *F. macrophylla* and *G. sepium*. Overall, Shelton (1994) concluded that 'there are no varieties (of *L. leucocephala*) for the very acid oxisols of tropical South America'.

Other species of leucaena may tolerate acid soils. Hutton (1990) found that *L. diversifolia* possesses considerable acid soil tolerance. Hutton (1981) identified two diploid *L. diversifolia* lines which grow vigorously in an acid oxisol (pH 4.5) with 90% aluminium saturation, and used them as the basis for breeding acid-soil tolerant *L. leucocephala x L. diversifolia* hybrids. Hybrids of *L. leucocephala x L. diversifolia* were considerably more tolerant than *L. leucocephala* cv. Cunningham of acid soil conditions in Malaysia (Table 1).

Table 1. Height of *Leucaena leucocephala* cv. Cunningham and four *L. leucocephala* line $11 \times L$. *diversifolia* lines 25 (diploid) or 31 (tetraploid) after 13 months growth on two acid soils in Malaysia (after Hutton and Chen 1993).

	Mean tree height (cm)		
Line	Kuala Linggi*	Serdang*	
Cunningham	148	163	
11 x 25 F3 62/6	265	193	
11 x 25 F3 62/12	229	226	
11 x 25 F3 39/2	200	218	
11 x 31 F3 30/1	221	242	

* The surface soil (0-20 cm) at Kuala Linggi and Serdang had pH 3.5 and 4.6 in water, and 80% and 60% Al saturation, respectively.

In contrast with the overall perception that *L. leucocephala* grows poorly on acid soils (eg. Hutton 1981; Shelton 1994), Oakes and Foy (1984) found considerable variation among 117 lines of *L. leucocephala* grown in an acid, aluminium-toxic Tatum subsoil (typic Hapludult). The lines on this soil gave relative root yields (root masses at pH 5.3 relative to that at pH 4.1) ranging from 0.34 (highly sensitive) to 2.46 (highly tolerant). Overall, however, Oakes and Foy (1984) found that *L. diversifolia* was most tolerant to acid soil conditions. *Leucaena leucocephala* and *L. pulverulenta* were intermediate in tolerance, and *L. lanceolata* and *L. retusa* most sensitive.

High production of leucaena is not possible in oxisols and ultisols without the selection or breeding of acid-soil tolerant genotypes (Hutton and Chen 1993). However, it must be remembered that selection or breeding for tolerance to one acid soil factor will not necessarily ensure tolerance to another factor. It is necessary, therefore, to determine the factors responsible for poor growth, and select for tolerance to those factors.

Research Priorities

It is our view that the major limitation imposed by acid soil infertility needs to be overcome to enable use to be made of the huge area of acid but infertile soils in the potentially highly-productive humid tropical and subtropical regions. Many of these acid soils could be used for food production with appropriate technology. Incorporating leucaena into farming systems (e.g. through alley cropping) would help improve the sustainability of food production in such regions, especially given that between 1975 and 1990 some 159 million ha of forest and woodland (often on steep erodible land) was cleared in developing countries to meet increased food needs (von Uexktill and Mutert 1993).

Is soil amelioration appropriate? As lime and gypsum are costly to obtain and apply, this option is probably not worthwhile on virgin soils that are highly acid and have a high buffering capacity (overcoming the high levels of phytotoxic aluminium that interfere with calcium, magnesium and phosphorus nutrition requires large quantities of lime). Gypsum may provide some improvement in growth but only in certain situations (McCray and Sumner 1990).

Selection and breeding of leucaena for acid-soil tolerance have already shown benefits (Hutton 1990; Hutton and Chen 1993) and hold great promise for overcoming the limitations to leucaena production on acid soils. Particular attention should be paid to inter-specific crosses, such as *L. leucocephala x L. diversifolia.* Several traits will probably need to be incorporated for genotypes to be successful, with particular attention being paid to tolerance of high aluminium and low calcium and phosphorus. As appropriate agronomic and nutritional traits will also need to be incorporated, this selection and breeding process will not be a trivial exercise.

Of itself, the development of cultivars adapted to acid soils will not solve the production problems of acid soils in the tropics and subtropics. Indeed, the constant use of such cultivars will result in continued acidification, since they are able to exploit the soil for basic cations at low pH where other crops fail. However, the development of leucaena with roots able to penetrate acid subsoils will improve production while at the same time decreasing the leaching of nutrients from the soil profile, thus improving the sustainability of systems in which leucaena is a component.

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Establishment and Early Growth of Leucaena

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Abstract

The paper reviews agronomic practices or factors that control and assist the establishment and early growth of leucaena, particularly *L. leucocephala*. Genotype variation, psyllid resistance and the presence or absence of mycorrhizae in the soil are two factors that cause some seedlings to be more vigorous than others. Other factors can be controlled by growers: these include seed treatments; fertilizing; weed control; planting method; sowing depth, rate and time of planting; plant spacing; and grazing. Priorities for research into seedling establishment are listed.

LIKE many tree species, leucaena (*L. leucocephala* (Lam.) de Wit) is slow to establish in comparison to herbaceous species. In many locations plants are not considered to be fully established for 12 to 18 months or longer. Small plants are vulnerable to weed competition, predation and defoliation during establishment, and cannot be used for a long time. All these aspects are major disincentives to leucaena growers.

Slow establishment of tree legumes can be partly attributed to rooting characteristics. Two-year-old leucaena has been reported to have as little as 0.5 cm/cm^3 in the surface 50 cm of soil (Swasdi-phanich 1993), whereas grasses may have 100-4000 cm/cm³ (Atkinson 1980). Low root densities make it difficult for plants to access nutrients (Bowen 1981) so clearly grasses have a competitive advantage during early growth.

Many researchers have studied the effects of factors such as genotype variation, seed treatment, planting method, nutrition and post-emergence management on leucaena establishment. This paper reviews current knowledge on establishment technology and highlights areas where more information is required.

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Genotype variation

Within the *Leucaena* genus the vigour of young seedlings varies with genotype. In glasshouse and field experiments in Hawaii, shoots of *L. pallida* and its hybrids were from 76% to 135% taller and 105196% heavier than *L. leucocephala* cv. Cunningham. *L. diversifolia* was least vigorous in the first three months of growth (Sorensson et al. 1994). Subsequent work has confirmed the superior seedling vigour of *L. pallida* lines compared to *L. leucocephala* lines, K636 showed greater seedling vigour than Cunningham (Sorensson et al. 1994).

Psyllids

Much of the research on establishing leucaena was done before 1986, the year when the leucaena psyllid became widely distributed around the world. Noone knows how the psyllid affects germination and establishment, or if previous research results are still valid. Palmer et al. (1989) have studied psyllid effects on *L. leucocephala* yield and consider that, because psyllids may do so much damage in the plants' first year, these pests could be a major factor hindering establishment and reducing yield.

Seed Treatment

Scarification

Leucaena's impermeable seed coat gives fresh seed a high level of exogenous dormancy. There is little endogenous dormancy and fresh seed will germinate if the testa is ruptured. In an investigation of safer and simpler methods than acid scarification, Gray (1962) compared various hot water treatments. Immersion in water at 80°C for two minutes, followed by rapid drying, consistently gave long shelflife and full germination.

However, hot water scarification of large seed lots is difficult and impractical in many situations. Mott et al. (1982) reported that exposing dry leucaena seed briefly to very high temperature (140°C for 30-60 seconds) raised germination from 47% to 67% in one sample tested and from 8% to 60% in another sample. This technique could be provided commercially in large heat drums. However, precise control of temperature and time is needed to prevent the seed being destroyed. For developing countries, use of hot water and sun-drying seems more appropriate.

Rhizobium inoculation

Effective nodulation is essential for vigorous growth in leucaena and much research has examined the effectiveness of different strains of rhizobium. Diatloff (1973) found height, survival and greenness of plants were greatly increased by inoculation with strain CB81, even at 15 months after planting where appropriate rhizobia were absent from the soil. Strain NGR8 was less reliable, especially on acid soils. On acid soils in Australia, Norris (1973) found alkali-producing CB81 was effective with or without lime, whereas the acid-producing strain NGR8 formed nodules only when seed was limepelleted. He recommended use of CB81 types and lime pelleting in all plantings on acid soils. Different strains may be most effective in acid or alkaline soils.

Trinick (1968) reported that *L. leucocephala* is quite specific in its rhizobium requirement and nodulates effectively only with a fast-growing rhizobium type which also cross-infects *Acacia farnesiana*, *Mimosa* and *Sesbania*. However, some rhizobium strains that nodulate these alternate hosts do not nodulate leucaena.

A strain known as CB3060, or TAL1145 in the NiFTAL collection, has been selected at the CSIRO Cunningham Laboratory, Townsville, from *L. diversifolia* in semi-arid northern Australia. This strain is able to nodulate and fix nitrogen in quite acidic soils (pH 4 to 5) as well as being effective in more neutral soils, and is now the recommended commercial inoculant strain. Strains that appear to be even more effective are currently being field tested (R.A. Date pers. comm.).

In recent trials at six sites in northeast Thailand, the inoculant strain TAL1145 formed most of the nodules on *L. leucocephala* over the first 72 weeks

(Homchan et al. 1989). The inoculated plants were 66-280% larger than uninoculated plants, depending on site, with the uninoculated plants having few nodules at 20 weeks. After 72 weeks, the effects of inoculation on height and production diminished, but it was considered that improved early growth would have contributed to better establishment. Similar effects have been noted in Malaysia (Chee et al. 1989).

In Australia, a similar trend was found in a threeyear study by van Bushby. The inoculum strain progressively formed a smaller proportion of the nodules on leucaena growing in acid podzolic soils (pH 5.3). This dilution of introduced strains by indigenous rhizobia in the soil, which has also been reported with clover inoculum strains, may be the outcome of genetic exchange. This would result in nodules on older plants containing a mix of genetic material from introduced and indigenous rhizobia. Inoculation may therefore contribute to the genetic pool of indigenous soil rhizobia as well as assisting early establishment.

On alkaline soils in Indonesia, Piggin et al. (1987) demonstrated effective inoculation could be achieved practically in remote situations by mixing seed with soil from an area where leucaena had been long established, at a rate of 100 g per 6 g of seed (100 seeds) or per metre of furrow. *L. leucocephala* plants, sown at various times before and after the wet season rains, survived better in inoculated treatments (19% vs 9% survival) and were much taller (48 cm vs 23 cm) and greener than uninoculated treatments at the end of the first dry season, about a year after planting. This inoculation effect may have been due to both rhizobia and mycorrhizae, and could have been partly due to added nutrients, as about 1 t/ha of soil was used as the inoculum.

Where soils are acidic, local soil should not be used in potting mixtures when transplanting seedlings of *L. leucocephala.* Transferring seedlings in cores of a medium which favours nodulation can enhance establishment.

The rhizobium requirements of the lesser-known *Leucaena* species have not yet been studied.

Seedbed Conditions and Treatments

Mycorrhizae

Leucaena leucocephala is known to form mycorrhizal associations which are important for seedling and plant growth. Young seedlings are very dependent on rapid early mycorrhizal infection of their roots to supply adequate phosphorus. Ruaysoongnern (1989) found that leucaena grew poorly in a sterilised soil without vesicular arbuscular mycorrhizae (VAM), even if 50 kg/ha phosphorus was applied. Growth was only 5% of the growth made by plants in a non-sterilised soil where they formed mycorrhizal roots. Phosphorus concentration in young leaves was 0.31% in 'plus VAM' plants compared to 0.07% in 'minus VAM' plants, and nodules weighed 297 mg/plant (plus VAM) or 0 (minus VAM).

Brandon and Shelton (1993) reported that leucaena plants growing in a soil with naturally low VAM activity suffered a period of phosphorus deficiency until VAM infection increased to effective levels. This was not observed in soils with high VAM levels. The phosphorus deficiency observed in low VAM soils could be corrected by fertilising at very high levels (1200 kg/ha), but was exacerbated by competition from grass weeds.

Levels of VAM in soils depend on such factors as cropping and pasture history, cultivation, natural vegetation, acidity and waterlogging. Long term cultivation may reduce the level of VAM infections of leucaena in some soils. There is no practical method for inoculating leucaena with mycorrhizae. Similarly, nothing is known about the mycorrhizal dependence and requirement of the less-known *Leucaena* species. These are two areas needing further research.

Fertilizer

Leucaena generally responds well to fertilizer applied during establishment and early growth. Hill (1970) reported that nitrogen added at 30-60 kg/ha increased yield significantly during the first three months of growth in Papua New Guinea. However, it had no effect on nodulation, and responses varied between weeded and unweeded situations. Egara and Jones (1977) failed to obtain a yield response to nitrogen in a two-month pot study, but attributed this to the high nitrogen status of the soil. The seedlings were not nodulated at 66 days, so the plants were probably using external nitrogen.

Phosphorus fertilizer at up to 40 kg/ha had little effect on the emergence, survival or production of *L. leucocephala* in its first year on alkaline limestone or clay soils in Timor (Piggin et al. 1987).

Heavy applications of lime increase growth on acid soils. In Papua New Guinea, leucaena grown without lime yielded 8000 kg/ha in the first year, but plants given 10 or 20 t/ha lime, broadcast, yielded twice or three times as much. Smaller applications of up to 1 t/ha, drilled, gave no significant yield responses (Hill 1971).

On acid soils, aluminium and manganese toxicity can seriously affect nodulation and nitrogen fixation. Ruaysoongnern et al. (1989) showed that readily available phosphorus and calcium promoted nodulation and rapid seedling growth. They established critical nutrient concentrations in seedlings for nitrogen, phosphorus, potassium, calcium, sulphur and manganese.

Weed control

Leucaena seedlings are slow growing and may take 12 to 18 months to attain a height of 1.5-2 m. Weed competition severely restricts seedling growth, although seedlings are rarely killed by competition alone. Hill (1970) reported in Papua New Guinea that fortnightly weeding increased growth in the first three months by 30 to 100%, depending on nitrogen fertilizer treatment.

In trials in Timor, weeding had no effect on the emergence of leucaena but seedling survival and growth in the first 14 months were greatly increased after weeding. In ungrazed conditions, weeding increased plant populations from 29 to 43 seedlings/m and plant height from 70 to 135 cm. Grazing greatly reduced seedling survival and growth and masked any effects of weed control (Piggin et al. 1987). This work shows the importance of weeding regularly during early growth to achieve maximum survival and growth of seedlings. In most developing countries, cropped areas are weeded and fenced, whereas follow-up weeding and fencing are neglected in areas planted to trees. As it is common for tree-planting efforts to fail, leucaena may be better planted as a crop.

Weeds are unlikely to suppress leucaena by shading, according to the work of Egara and Jones (1977). Leucaena tolerates shade well, responding by increasing leaf area and maintaining shoot growth, even with only 35% of full illumination. However, total yield is somewhat suppressed. Leucaena seedlings commonly establish beneath parent plants to form thickets.

Many researchers have examined chemical control of weeds for leucaena. Brewbaker et al. (1985) reported that pre-emergence weeds could be controlled with trifluralin (0.5 kg a.i./ha) and alachlor (3 kg/ha) when incorporated, or with 2,4-D amine (6 kg/ha), dacthal (8-10 kg/ha) and oryzalin (3 kg/ha) when surface sprayed. The post-emergence herbicides fluazifop (2 kg/ha) and bentazone (2 kg/ha) were effective against grass and broadleaf weeds respectively, without being excessively toxic to leucaena (Shelton 1994). Jones et al. (1982) recommended hoeing until plants are 1 m tall, or applying dacthal, paraquat or 2,4-D amine preemergence to control weeds.

However, results with herbicides vary with factors such as weed flora, climate and soil type. Cooksley (1974) showed that, in the field, trifluralin, benfluralin and chlorthal had little effect on weed control or establishment and production of leucaena. Cooksley (1983) subsequently compared the effects of seven pre-emergence herbicides or hand-weeding on weed control and leucaena growth. None of the herbicides tested was consistently as effective as hand-weeding. Pratchett and Triglone (1990) reported that trifluralin and basagran had no effect on leucaena survival or yield at 225 days after sowing under irrigated conditions in north-west Australia, which suggests that weeds have little effect where moisture is not limiting. However, practical experience from central Queenland has clearly demonstrated the importance of good chemical weed control during establishment over extensive areas.

Jones and Aliyu (1976) found *Eleusine indica* reduced leucaena seedling production to 16-25% of the weed-free control in an experiment growing from 4 to 32 grass seeds and four leucaena seeds in pots. Trifluralin, dacthal and 2,4-D all diminished leucaena growth to some degree, although only trifluralin effectively controlled the weed. Activated charcoal alleviated the effects of herbicides on the leucaena. Jones and Aliyu (1976) recommended the use of herbicides and activated charcoal to facilitate the field establishment of leucaena.

Cooksley (1974) improved leucaena growth without affecting seedling numbers by burning windrows of logs before sowing, and by postemergence cultivation along planting rows. Effects were attributed to sterilisation of weed seeds, release of nutrients by burning and weed control by cultivation (Cooksley 1974). Falvey (1981) found leucaena's establishment and early growth were generally better after pre-sowing cultivation, although effects depended on soil type and weed flora.

Inadequate attention to weed control remains one of the major obstacles to more successful establishment of leucaena in central Queensland. Adequate technical knowledge is available, so the problem must be overcome by improved communication and extension programs.

Sowing method

Leucaena can be sown into a fully prepared seedbed or into cultivated or sprayed strips in existing native or improved pasture (Jones et al. 1982). It can also be sown beneath food crops such as maize and sorghum, where row spacing is wide enough to reduce early competition; this method is particularly appropriate in much of the developing world because weeds are eliminated several times in the crop by hand-weeding.

Piggin et al. (1987) investigated various sowing methods appropriate to developing countries on several sites and soils in semi-arid Timor. Seed was sown in the bottom of shallow furrows, on the soil

ridge formed below excavated furrows, or in shallow dibble stick holes. Emergence and survival of seedlings were measured for 12 months. There was reasonable emergence (50-60%) and survival (50-70%) from most methods although only 38% of seedlings emerged from the dibble stick holes on clay, and survival was relatively low for furrows (24%) and ridges (35%) on the limestone soil. Plant growth was usually better from the ridge sowings. Ridge sowings took 2.2 min/m whereas furrow and dibble stick sowings took 1 min/m. The authors suggested that ridge sowing would be appropriate where labour is abundant and rapid growth is desired. Furrow or dibble stick sowings would be appropriate where labour is a constraint. Dibble stick plantings would be particularly useful on steeper areas prone to erosion because of minimal soil disturbance.

Sowing with a precision drill on a fully prepared seedbed is recommended for establishment over extensive areas in developed countries like Australia, where low-labour, mechanised farming is practiced.

Sowing rate

Sowing rate depends on the desired plant population density in the field, combined with such factors as seed weight, seed viability, row spacing and seedling survival.

According to Jones et al. (1982), sowing rates under rainfed conditions could range from 0.5 to 5 kg/ha, depending on row spacing and seedbed conditions. This is equivalent to 4-40 seeds/m of row (for rows 4 m apart), as there are about 20 000 seeds/kg. Under irrigation, Pratchett and Triglone (1989) recommended sowing 10-13 kg seed/ha when sown in 3-4 m rows.

Piggin et al. (1987) in Timor, at several sites and soil types, compared sowing rates of 10, 50 and 100 kg/ha fertilised at 0 or 40 kg phosphorus/ha. They found that phosphorus fertilizer had no effect on either percentage emergence or on seedling survival at 12 months; that emergence fell from 75% at a sowing rate of 10 kg/ha to 25-50% as sowing rate increased to 100 kg/ha; and that survival was about 25% on limestone soil and 70% on clay soil, at all sowing rates.

Piggin et al. (1987) concluded that for rows spaced 1 m apart with 10 cm between plants (10 plants/m) sowing rates would need to be about 25-50 kg/ha on limestone soils and 10 kg/ha on clay. With a spacing of 20 cm between plants (5 plants/m), sowing rates could be reduced to 10 kg/ha on limestone and 5 kg/ha on clay. While these results cannot be transferred directly to other environments, they do suggest that some detailed information on plant population dynamics is required if sowing rates in any situation are to be predicted accurately. In general, however, as row spacing is increased, sowing rate is reduced proportionally.

Sowing depth

Jones et al. (1982) recommended sowing at a depth of 2.5 cm with a press-wheel planter, and at depths up to 6 cm with a combine planter.

Piggin et al. (1987) compared sowing depths for *L. leucocephala* in alkaline, sedimentary soils in Timor. They found best emergence (80% of viable seed) from seed sown at 5 cm, with reduced emergence (20-25%) from seed sown at 0 cm or 10 cm, and very poor emergence (0.8%) from seed sown at 15 cm. Seedlings from all depths survived equally well (about 53%).

Row or plant spacing

Jones et al. (1982) recommended row spacings of 1.5-5 m, with wider spacing where operations are mechanised and the area is grazed, or narrower spacings where hand operations and cutting are practiced. In developing countries leucaena is commonly used from narrow plantings or dense natural forests.

In Australia, more research is needed to define appropriate row spacings and plant densities for different situations. In low rainfall areas it is argued that wide spacing (greater than 10 m apart) allows trees to fully exploit limited moisture. However, narrow spacings increase the proportion and total yield of edible forage available for ruminants.

Time of sowing

The optimum time of sowing depends on locality. Jones et al. (1982) recommended sowing into moist soil after the first rains of the growing season in Queensland. Then weeds would be killed by cultivation and leucaena would emerge with adequate moisture and minimum weed competition. However, early growth is closely related to minimum air temperature, so time of sowing can be chosen more precisely. For example, Cooksley (1986) suggested planting when daily mean minimum temperatures are likely to be above 15°C.

In semi-arid Timor where the wet season is from December to March, Piggin et al. (1987) found that *L. leucocephala* could establish quite successfully from furrow sowings in August, October, December or February. This means that sown seed can survive in the field for at least four months before the wet season and still germinate satisfactorily. It is of practical significance, at least in the semi-arid tropics, that leucaena planting programs can be completed well before the wet season, because available labour must be diverted to cropping operations once the wet season arrives.

Transplanting

Leucaena establishes and grows rapidly when planted as seedlings, but the operation costs more and labour must be available. The seedlings can be raised in pots or polythene bags (polybags) or in shallow beds. They are transplanted when taller than 15-30 cm. Seedlings from densely sown beds can be transplanted as 'bare stems' (without soil) in moist conditions, to minimise costs. In Timor, seedlings transplanted from polybags established satisfactorily when planted early (December) or late (February), but they survived and grew better from the late planting (Piggin et al. 1987).

Piggin et al. (1987) compared establishment of *L. leucocephala* from various seed and vegetative methods in Timor. Seedlings from polybags survived best (83%) in the first 12 months, and had grown best (166 cm tall) by 7 months. Bare stem transplants did not survive so well (48%) or grow as tall (68 cm) and were generally no better than furrow-sown seed, of which 20-25% emerged and 50-60% survived, producing seedlings 50-90 cm tall.

There are conflicting reports on propagation from stem cuttings. Piggin et al. (1987) and Litzow and Shelton (1992) were unable to establish stem cuttings in the field, but Duguma (1988) and Bristow (1983) reported successful establishment from cuttings. These differences may be due to planting material and moisture availability but are probably due to inhibition of root initiation. Success is more likely where there is abundant moisture.

Stem grafting has been used successfully to propagate plants. This is an important topic for further research, and is particularly relevant to farmers who want to propagate elite hybrids and seedless triploids.

Defoliation and grazing

Seedlings are very palatable to domestic and wild animals, and establishment is usually restricted unless grazing is controlled. Although not usually a problem in developed countries, this aspect may present severe difficulties in many developing countries where livestock are often unrestrained. In a trial in Timor to investigate the effects of grazing, sowing rate, phosphorus fertilizer and weeding, Piggin et al. (1987) reported that grazing reduced plant numbers and height at 9-14 months after planting. The grazing pressure was severe, but at normal Timorese levels. Grazing obliterated the effects of sowing rate and weeding that had been evident in ungrazed plots. At 17 months after sowing, in the second dry season, all the grazed plants were dead. These results illustrate the importance of reducing or excluding grazing during the establishment phase of leucaena and of planting in areas where livestock can be controlled. Once the plants are 1–1.5 m tall, light grazing can stimulate branching. Regular grazing is possible usually in the second or third year.

Recommendations and Conclusions

For successful establishment leucaena should germinate rapidly and evenly and make its early growth quickly in weed-free conditions. This paper has reviewed factors and practices which promote that situation. However, most information on establishment and early growth concerns only *L. leucocephala* and was collected before the leucaena psyllid became widespread. Some practices will need to be re-evaluated in the presence of psyllids, and all practices need to be tested on other *Leucaena* species identified as having good potential productivity, nutritive value, psyllid resistance, and acid and cold tolerance. Research and development needs for improved leucaena establishment are listed in Table 1.

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Issue	Priority	Research need L. leucocephala	Priority	Research need Other <i>Leucaena</i> spp.		
Genotype variation Psyllids	medium medium	accessions with high vigour accessions with high tolerance	high high	species with high vigour species with high tolerance		
SEED						
Scarification	low	extend known methods	medium	check application of known methods		
Rhizobium	low	quick nodulation	high	define needs and effects		
SEEDBED						
Mycorrhiza	medium	investigate needs; develop practical inoculation systems	medium	define needs and effects		
Fertilizers	low	define nutrition needs	medium	check and define nutrition needs		
Weed control	low	extend known methods	medium	check known methods apply		
Sowing method	low	extend known methods	low	check known methods apply		
Sowing rate	low	define local interactions and psyllid effects	low	check and define local interactions		
Sowing depth	low	extend known methods	low	check known methods apply		
Row & plant spacing	medium	effects on production, water use, etc.	medium	effects on production & management		
Time of sowing	medium	define local needs & interactions & psyllid effects	medium	check & define local needs & interactions		
Transplanting	medium	require vegetative propagation methods	medium	check ways to propagate hybrids		
Grazing or defoliation	low	extend known methods	medium	check known methods		

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Novel Methods for the Vegetative Propagation of Leucaena (Leucaena leucocephala) under Laboratory Conditions

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Abstract

Stems, approximately 3 mm diameter, of young, potted leucaena (cv. K8) seedlings 2-4 months old, were air-layered indoors onto small, moist foam-rubber cubes of about 27 cm³, enclosed within clear polythene sheets or sleeves. Root induction was rapid and roots appeared through the foam rubber about 18 days after initial layering during summer (outdoor temperature around 28°C). Young roots were allowed to harden before layers were cut off 10 days later (i.e. at 28 days). Vegetatively propagated plants were ready for transplanting indoors within a month. The newly-potted plants were further hardened for about 10 days before planting outdoors. A modified version of this technique (the hydro-air-layer) used plain water instead of the solid medium (foam rubber) inside the polythene: rooted plants were ready within the same time using this method, too. The numerous advantages of these methods include ease of use and the possibility for rapid clonal multiplication using simple, inexpensive and unsophisticated techniques.

THE need for a simple and successful vegetative propagation technique for leucaena is widely acknowledged, as much of the promising new interspecific hybrids being developed are seedless, or virtually so (Brewbaker and Sorensson 1990).

Asexual propagation assumes particular significance for elite or 'super' trees of natural or human-made hybrid origin. They may show superior growth vigour, or psyllid resistance, or cold hardiness, but either do not breed true to type, or set few seeds (partially sterile clones) or no seed at all (sterile or seedless clones). For example, individuals displaying special features occur naturally and commonly in *L. diversifolia* K156 plantations. Humanmade interspecific hybrids are now possible (Sorensson 1993) and are being released increasingly for commercial purposes. Potentially valuable characteristics, occurring naturally in leucaena plants in farmers' fields in remote areas, could be lost forever if not clonally propagated and saved.

Success in saving and perpetuating such plants may depend upon the development of a simple,

cheap, rapid, reliable and accessible method of vegetative propagation which does not involve sophisticated technology as in tissue culture. Mastery of a simple field technique for cloning is essential before there can be seedless plantations (Brewbaker 1988). Superior genotypes cannot be kept genetically pure unless breeders use vegetative propagation (Litzow and Shelton 1991) and the commercial production of hybrid seed may be possible only if one of the parent genotypes is cloned (Litzow and Shelton 1991).

Numerous attempts at vegetative propagation of leucaena have been reported in the literature. These include:

- micro propagation by tissue culture (Venketeswaran and Ghandi 1979, Peasley and Collins 1980, Glovak and Greatbatch 1982, Nagmani and Venketeswaran 1983, Ravishankar et al. 1983, Datta and Datta 1984, Dhawan and Bhojwani 1985, Goyal et al. 1985, Toruan-Mathius 1992);
- propagation by stem cuttings (Hu and Chih-Cheng 1981, Bristow 1983, Duguma 1988, Litzow and Shelton 1991);

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- propagation by grafting (Versace 1982, Brewbaker 1988, Singh 1989); and
- tissue layering (Ghatnekar et al. 1982).

However, vegetative propagation remains routinely unsuccessful (Brewbaker 1988). The asexual propagation of leucaena is generally held to be difficult (Litzow and Shelton 1991, Toruan-Mathius 1992).

This paper presents some novel methods for vegetatively propagating *Leucaena leucocephala* under laboratory conditions, developed at the University of Mauritius by the author in early 1987. The methods overcome some of the difficulties normally associated with this mode of propagation. The propagation of leucaena seedlings on foam rubber ('hydro foam' or 'hydroponic' seedlings) has been reported previously (Osman 1986). Here I describe the vegetative propagation of leucaena by an air-layering technique using foam-rubber (foamair-layer) as the rooting medium, and by a modified technique (hydro-air-layer or hydro-layer) without any solid medium (no foam-rubber). The cultivar used was the Giant K8.

Materials and Methods

Foam-air-layer

The method consists of ring-barking about 5 mm of the stems (about 3 mm in diameter) of potted, 2-4 month old leucaena seedlings, 20-25 cm from the tip. The ring-barked area is then inserted into a small piece of thoroughly moistened, roughly cubical foam rubber (about 27 cm³) slit centrally on one side about half way into the block. The foam is tied loosely into position, then wrapped neatly with a small piece (about 12 cm x 20 cm) of clear polythene (plastic) sheet that is attached securely to the stem above and below the block.

The basic arrangement may be modified; for example

- (i) the foam block may be prestapled, stitched or glued (with waterproof glue) into the middle of the polythene sheet to avoid having to tie the foam after the shoot has been inserted
- (ii) clear polythene sleeves (roughly 8 cm in diameter and 12 cm long) may be used instead of the sheet. They are slid on from the tip of the stem, with some care, though this may be a bit tricky with small diameter sleeves. Sleeves are useful when there is no foam or other solid medium to hold the water normally held by the foam (see 'hydro-air-layer'/'hydro-layer' below)
- (iii) it may be easier to tie the polythene with plastic covered wire or binding wire instead of string

(iv) as the polythene chamber must be kept moist inside, various precautions may be taken to minimise water loss. For example, the bottom of the polythene may be tied securely at two points close to each other below the foam, to prevent leakage by gravity. Alternatively, the top of the sheet or sleeve may be first tied around the stem just below the foam. Then the lower end of the polythene is pulled up over the foam so it becomes the top end, and tied in place around the top of the stem. The polythene now at the lower end is allowed to sag a little so that water can collect in the depression without leaking.

In our investigation the stems were semi-woody. No rooting aid or hormone was used. Root induction was rapid at the prevailing outdoor summer temperature (28-30°C, a few degrees lower indoors), with roots appearing through the foam from about 18 days. The roots then developed quickly and profusely. The layers were severed a week or so later to allow the roots to harden before transplanting, but when the layers were cut the leaves and tips wilted. The new plants became established in indoor pots easily, rapidly, and without problems. Before planting outdoors the newlypotted plants were allowed another 10 days to harden. Depending on progress, even these new plants became layerable a few weeks after potting, while the original plants from which the layers were produced also became layerable when sufficiently developed.

Seedlings kept indoors commonly have long stems which may be multi air-layered simultaneously at convenient intervals along the stem. In our investigation, plants were layered at only two points at a time, though more than two layers at a time appear possible. Where two layers were made, roots appeared in the top layer first, probably due to apical dominance. The lower layer was cut about a week after the top one. Plants can be quickly multiplied by such a system. In plants with two layers, some pinnules of the lower leaves turned yellow, and then fell, in the early stages.

Hydro-air-layer or hydro-layer

In a modified version of the basic technique, I eliminated the foam altogether and used no solid medium, only plain tap water, inside the polythene chamber. Roots formed freely after a few days and layers were ready in about the same time as layers raised on foam. This modified technique allows root growth and development to be observed through the transparent polythene — useful in root development studies.

Air-layering without ringbarking

It is of interest to record that one intact (i.e not ringbarked) bare-rooted seedling, with its roots completely submerged in plain water in a culture jar, was induced to root where the stem was held in position by foam placed at the mouth of the container, about 10 cm above the plant base. The foam was kept wet by regular watering or by simply tilting the jar or raising the level of water inside. A wick could be used instead, with one end squeezed between the jar wall and the foam and the other dipping in the water. New roots appeared through the foam in 15 days. This observation suggests young leucaena stems root readily.

Layering in the field

In the field, young shoots or regrowth of established plants could not be layered successfully by the methods described above. This was probably due to the much higher temperatures inside the polythene and the stronger light outside. Layers failed to produce roots even when shaded with newspaper. The investigation was not pursued further. However, a young but much larger stem (coppice) on a well established *Calliandra calothyrsus* plant produced vigorous roots readily and profusely when hydrolayered.

Discussion

Although I used a small number of plants, all foamand hydro-layered plants rooted readily, so the rate of success was high. However, the rate of success has to be tested under large-scale operation. The field production of layers also needs be investigated as that would allow many more plants to be produced from the numerous young shoots and regrowths (coppices) formed on established trees. In field propagation, multibranched trees can be coppiced at a comfortable working height and induced to produce a large number of young shoots continuously by periodic cuts combined with good management (watering and fertilizing).

Several shortcuts may save time in the layering operations and shorten the interval when the layers are forming. For example:

- (i) instead of ringbarking, it may be enough to simply scrape the bark with a penknife or file. However, this requires testing. In a well tuned system it should be possible to complete each layering in less than a minute.
- (ii) under favourable conditions, it may be possible to sever layers earlier, eliminate the root hardening stage and harden potted plants for about one week. Thus a layer could be

produced in about two to three weeks, and a potted plant for field planting in about three to four weeks.

(iii) miniaturising the process by using shorter lengths of stem and smaller foam pieces may allow roots to come through more quickly.

The methods described here have several advantages. They are simple, low cost, rapid, accessible and have given a high rate of success. Both types of layers are very light and easily transportable even over long distances. As well as providing several other advantages already reported for 'hydro foam' seedlings (Osman 1986), the foam protects the roots and reduces transplanting shock. Foam costs little but can be dispensed with where unavailable. However, on-foam layers do not have to be potted in soil for hardening before setting out in the field. During hardening, such layers can just be placed in a tray of water as for 'hydro foam' seedlings (Osman 1986).

The techniques described in this paper for *L*. *leucocephala* now need to be tested on the numerous other leucaena species and their crosses.

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A Review of Wood Quality in Leucaena

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Abstract

Leucaena wood quality is reviewed in relation to different end-uses. The majority of published data refers to industrial uses and to one species, *L. leucocephala*. Little is known for other species. The limited data available, together with local knowledge from Mexico and Central America, indicate that considerable variation in wood quality exists across the genus. Several lesser-known species are superior to *L. leucocephala* in terms of wood density and durability, making them preferable for fuelwood, posts and construction. High wood quality is apparently negatively correlated with leaf production. The need for more detailed assessment of leucaena wood quality across species, sites and silvicultural regimes is emphasised.

IN spite of the relatively recent international interest in leucaena, for centuries the indigenous people within its native range have valued its wood for its quality and range of products. Without exception, in Mexico and Central America leucaena species are widely preferred, used, protected, managed and even cultivated for wood production. Leucaena leucocephala is generally described as being strong, light-weight, easy to work, and able to give an attractive finish (National Research Council, 1984; Rao, 1984; Van Den Beldt and Brewbaker, 1985). These qualities make Leucaena wood suited to a wide range of uses, ranging from the traditional small-scale use by farmers and smallholders to the more recent utilisation by large scale industries for pulp and for energy generation. Thus leucaena is one of the few trees from which wood is used for both industrial and non-industrial purposes. Each end-use requires specific properties (Table 1). The combination of a range of desirable qualities in Leucaena wood has undoubtedly contributed to its widespread popularity.

Although wood quality is thus clearly of fundamental importance to the utilisation of Leucaena, there is little published information on this aspect compared to the very extensive literature assessing yield or fodder quality. Moreover, most Table 1. Major end-uses of leucaena wood and the properties provided for each.

End-use of Leucaena wood	Properties provided by Leucaena
Fuelwood (domestic)	Low smoke production
	Low spark production No unpleasant smell
	Ease of drying and splitting
	Good heat production
Fuelwood (industrial)	Good heat production
House construction	Good compressive and tensile
	strength
	Medium sized straight poles
	Good durability and/or ability
	to accept preservatives
Fence posts	Good durability and/or ability
	to accept preservatives Relatively straight stems
Pulp	High holocellulose content
ıup	Low extractive content
	Bark easily removed
Charcoal	Good recovery value
	High heating value
Crafts	Easy to work
	Attractive finish
	Density of medium or above
Parquet flooring	Machines well Attractive finish
Furniture	Good tensile strength
i unntuit	Attractive finish
	Easy to work
	•

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evaluation of wood quality has concentrated on industrial uses in Southeast Asia, with little published on small-scale rural use, even though this is still the most important aspect of Leucaena wood utilisation. As most studies have emphasised the use of Leucaena wood when grown as an exotic species, the information relates almost exclusively to just one species, *L. leucocephala*, with virtually nothing available on use or characteristics of the wood of other *Leucaena* species. That leucaena growers and breeders have attached little importance to wood quality is well illustrated by the fact that few, if any, planting or breeding programs dealing with the genus have been guided by wood quality studies.

We believe, therefore, that the evaluation of wood quality must be incorporated into leucaena improvement programs, to guide researchers towards understanding how to improve not just the yield but also the quality of wood produced. **To** do this, researchers must assess the characteristics that determine wood quality, and understand the patterns of variation and inheritance both within and between leucaena species.

Review of Wood Quality in Leucaena Fuelwood

The value of wood as a fuel is determined principally by its specific gravity (SG), with Panshin and de Zeeuw (1970) recording little variation among tree species in the heat produced by a unit weight of oven-dry wood. Van Den Beldt and Brewbaker (1985) reported L. leucocephala as being of medium density. MacDicken and Brewbaker (1982) measured an SG of between 0.45 and 0.55, a value that compares favourably with other commonly grown fuelwood species such as Gliricidia sepium (SG = 0.5-0.6; Withington et al. 1987), Albizia spp. (0.45-0.59; Chundnoff 1984), Calliandra calothyrsus (0.51-0.78; National Academy of Sciences 1980) and Prosopis juliflora, considered one of the best fuelwood species (0.7; National Academy of Sciences 1980).

The characteristics of a good domestic fuelwood, however, must include not only a high value of SG, but also ease of splitting and drying, and good burning qualities. Wood of *L. leucocephala* meets these requirements as it is thornless, generally easy to cut and dry, burns with a steady flame, and produces little smoke, few sparks and only a small amount of ash (Pound and Martinez 1983; Brewbaker 1987; Van Den Beldt and Brewbaker 1985). Furthermore, Leucaena's rapid growth and ability to coppice have increased its suitability as a fuel. Within their native ranges, all *Leucaena* species are valued greatly for their ability to produce good or excellent quality fuelwood (the exception is *L. esculenta*, which is not used for wood only because trees are protected for pod production). Yet little published information is available on the quality of this fuelwood. The only data on the comparative performance, in terms of wood yield and quality, of the complete range of *Leucaena* species come from an evaluation trial established by the ODA/COHDEFOR Forest Conservation and Tree Improvement Project CONSEFORH, on a seasonally dry tropical site in central Honduras.

In this trial, specific gravities were determined at age two for each species (Table 2). The highest values were recorded for L. collinsii ssp. zacapana (0.76 and 0.71), L. retusa (0.73), L. greggii (0.70) and L. shannonii ssp. shannonii (0.69), and for some lesser-known species (Stewart et al. 1991). The generally high values found in this study may reflect the harsh site conditions with a dry season lasting five to six months. In some cases, the recognition of the high quality of fuelwood of these species has led to the protection and management of natural populations specifically for fuelwood production by pruning or coppicing (Hughes 1993). For example, natural regeneration of L. collinsii ssp. zacapana, in more or less pure-species secondary bush fallows in the Motagua Valley in Guatemala, is managed on a three to five year coppice rotation specifically for firewood. Notably, L. leucocephala and hybrids bred primarily for leaf production produced relatively low SG values, ranging from 0.61 for KX1 to 0.50 for KX3+. This finding may explain why, despite the widespread occurrence of L. leucocephala throughout much of Central America and Mexico, it is often other species of leucaena that are preferred as fuelwood. Clearly the species of greatest interest for wood production are likely to be different from those preferred for leaf production.

Wood from thinning during the trial was offered to local families. These people were then asked for their views on various wood-burning characteristics. Although a relatively small-scale study, the finding that all species produced good fuelwood emphasises the need for a comprehensive evaluation of fuelwood quality for a range of leucaena species.

Leucaena makes excellent charcoal with recovery values of between 25-30% (Brewbaker 1987), and a heating value of some 7 000 kcal/kg, about 70% of the heating value of fuel oil (National Research Council 1980).

There has also been recent interest in using leucaena to produce industrial energy. With a

calorific value of between 4 200 and 4 670 kcal/kg (Bawagan and Semana 1978; Brewbaker 1987; MacDicken and Brewbaker 1982; Pound and Martinez 1983), leucaena is comparable to other fast-growing non-resinous hardwoods. Proposals for potential end-users of energy provided by leucaena include sawmills, electric generators, rail-road locomotives, food driers and power stations (Bawagan and Semana 1978; National Research Council 1980). Only in the Philippines, however, have extensive dendrothermal energy schemes based on leucaena been developed, and with very mixed success (Brewbaker 1987).

Construction

Although it has acceptable strength characteristics, and does accept water-based preservatives, *L. leuco-cephala* has little value for heavy construction because of its low durability and its susceptibility to termite attack (Bawagan 1983). However, several other species of *Leucaena* are highly prized within their native ranges for building purposes. For example, *L. salvadorensis* is greatly valued for its excellent strength and high durability, which make it particularly valuable as corner posts in houses (Hellin and Hughes in press). Other species, notably

Table 2. Specific gravity measurements taken from a two year old species and provenance evaluation trial of leucaena, CONSEFORH, Honduras (from Stewart et al. 1991).

	Mean specific gravity
L. collinsii ssp. collinsii (Narcisco Mendoza, Chiapas, Mexico)	0.64
L. collinsii ssp. collinsii (Chacaj, Huehuetenango, Guatemala)	0.58
L. collinsii ssp. zacapana (Puerto de Golpe, Progreso, Guatemala)	0.71
L. collinsii ssp. zacapana (Gualan, Zacapa, Guatemala)	0.76
L. collinsii ssp. zacapana (El Carrizal, Chiquimula, Guatemala)	0.71
L. diversifolia ssp. stenocarpa (Zambrano, Francisco Morazan, Guatemala)	0.57
L. diversifolia ssp. diversifolia (Coral Falso, Veracruz, Mexico)	0.54
L. diversifolia ssp. diversifolia (Xalapa, Veracruz, Mexico)	0.49
L. diversifolia ssp. diverstfolia (K156) (Hawaii, USA)	0.53
L. diversifolia ssp. diversifolia x L. pallida (KX1) Hawaii, USA)	0.61
L. esculenta ssp. esculenta (Pachivia, Guerrero, Mexico)	0.60
L. esculenta ssp. esculenta (Tiringucha, Michoacan, Mexico)	0.59
L. esculenta ssp. matudae (Mezcala, Guerrero, Mexico)	0.62
L. esculenta ssp. paniculata (Chapulco, Puebla, Mexico)	0.64
L. greggii (El Barrial, Nuevo Leon, Mexico)	0.70
L. lanceolata ssp. lanceolata (San Jon, Oaxaca, Mexico)	0.64
L. lanceolata ssp. lanceolata (Escuinapa, Sinaloa, Mexico)	0.58
L. lanceolata ssp. sousae (Cacalote, Oaxaca, Mexico)	0.60
L. lanceolata ssp. sousae (Puerto Angel, Oaxaca, Mexico)	0.58
L. leucocephala (K8) (Zacatecas)	0.57
L. leucocephala (K636) (Saltillo, Coahuila)	0.59
L. leucocephala x L. pallida (KX2 88-1) (Hawaii, USA)	0.55
L. leucocephala K8 x L. diversifolia ssp. diversifolia (KX3) (Hawaii, USA)	0.54
L. leucocephala K636 x L. diversifolia ssp. diversifolia (KX3+) (Hawaii, USA)	0.49
L. macrophylla ssp. macrophylla (Vallecitos, Guerrero, Mexico)	0.60
L. macrophylla ssp. nelsonii (San Isidro, Oaxaca, Mexico)	0.56
L. multicapitula (Los Santos, Azuero, Panama)	0.50
L. pulverulenta (Altas Cumbres, Tamaulipas, Mexico)	0.59
L. pulverulenta (South Texas, Texas, USA)	0.61
L. retusa (Sierra La Encantada, Coahuila, Honduras)	0.73
L. salvadorensis (La Garita, Choluteca, Honduras)	0.63
L. salvadorensis (Yusguare, Choluteca, Honduras)	0.62
L. shannonii ssp. shannonii (Comayagua, Comayagua, Honduras)	0.69
L. shannonii ssp. shannonii (Champoton, Campeche, Mexico)	0.67
L. shannonii ssp. magnifica (El Rincon, Chiquimula, Guatemala)	0.67
L. shannonii ssp. magnifica (Quetzaltepeque, Chiquimula, Guatemala)	0.64
L. trichodes (Cuicas, Trujillo, Venezuela)	0.52
L. trichodes (Jipijapa, Manabi, Ecuador)	0.60

L. collinsii ssp. collinsii, L. collinsii ssp. zacapana and an un-named species (L. sp. nov 1), also produce wood that is more durable than that of L. leucocephala, and therefore highly valued for both fence and house construction. (Durability results from the early and abundant heartwood production of these species compared to L. leucocephala.)

Pulpwood

As L. leucocephala ssp. glabrata has excellent pulping qualities, it is one of the most valued tropical hardwoods for paper and rayon manufacture (National Research Council 1984; Brewbaker 1987; MacDicken and Brewbaker 1982). The bark is easily removed, and low levels of extractives and lignin combined with a high holocellulose content produce a high pulp yield of between 50% and 52%. However, compared to other softwood pulps, it has a low tearing strength and folding endurance, and only average tensile strength. These latter characteristics restrict its use to the production of printing and writing papers, although it could be blended with other pulps to make other products (Bawagan 1983; Bawagan and Semana 1978; Brewbaker 1987; Tang 1981; Van Den Beldt and Brewbaker 1985).

Other uses

The close-grained texture of leucaena wood, combined with its ease of working, uniformity of grain and attractive appearance, make it suitable for the manufacture of furniture and crafts. The high SG, fine grain and high proportion of heartwood in *L. salvadorensis* and *L. collinsii* make wood of these species valuable for turning. Other minor uses include production of props for banana plantation (Virtucio 1978), poles for rural electrification (Bawagan and Semana 1978), mine props (Brewbaker 1987), laminated board (Jai et al. 1982) and particle board (Van Den Beldt and Brewbaker 1985).

The Relationship between Silviculture and Wood Quality

The site conditions and silvicultural regimes used to manage leucaena have a direct bearing on its wood quality and hence on its final products. Grown in a woodlot or plantation, spacing and management can influence factors such as proportion of sapwood to heartwood, stem straightness, degree of compression wood, and number and occurrence of knots, all factors which effect the potential end use of the wood (Van Den Beldt 1983). Grown on a short rotation, *L. leucocephala* wood often has a high proportion of juvenile wood, rendering it suitable only for low quality products (Pound and Martinez 1982). Tang and Ma (1982) found that all test properties, including SG, compression strength and modulus of elasticity, reduced with increasing population density from 2500 to 10000 stems/ha. Close initial spacing, followed by pruning of lower branches and successive thinning, helps to produce straight, clear boles while maintaining strength characteristics (Bhatia et al. 1985; Rao 1984). Regular pruning of *L. salvadorensis* is used in traditional agroforestry to produce corner posts for house construction (Hellin and Hughes, in press).

Conclusions

Reviewing the available information not only demonstrates the great value of leucaena wood in providing a wide range of products, but also illustrates the misplaced reliance on only one introduced species, *L. leucocephala*, to meet these needs. While *L. leucocephala* does possess characteristics that make it suitable for many uses, the species has significant limitations. As the latter are rarely presented, the need to evaluate the properties of other *Leucaena* species has not been obvious.

However, anecdotal evidence from the natural ranges of the other species in Central America and Mexico, together with the results comparing growth and performance of the complete range of *Leucaena* species (Stewart et al. 1992), suggest considerable variation in wood quality across the genus. More specifically, *L. leucocephala* is often not the preferred, or most suitable, species for many enduses. Several other *Leucaena* species, most notably *L. salvadorensis, L. collinsii* ssp. *collinsii, L. collinsii* ssp. *zacapana* and *L sp. nov 1*, have wood superior to that of *L. leucocephala* in terms of durability, strength and fuelwood characteristics.

We believe that any assessment of the value of leucaena for widespread planting must include an evaluation of the quality of its wood in relation to the proposed end-product. Researchers must consider wood quality at the same time as assessing the more standard characteristics such as wood and leaf yield. Only by doing this will full assessment be possible of leucaena's usefulness in meeting the needs of both small- and large-scale growers. This approach will require detailed assessment of wood quality for the complete range of *Leucaena* species, considering the likely range of end-uses of the wood, and giving due prominence to the non-industrial uses of fuelwood and local construction (Hughes 1989).

More detailed assessments of specific gravities, heartwood production, fuelwood characteristics and durability should be included in future trials. Such information would enable more comprehensive assessment of the value of *Leucaena* species when planted in different situations. Combined with data on yield, this information will facilitate more appropriate decisions on choice of suitable species and provenances for future planting programs.

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The Nutritive Value of Leucaena Species

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Abstract

The nutritive value of Leucaena species and hybrids is a function of chemical composition of foliage and the extent to which it meets the nutritional needs of animals for maintenance and production. The protein content of all Leucaena species was found to be high (14-30%) when compared with the minimum requirements of ruminants (8-15%). With the possible exception of sodium, copper and zinc, Leucaena leucocephala (Leucaena) is a rich source of macro- and micro-elements for the ruminant animal. In comparison with grasses and other forages, leucaena has low cell wall (NDF and ADF) contents and high digestibilities, although those species with high tannin contents appear to have lower digestibilities. Leaflets are lower in ligno-cellulose (ADF) than rachae, and L. leucocephala has higher leaf digestibilities than L. pallida and L. diversifolia. Mimosine is the most important anti-nutritive compound in Leucaena species, but the presence of bacteria capable of metabolising mimosine has overcome this major limitation to leucaena use in unadapted ruminants. Tannins may have antinutritive properties in some species and this topic requires further research. With the exception of L. esculenta, the leucaena species tested had a high acceptability by animals. Leucaena has great value as both a sole feed and as a supplement to low quality straws and hays. However, the mimosine content of leucaena continues to limit its use in non-ruminant diets, where usually, no more that 10-20% can be used in a ration. A protocol for evaluating leucaena leaf as a supplement or sole feed and in intensive feeding and grazing systems is presented. L. leuco*cephala* is a high quality forage in many different animal feeding systems and should be a benchmark for testing species and hybrids. The palatability and nutritive value of new selections from agronomic trials must be determined before being released into farming systems.

CULTIVARS and accessions of *L. leucocephala* (leucaena) are the most commonly propagated of the 16 species of leucaena. Consequently there is much information available on its productivity and nutritive value in feeding systems (Jones, R.M. 1994). Leucaena is now widely spread through most tropical and sub-tropical regions of the world and provides an important source of feed for ruminant livestock. The limitations of leucaena (slow establishment, poor tolerance to acid soils, high mimosine content, susceptibility to psyllid attack) have been well-documented. These limitations have prompted research into other fodder tree species, and more

recently, into the other *Leucaena* species and their hybrids (see Shelton and Jones, these Proceedings). As a feed for animals, fodder trees must provide high yields of edible leaf and stem which are both palatable and of high nutritive value for stock. The following paper reviews information on the nutritional attributes of *Leucaena* species and hybrids with a view to making better use of this valuable genus in grazing systems.

Defining Nutritive Value

The nutritive value of a feed is determined by its ability to provide the nutrients required by an animal for maintenance, growth and reproduction, and is a function of the feed intake (FI) and the efficiency of extraction of nutrients from the feed during digestion (digestibility - D). Feeds of high nutritive value promote high levels of animal production (liveweight gain, milk yield etc.). There is no simple predictor of nutritive value of tree legume forage.

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Nutritive value of tree leaves may vary with the manner of usage. For example, some foliage may be more valuable as a supplement or protein source than as a sole feed. Chemical composition alone is an inadequate indicator of nutritive value since the availability of nutrients from forages is variable. In vitro digestibility (IVDMD) measurements provide information only on potential nutrient availability. Digestible dry matter (or energy) intake (FI x D) may also be a poor predictor of potential productivity since the composition of nutrients absorbed is not described.

Leng (1986) has proposed that feed quality ought to be assessed in terms of the balance of nutrients supplied in relation to animal needs for productive function. In this scheme, feeds would be ranked on voluntary consumption potential; potential digestibility (fermentative digestion); rates of microbial synthesis in the rumen relative to volatile fatty acids (VFA) produced (fermentation protein/energy ratio — P/E); propionic acid (glucogenic) synthesis relative to total VFA synthesis (fermentative glucose/ energy ratio — G/E); and ability to provide by-pass nutrients (protein, lipid, starch) for absorption in the small intestine (absorbed P/E and G/E ratios).

This information is available for many temperate and some tropical grass and legume forages, but there is no comparable information for fodder trees fed as a sole dietary source. Comparable information, together with voluntary feed consumption and feed digestibilities may be needed if meaningful comparisons are to be made among *Leucaena* species and cultivars.

Chemical Composition of Leucaena Species

Table 1 shows reported values for the protein, ash, neutral detergent fibre (NDF), acid detergent fibre (ADF), lignin content and IVDMD for some *Leucaena* species and their accessions and hybrids. This information permits some prediction of differences in nutritive value between species.

Sample preparation

Some of the variability seen among species from various studies may be due to different methods of sample preparation and analysis. For example, drying method has a significant effect on values obtained in our laboratory. Oven-dried samples have lower total nitrogen, tannin and IVDMD than freeze-dried samples (Bray 1993). Each laboratory seems to have its own method for determining IVDMD which makes comparisons between laboratories difficult.

Protein content

All Leucaena species contain comparatively high concentrations of protein (> 150 g/kg) when compared with tropical grasses and cereal straws (30-100 g/kg), making them valuable protein supplements to low quality forage diets. Plant proteins are digested in the rumen to provide energy, amino acids and ammonia for microbial protein synthesis. The microbial population in the rumen has a minimum requirement for ammonia (70 mg/L) and decreased microbial activity (digestion) follows when values fall below this level. Feeds containing less than 8% crude protein are generally considered nitrogen (N) deficient and on this criterion all *Leucaena* species are capable of meeting the minimum N requirements for ruminants. Some feed proteins may escape digestion (by-pass proteins) in the rumen and provide additional protein for absorption in the small intestines. It is this supplemental protein which promotes high levels of production. Protection against digestion may be afforded by heat denaturation of proteins during drying or by complex formation with tannins during mastication and ruminal metabolism. Where tannin-protein complexes are dissociated in the small intestine, additional protein of high biological value is available for use by the animal.

Bamualim et al. (1984) found that when fresh *L. leucocephala* (cv Cunningham) leaf was fed to goats as a supplement to low quality straw, 34% of the leaf protein passed undigested through the rumen. When dried leucaena was fed to sheep as a supplement, more than 60% of the protein by-passed rumen fermentation. Gupta et al. (1992) found similar values (67%) for cattle given dried leucaena. More extensive study of the effects of tannin level and *Leucaena* species on plant protein digestion in the rumen are required, so that by-pass protein capabilities may be assessed.

Tannins may have either beneficial effects (increased by-pass protein, decreased ammonia loss) or detrimental effects (depressed palatability, decreased ammonia availability, decreased postruminal protein absorption), depending on the concentration and nature of tannins in the feed. The tannin contents of *Leucaena* species vary from 14 to 170 g/kg in the plant dry matter (Table 2). The significance of tannins in leucaena is discussed in detail by Wheeler et al. (these Proceedings).

Ceil wall and lignin content of leucaena

The digestibility of plant material is related to the time the feed is retained in the rumen and to the proportion and lignification of the plant cell walls (NDF). Studies with other fodder trees have shown

Leucaena species	Crude protein (N x 6.25)	Ash	Neutral detergent fibre	Acid detergent fibre	Lignin	IVDMD %	References
L. collinsii						81	7
L. diversifolia	223	57	368	209	116	52-72	4
K156	204-276	62-85	334-383	200		52	1,2,4,5
CP146568	197		347	210		56	5
L. esculenta	194-249	72	506-524			53-69	1,3,7
L. lanceolata	165	130	342	228	77	65	4,7
L. leucocephala	190-140	77	407	121	68	51-78	1,7,9,10
K8	178-269	59-158	334-494	312	65	46-54	2,3,4
K28	165-191	138	336-404	199-340	85		4,10
K500	212-270	61	312-333	175-241	2	65	5,6
K584	238	(1	494	101	93	50 64	3
K636	198-278	61	336-473	181		50-64	2,3,5
L. macrophylla	218	108	332	271		77	4,7
L. pallida	170-228		369-527			69 53	7
K376 K803	170-228		309-327	209		55 61	3,5
K805 K806	225		565	209		01	3
K953	194		368	204		59	5
K806*K748	169		377	212		55	5
L. pulverulenta	192-250	76-141	433	327-376		48-60	5 3 5 4 7 7
L. retusa		/0111				74	7
L. salvadorensis						75	7
L. shannonii	202	121	356	222		74	4,7
L. trichodes	193-240	74-98	398	287		50-77	4
Hybrids							
L. diversifolia x	a / =					50	2
L. pallida (KXI)	247		505			53	3
L. leucocephala x							
L. diversifolia							
KX3 K743	193-235		342-5 11	194		58	2 5
L. pallida x	265	57	395	194	87	44	3,5 2
L. leucocephala	205	57	393		07		2
KX2							
KX2 K376*K8	244		498			50	3
K806*K636	163-186		344-372	198-207		56-58	5
K748*K636	177		350	190 207		62	5
L. leucocephala x	171		373	212		61	5
L. pulverulenta	1 / 1		575			~ -	e e
K75 22/9-5	156-299	66-99	342-366	279	101	50	2,4
					78	45	2
K75 22/9-5 K340D 22/9-7	260	68 68	371	217			

Table 1. Chemical composition (g/kg dry matter) and in vitro dry matter digestibilities of *Leucaena* species, accessions and hybrids.

1. Malini et al. 1989. 2. Gupta et al. 1991. 3. Austin et al. 1990. 4. Gupta et al. 1986. 5. Castillo 1993. 6. Robertson 1988. 7. Sorensson et al. Unpublished. 8. Brewbaker 1987. 9. Pound et al. 1983. 10. Akbar et al. 1984

Leucaena species	Sulfur	Potassium	Sodium	Calcium	Magnesium	Tannin	Mimosine	References
L. diversifolia		2.0		20	8		41	1, 4
K156				19	8	99		4, 5
CP146568						141		5
K156						41	12-111	1,3,
78-49				19	7		34	2,4
L. esculenta		1.2-2.0		21	18			1
L. lanceolata					6			4
K10				18	14		26	4, 10
L. leucocephala	2.0	1.0-2.3	0.3	21-24	9	14	25-40	1, 7, 8, 9
K8	2.9	1.9-3.5	0.3	29-35		54	30-170	2,3,4,10
K28	2.9	2.4	0.4	28-35			21	4,10
K500	2.6	3.0	0.4	24		37-67	31	5,6,10,12
K584		1.8						3
K636		1.6				55-61	170	2,3,5
cv. Peru	2.7	2.8	0.4	23	11			10
L. macrophylla								
K384				23			54	4
L. pallida								
K376		1.9				89		3.5
K803						63		5
K806		1.9						3, 5 5 3 5 1
K806*K748					8	108		5
L. pulverulenta				21			25-39	1
L. shannoni				26				4
L. trichoides				20			40	1
Hybrids								
L. leucocephala x								
L. diversifolia								
KX3						98		3, 5
K743						49	76	3, 5 2
L. pallida x								
L. leucocephala								
KX2								3, 5
K376*K8						100-124		3, 5 5
K806*K636						104		5
K748*K636						90		
L. leucocephala x								
L. pulverulenta				30-31	7-9			4
Ř75						48	26-154	2, 4 2
K340D						54	178	2
Minimum dietary								
requirements	1.5	1.2-2.4	0.7	4.0	2.0			11
(g/kg feed DM)								
Sing reed Ditty								

Table 2. Macro-element, tannin and mimosine content (g/kg dry matter) of some *Leucaena* species, accessions and hybrids.

1. Malini et al. 1989. 2. Gupta et al. 1991. 3. Austin et al. 1990.4. Gupta et al. 1986. 5. Castillo 1993. 6. Robertson 1988. 7. Sorensson et al. Unpublished. 8. Brewbaker 1987. 9. Pound et al. 1983. 10. Akbar et al. 1984. 11. NRC 1982. 12. Elliott et al. 1985

a negative correlation (r = -0.92) between lignin content and feed digestibility in nylon bags incubated in the rumen (Bamualim et al. 1980). Leucaena species with low cell wall content (i.e. high soluble cell contents) are generally of higher digestibility than those with high cell wall (> 450 g/kg dry matter — DM) and lignin contents. However, digestibility estimates vary greatly with technique used for sample preparation and incubation, so it is difficult to draw firm conclusions from the reported data in Table 1. The presence of tannins is now known to interfere with the detergent fibre and lignin estimation and researchers have developed new techniques to overcome these difficulties. All previous studies of cell wall composition in leucaena (and in other tannin-containing species) need to be re-evaluated in the light of this information.

Leaf Morphology and Nutritive Value

Leaf morphology affects composition by controlling the ratio of fibrous lignified or cutinised (indigestible) tissue to photosynthetic (digestible) tissue. An important feature of leucaena leaf (leaflets) is the relatively low fibre content, and although NDF contents are quite variable, lignocellulose as measured by ADF is in the range of 1%20% (Lowry et al. 1992).

Two features give rise to the low fibre content of leucaena leaflets: the pinnate habit and the mobile pulvinus.

The pinnate habit is of significance to herbivores. In the compound leaf there is a separation of photosynthetic and vascular tissue, so that the leaflets alone provide higher quality feed than the leaf as a whole. For six species of *Leucaena*, Lowry et al. (1992) found that leaflets contained 19.7±7.2% ADF and that rachis/rachillae contained 46.8±4.4% ADF. Bray (1993) observed that, for L. leucocephala (K500, K636) and its hybrid with L. pallida (K748*K636), the rachis and stem generally had lower in vitro digestibilities (46-51%) than the leaflets (56-63%)). In the same study, the IVDMD of rachis and leaflets from L. diversifolia (CP146568) and L. pallida (K806*K748) were similarly low (45-50%). These two species are characterised by high tannin contents which may have depressed digestibility (Castillo 1993).

The mobile pulvinus is the second aspect related to low fibre content. Leucaena and many tree legumes show 'sleep movements', with leaflets that fold about the rachis at night. Species that show leaf folding tend to have less fibrous (lignified) leaflets. Lowry et al. (1992) found that, in 23 pinnate species of tree legumes, those with folding leaves had ADF values of $22.6 \pm 6.8\%$ compared to 35.2

 \pm 5.3% for species with fixed leaves. The mobile pulvinus also allows leaflets to detach more readily on drying, making it easier to prepare clean leaf meal.

It would seem that leaf characteristics, such as proportions of rachis and leaf folding may be useful guides to nutritive value in some *Leucaena* species.

Macro- and Micro-Element Content of Leucaena Species

The levels of the macro-elements of nutritional importance for ruminants in different Leucaena species and accessions are summarised in Table 2. Jones and Jones (1983) found no major differences in the concentrations of various elements (nitrogen, phosphorus, potassium, magnesium, calcium and sodium) in leaves and stems of L. leucocephala cultivars Peru and Cunningham. Similarly, after a survey of 20 promising Leucaena genotypes, Austin et al. (1992) concluded that all had adequate levels of macro-elements for ruminant nutrition, with the possible exception of sodium. These workers also surveyed trace-element concentrations: compared to National Research Council (1984) recommendations, Leucaena species contained only marginal levels of copper (6.8 ppm, requirement 8 ppm) and zinc (24.2 ppm, requirement 30 ppm). In a review of the mineral composition of *L. leucocephala*, Kleinjans (1984) also found low sodium and zinc levels.

However, while values less than predicted requirements may indicate deficiency, values greater than prescribed do not necessarily indicate adequacy, as element availability may vary between different plants and tissues. For example, sulfur in plants is largely associated with proteins, and availability of sulfur in the rumen is decreased when protein-tannin complexes form. Phosphorus levels in leucaena appear adequate, but availability may also be affected by interactions with other plant metabolites. Calcium, potassium or magnesium are not likely to be limiting in leucaena. Although sodium levels appear low, deficiencies of sodium are unlikely when other feeds are consumed with leucaena. As a general recommendation, therefore, leucaena foliage may be seen as a comparatively rich source of macro- and micro-elements for ruminant diets, and also of carotene for vitamin A synthesis.

Anti-Nutritive Factors

Anti-nutritive factors assume greater significance when tree leaves are the sole or major component of the diet. Although *Leucaena* species contain an array of secondary plant metabolites (Lowry et al. 1984), the major compounds that affect nutritive value are the non-protein amino acid mimosine and tannins. Acamovic et al. (1986) found that leucaena leaf meal and seeds contain saponins (haemolytic glycosides) at a level comparable with soyabean meal. Saponins are known to have adverse effects on growth and cholesterol metabolism in nonruminants. The implications of tannin in leucaena is discussed by Wheeler et al. (these Proceedings).

All Leucaena species are characterised by variable mimosine contents (Table 2). Although highly toxic to non-ruminants, mimosine does not act as a feeding deterrent to either ruminants or psyllid insects. Mimosine content varies with tissue sampled, from 8-12% in actively growing shoots, 4-6% in young leaves and 4-5% in young pods and seeds (Bray these Proceedings). Arora et al. (1986) found that mimosine contents varied with temperature, being higher in winter than in summer. Bray et al. (1988) reported large variations in mimosine content between harvests for L. leucocephala, L. diversifolia and the hybrid L. leucocephala x L. pulverulenta. In a survey of 306 accessions of Leucaena in search for high proteinlow mimosine plants, a protein range from 11.1-31.4% and a mimosine range from 0.2-7.0% was reported (Saunders et al. 1987).

Mimosine acts in animal tissues by interfering with cellular mitosis, and the symptoms of toxicity are alopecia, reduced appetite and finally death. Hence leucaena leaf meal is of limited value in nonruminant diets (< 10% dry matter). R.J. Jones (1994) recently reviewed the effects of mimosine on ruminant health and productivity. Mimosine may be metabolised to DHP (3-hydroxy-4 (1H)-pyridone) in leaf tissue and in the rumen. In ruminants adapted to leucaena consumption, specialised rumen bacteria may degrade DHP further to harmless compounds. Where leucaena is fed to unadapted stock at rates of more than 30% of the diet, DHP may act as potent goitrogen and result in hyperthyroidism and death. However, it is now possible to inoculate ruminants with DHP degrading organisms and thus overcome potential toxic effects. Although mimosine content appears no longer to be a limitation on leucaena use by ruminants, low mimosine varieties may still be valuable as low fibre-high protein feeds for non-ruminants.

Voluntary Intake and Palatability of Leucaena Species

There are no laboratory techniques to predict voluntary feed intake in ruminants, so feeding trials must be conducted to obtain this important information. Voluntary intake of forages by ruminants is a function of animal preference (palatability) and physiological control of rumen fill (residence time). Palatability of a feed has been related to both physical characteristics (hairiness, bulk density) and the presence of compounds which may affect taste and appetite (volatile oils, alkaloids, tannins, soluble carbohydrates). There have been only a few studies of the voluntary consumption of fresh leucaena as a sole feed, summarised in Table 3. Care must be taken with the interpretation of these results as low intakes may have been associated with mimosine toxicity in unadapted animals. However leucaena intakes do appear higher than those found for grass forages.

 Table 3. Voluntary consumption of fresh leucaena as a sole feed.

Species	Voluntary intake (gDM/kg LW)	in vivo DMD%	Reference
Sheep	31.9	63.2	Yates (1982)
Goats	35.6	68.0	Yates (1982)
	24-28	54.0	Devendra (1982)
	27-40	71.0	Upadhay (1974)
Cattle	not recorded	54.8	Gohl (1981)

Palatability (or edibility) is that aspect of voluntary feed intake controlled by animal preference, and may be a major determinant of leaf intake in some fodder tree species. Osman (1982) showed that the edibility of L. leucocephala varied with plant age and with seasonal temperature and moisture conditions. He found that the edible fraction fell from 96% at 90 days to 84-92% at 120 days after regrowth commenced. Osman (1986) also recorded that the percentage of leaf fell from 66% of DM yield at 60 days to 40% at 150 days. Austin et al. (1991) investigated cattle's acceptance of eight Leucaena species (L. leucocephala K8, K636, K584; L. pallida K376; L. esculenta K948; and Hybrids KX1, KX2, KX3). They found that, during a short grazing period, more than 30% DM was removed from all species except L. esculenta, which was poorly accepted (<5%). Leucaena is one of the most palatable of fodder tree species, and care needs to be taken that this attribute is not lost during selection for environmental adaptations such as disease and pest resistance, and cool and acid-soil tolerance.

Digestibility

The importance of digestibility as an indicator of feed quality arose from studies with grass forages

where there is often a close relationship between feed intake and digestibility. In this case, digestibility directly reflected digestible nutrient intake and was therefore a useful measure of quality (Minson 1982). However, no similar relationship is found between intake and digestibility for either forage legumes or for fodder tree leaves. With these, intake appears determined primarily by the shape, size, fragility and palatability of the leaf material consumed. For example, Lowry (1989) found that sheep offered Albizia lebbek leaf consumed more mature fallen leaf (in vivo DMD 43%) than fresh leaf (DMD 64%). Similarly, goats consumed similar amounts of leucaena (35.6 g/kg LW) and gliricidia (32.6 g/kg LW) despite large differences (68.0 and 56.3% respectively) between in vivo digestibilities (Norton 1994a).

Digestibility may be determined by the residence time of leaf in the rumen, high rates of passage and low digestibility being associated with high voluntary feed intakes. For this reason in vitro digestibility (IVD) and in sacco digestibility (ISD) techniques alone may be of limited value as predictors of nutritive value for fodder tree leaves. Norton (1994a) cited examples where IVD measurements failed to rank feeds in terms of in vivo digestibility. Care is thus required if nutritive value is to be judged on these techniques alone.

Leucaena Leaf as a Supplement

The discussion so far has concentrated on the composition and potential nutritive value of leucaena as a sole feed. However, its most common usage is as a supplement to other feed sources. Where leucaena availability is limited, there is a need to make optimum use of this valuable resource. As a supplement many of the detrimental effects are minimised, and a high quality legume may stimulate the intake and utilisation of low quality forages by correcting essential nutrient deficiencies (Norton 1994b). The level of supplementation for optimum use of fodder tree leaves varies with animal species and composition of the basal diet. As a general rule, no more than 30-50% leucaena in the diet (or 0.8-1.2% liveweight) is required for optimum performance of cattle, sheep and goats on low quality diets (Norton 1994b). Leucaena leaf has also been used successfully to increase the protein content and nutritive value of tropical silages (Tandraatmadja et al. 1993). In this context, the ability of leucaena to provide by-pass protein is important, and even some Leucaena species with high levels of tannins may be beneficial as supplements, providing the tannin-bound proteins are released for digestion in the small intestines.

Leucaena in Diets for Mono-Gastric Animals

Several studies have investigated the potential for using leucaena leaf meal in pig and poultry rations. However, some form of pre-treatment is usually necessary to overcome the toxic effects of mimosine. In Thailand, 25% water-soaked Leucaena leaves were incorporated successfully into grower pig rations (Kanto 1991), and Hongo et al. (1990) found that up to 30% molasses ensiled leucaena leaves could be added to pig rations without penalising growth. However, low digestibility of proteins and low ME content compared with legume grains suggests that there is only limited scope for the use of these meals in commercial diets for nonruminants (D'Mello 1992).

Assessment of Nutritive Value

There is a need to establish a protocol for characterising the nutritive value of tree legumes, given that chemical composition and in vitro digestion techniques may have significant limitations in the prediction of potential nutritive value. Detailed information on nutritional value can only be obtained from feeding trials where feed intake is measured. After selection of promising species/ cultivars for superior nutritive value in feeding trials, these should then be evaluated with sheep, cattle or goats in grazing trials.

Intensive feeding trials

Both fresh and dried edible fractions of leucaena should be used for evaluation. When animals are held in metabolism cages, nutrient balances can be calculated. Where available, rumen- and abomasally-fistulated animals may be used to determine available and absorbed P/E and G/E ratios (as described earlier). The value of the leucaena species being tested as a supplement and as a sole diet is measured by offering animals the test diet at 0 (basal low quality roughage), 1% and 2% of liveweight and ad libitum, with the basal roughage diet provided ad libitum for all treatments (except where the leucaena is being used as the sole diet). The numbers of animals and length of trial should be long enough to obtain meaningful data on growth rate (say 6-8 weeks).

Grazing trials

The time taken for the establishment and growth of fodder trees and the need for large committed areas of land make grazing trials expensive. Such trials may take up to five years to complete, and the experimental design must be sufficiently robust

for statistical interpretation. The objectives of a grazing trial must be clearly defined. These may seek to evaluate Leucaena species under continuous grazing (sole feed) or as a strategic supplement in an integrated pasture system. In the latter case, intermittent grazing for various periods will generate different levels of supplementation. A minimum design would require replicated areas (1 ha each, 3 m row spacing, undersown with grass) grazed by a minimum of three or four steers (30 sheep or goats) for each species under test. Where possible, the effects of varying stocking rate should also be evaluated, since stocking rate is usually the major determinant of animal productivity from grazing systems. Before the trial begins, the tree canopy should be sufficiently well developed to withstand continuous grazing for at least 100 days (4000 kg edible leaf dry matter/ha). Where animals have not previously been exposed to leguminous trees as a source of forage, they may take up to three months before consuming significant amounts of tree leaves. A minimum grazing period of one year would ensure that any seasonal changes in nutritive value and acceptability are recorded. The experimental design of grazing trials should also accommodate the need to remove animals before permanent damage to the trees occurs through overgrazing. Where value as a supplement is being tested, animals may be held for varying times (days/week or weeks/month) on the plots, and for the remaining time be depastured in adjacent areas of grass pasture with control animals. Although there may be many different grazing management systems that could be developed for leucaena in the tropics and subtropics, clear guidelines must be established for the objective measurement of system productivity and sustainability.

Conclusions and Priorities

In this review of the nutritive value of *Leucaena* species and their hybrids, we have found a significant amount of information on the composition and nutritive value of L. leucocephala, but much less on other *Leucaena* species. Where IVDMD is used as a guide to nutritive value, some studies have shown L. diversifolia, L. esculenta and L. pallida to be lower in nutritive value than L. leucocephala. However, more information on palatability and intakes of these species is needed before any final judgment can be made. Both drying and the presence of tannins can decrease protein digestion in the rumen. More quantitative data must be collected on the extent' to which proteins from the different Leucaena species bypass rumen fermentation and contribute to increased intestinal protein availability. The distinct differences between species' cell wall content also merits further study.

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Condensed Tannins in Leucaena Species and Hybrids and Implications for Nutritive Value

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Abstract

Condensed tannins are secondary metabolites which have a role in the protection of plants from predation by insects and herbivorous animals. Tannins form insoluble complexes with proteins and plant cell walls during mastication and digestion, and are thought to make feed less palatable and nutrients less available to ruminants. Tannins in leucaena protect proteins from ruminal digestion (35-60%). Recent studies suggest that bacteria capable of disrupting the tannin-protein complex can be introduced into the rumen to reduce the detrimental effects of tannins. There have been few studies of the tannins in *Leucaena* spp., and methods of sampling and analysis should be standardised to validate future comparisons. *Leucaena leuco-cephala* has moderate levels of tannins (1.4-7.9%) while *L. pallida, L. diversifolia* and hybrids have higher levels. High tannin levels are associated with psyllid resistance, lower palatability and lower nutritive value. This topic requires further study. Future research should aim to identify and characterise the chemical structure and biological activity of leucaena tannins.

TANNINS in plant tissues are secondary metabolites and are widely distributed throughout the plant kingdom. These polyphenolic polymers are of relatively high molecular weight and have the capacity to form complexes with carbohydrates and proteins. Tannins are usually classified into two major types, hydrolysable and condensed tannins. Hydrolysable tannins (HT) are highly toxic to animals and produce gallotannins or ellagitannins from acid hydrolysis. Condensed tannins (CT) are hydrolytically cleaved to anthocyanidins and related compounds, and are more correctly called proanthocyanidins or polyflavanoids. Low molecular weight CT oligomers are now known to be more reactive, with higher protein precipitating capacities, than high molecular weight polymeric tannins (Butler 1992).

Tannins have complex structure and chemistry. They are specific to each plant in which they are found, varying from each other in flavonoid stereochemistry, molecular size and polymeric form.

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Comparatively little is known about the structure and activity of tannins in plants of agricultural importance, except for sorghum, *Lotus* spp. and *Onobrychis viciifolia* (sainfoin). There is a specific need for more information on the tannins of high protein legume forages, particularly tree legume species of the genera *Leucaena*, *Calliandra* and *Albizia* which are showing promise in tropical and sub-tropical environments and are high in CT.

Condensed tannins protect plants from being eaten by animals and insects (Cheeke and Shull 1985). Condensed tannins also affect animals by making feed less palatable (Barry and Duncan 1984), by reducing the availability of feed protein through the formation of insoluble protein-tannin complexes, by inhibiting digestive enzymes, and possibly by direct toxicosis. There is also equivocal evidence that tannins prevent unadapted insects from feeding on plants (Griffiths 1991). On the other hand, there is substantial evidence that tannins may also benefit animal production by protecting dietary protein against digestion in the rumen. These effects appear to depend on the level and composition of tannin in different plants.

This paper reviews information presently available on the biological and nutritional significance of tannins in *Leucaena* species and hybrids.

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Measurement

Many techniques purport to measure CT in plant material, but most have some deficiency that limits general use. The efficiency of the procedures used to extract and isolate CT is critical. Interfering substances such as phenolics and pigments must be removed before starting tannin analysis. Techniques using Folin-Denis or Folin-Ciocalteau reagents cannot distinguish between tannin and non-tannin phenolics. Burns (1963) first described a method using vanillin plus hydrochloric acid (HCI) which forms complexes with tannins and gives a pink colour. Although very specific for CT, this method also detects monomeric and polymeric flavonoids which are not pro-anthocyanidins. Broadhurst and Jones (1978) used the standards cathechin and tannic acid (HT) to calculate tannin content, but neither of these standards has the same biological activity as CT, and neither bears much relation to the CT usually found in plant tissue. Tannin values based on cathechin overestimate true values. Bate-Smith (1954) used n-butanol plus HCI to convert proanthocyanidins to cyanidins which have a pink colour in solution. Whilst this method also has some limitations, it has been adopted as the preferred method for tannin analysis. However the problem of standards still remains.

The standards used in our laboratory are purified tannins extracted from the plant under study, e.g. Lotus pedunculatus, Desmodium intortum, Leucaena leucocephala and Leucaena pallida. These standards have been further analysed by High Performance Liquid Chromatography to determine their molecular size and distribution and flavonoid composition. We have found that even within the Leucaena genus, there are differences in the composition, and possibly in the reactivity, of the tannins isolated from L. leucocephala and L. pallida. So it is possible that knowledge of a tannin's concentration alone is inadequate to describe its biological activity. More research is needed to discover the characteristics and biological activity of the tannins from lesser-known leucaena species and hybrids. It is also clear that without relevant standards we cannot meaningfully compare tannin content between plant species.

Near infra-red spectroscopy (NIRS) has been used to screen rapidly large numbers of samples for CT content. This technique relies on calibrating the machine against standards with known tannin content, and has been shown to predict accurately the relative tannin contents in plant material ($r^* =$ 0.92). However, to rank samples with respect to tannin content has no biological meaning, and the problem of choosing standards remains the same.

Tannin Concentrations in Species and Hybrids

The literature reports great variability in CT content between Leucaena species and their hybrids. However, as explained above, direct comparison of CT contents may be meaningless because of the many different sampling procedures and assay methods. Compared to freeze-drying, oven-drying can greatly reduce tannin content (Price et al. 1979). Indeed, in some plant species, tannins apparently disappear completely during oven-drying (Ahn et al. 1989). Tannin content also varies in different plant parts (Bray 1993). Tannins are synthesised in specialised cells or organs and their concentrations vary markedly during the life of the plant, depending on environmental conditions (Baas 1989). In leucaena leaves, tannins are generally contained in the pallisade cells of leaflets.

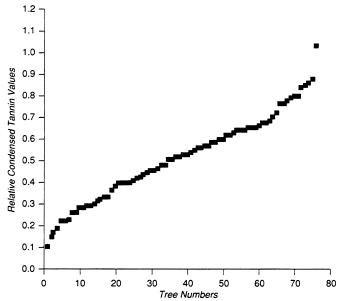


Figure 1. Distribution of relative condensed tannin values by tree number for 76 segregating KX2 trees of the hybrid of *L. leucocephala* (K8) with *L. pallida* (K378) at the Redland Bay Research Station.

Among leucaena species, L. leucocephala appears to have moderate tannin content (1.4-7.9%) while L. pallida and L. diversifolia have higher contents (Table 1). Levels in segregating hybrids are extremely variable (Fig. 1). Bray (1993) measured the percentages of CT in the edible fraction of L. leucocephala, L. pallida, L. diversifolia and two hybrids. Samples were oven-dried, and values were lower than would be expected from freeze-dried material. The tannin contents, averaged over five leucaena lines, were 2.8% in leaf, 4.6% in rachis and 2.2% in stem. The distribution of CT in plant parts varied with species. In L. diversifolia levels of CT were very high in rachis, but not in leaves, while the hybrid L. pallida K748 x L. leucocephala K636 had uniformly high CT concentrations in all fractions (Fig. 2).

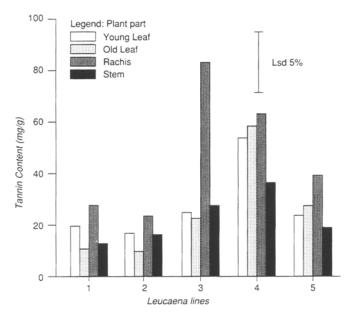


Figure 2. Condensed tannin content in oven-dried samples of the plant parts of five leucaena lines (1 = L. *ieucocephala* K500, 2 = L. *leucocephala* K636, 3 = L. *diversifolia* CP146568, 4 = L. *pallida* K748 x *L. leucocephala* K636, 5 = L. *pallida* K806 x *L. pallida* K748).

The effects of climate, soil, stage of maturity and management on condensed tannin content of leucaena are not known, and should be studied. There are also reports that CT levels increase when plants are browsed by animals.

Tannins and Protein Metabolism

Crude protein levels in Leucaena species are high, from 17 to 27% in edible foliage (Table 1). There may be slightly less crude protein in some species, such as *L. pallida* and *L. diversifolia*, but this may be because the edible fractions of these species have a higher proportion of stem. Within the edible fraction, Bray (1993) has found that protein levels are highest in young leaf (28-36%) and lowest in rachis and stem (9-18%).

However, a feed's measured crude protein content may not indicate protein availability to a feeding animal. Protein is degraded in the rumen and synthesised into microbial protein. Excess nitrogen is released as ammonia, which in turn is converted to urea and excreted in urine. Hogan (1982) reviewed a range of forages and found an average of 25% of crude protein passed through to the small intestine. Losses were greatest from high protein feeds such as legumes. However, in studies with L. leucocephala, a larger proportion of protein bypasses rumen fermentation, with 60% of the protein in dried forage or 35% of the protein in fresh forage reaching the small intestine (Norton these Proceedings). Tannins may be involved in these high levels of protection against ruminal digestion.

Condensed tannins are released when plants are chewed, and bind with feed and salivary proteins to form complexes that are insoluble at rumen pH. High tannin concentrations also reduce the activity of microbes in the rumen. The protective action of tannins may depend on their molecular form (monomeric vs polymeric). Evidence suggests that tannins become more highly polymerised and less reactive as tissues age.

Although significantly increased digestion of the protein occurs in feeds containing 1-4% tannin, because more of the protein is delivered to the small intestines, feed tannin contents of 6% or more may reduce intake, rumen digestibility and post-ruminal absorption (Barry and Duncan 1984). Tannins released from one plant species in a diet may sometimes bind proteins from other tannin-free species in the diet (Waghorn and Jones 1989; Yu et al. 1991). Some *Leucaena* species have high tannin contents which may make them useful as a 'tannin supplement' to protect the dietary protein in companion feeds.

Studies on the relationship between CT content and protein digestibility in the rumen have shown mixed results. Ahn et al. (1989) found that nitrogen digestibility through nylon bags was poorly correlated with the tannin content in various tree legume species. Species with no tannin were highly digestible, but *Calliandra calothrysus* (8% CT) had low digestibility. A different nylon bag study at the University of Queensland compared the protein digestibility of 17 selected F3 segregating hybrids of the interspecific cross *L. leucocephala x L. pallida*. Protein digestibility was highly correlated with tannin content, but only when trees were separated into psyllid susceptibility classes (Fig. 3).

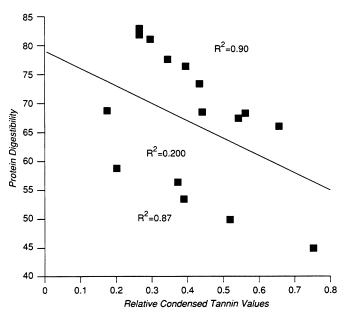


Figure 3. Comparison of relative condensed tannin values with protein digestibility for 17 segregating KX2 trees of the hybrid of *L. leucocephala* (K8) with *L. pallida* (K378).

Species/Hybrids	Parameter (DM basis)					
	CP(%) ¹	$NDF(\%)^1$	$ADF(\%)^1$	IVDMD(%) ¹	$CT(\%)^2$	
L. leucocephala						
K500	21.2	31.2	17.5	65.2	6.7	
K636	19.8	33.6	19.1	64.2	6.1	
A25221	27.4	31.5	17.8	71.0	5.9	
CP161227	22.9	31.6	18.1	64.9	7.9	
Mean	22.8 a	32.0 d	18.1 b	66.3 a	6.6 d	
L. pallida						
K803	17.2	37.7	20.9	61.2	6.3	
CSIRO composite	17.6	37.5	20.9	54.0	11.1	
K375	16.7	36.9	19.8	52.9	8.9	
K806 x K748	16.7	37.7	21.2	55.0	10.8	
К953	19.4	36.8	20.4	58.9	5.4	
Mean	17.5 c	37.3 a	20.6 a	56.4 c	8.5 c	
L. diversifolia						
K156(4n)	21.4	33.4	20	52.5	9.9	
CPI46568(2n)	19.7	34.7	21	56.0	14.1	
Mean	20.6 b	34.1 c	20.5 a	54.2 c	12.0 a	
KX2 Hybrids						
K376 x K8(F3)	16.3	34.4	20.7	56.1	12.4	
K806 x K636(Fl)	17.7	34.6	19.7	61.6	10.4	
K376 x K8(Fl)	18.6	37.2	19.8	58.0	10.0	
K748 x K636(Fl)	17.1	37.3	21.1	60.6	9.0	
KX3 Hybrid						
K156 x K636(Fl)	19.3	34.2	19.4	58.2	9.8	
Mean	17.8 c	35.5 b	20.2 a	58.9 b	10.3 b	
LSD Accessions						
(P<0.05)	2.72	1.94	1.21	3.53	1.46	

Table 1. Crude protein (CP), neutral detergent fibre (NDF) and acid detergent fibre (ADF) contents, in vitro dry matter digestibility (IVDMD) and condensed tannin (CT) level of 16 leucaena species and hybrids (after Castillo 1993).

Note: LSDs refer to individual accessions only. Group means within column with a common letter are not significantly different (P < 0.05) using SAS-GLM procedure

¹ Leaf fraction only

² Condensed tannin (Free-CT + bound-CT)

The upper group had a psyllid susceptibility rating of 5.6 (moderately susceptible) while the lower group was rated at 3.4 (moderately resistant). These two groups may represent different degrees of CT polymerisation.

More research is needed on protein-tannin formation and degradation in the ruminant digestive tract. We want to understand how and when tannins from different plants may improve or diminish the plants' nutritive value to grazing animals.

Other Effects on Nutritive Value

Studies on CT content in relation to in vitro dry matter digestibility (IVDMD) have given variable results. Castillo (1993) found a poor relationship (Fig. 4) while Fogarty (1993) obtained a close relationship (Fig. 5), but this may be because IVDMD data are inherently variable due to calibration and sampling procedures. The effect of tannin on cell wall digestibility may be related to the extent of tannin binding to protein and carbohydrate. That, in turn, may depend on where the tannin is located, its type and its concentration in the cell.

Tannins are reported to adversely affect palatability in some plant species, such as *Lotus pedunculatus* (Barry and Duncan 1984). However, Austin et al. (1991) found that *Leucaena pallida* and its hybrids with *L. leucocephala* are highly palatable. Ethiopian studies with *Calliandra calothrysus* showed that forage with high tannin and low IVDMD could still give reasonable liveweight gains in feeding trials (J. Tothill pers. comm.), which supports the view that IVDMD may not be a reliable method for ranking fodder trees for nutritive value (Norton, these Proceedings).

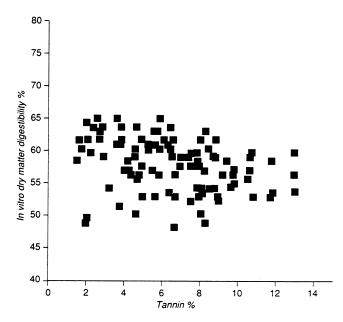


Figure 4. Relationship between in vitro dry matter digestibility and tannin content (%) (Castillo 1993).

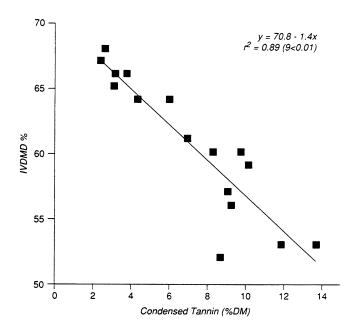


Figure 5. Relationship of leaf IVDMD % and leaf condensed tannin concentration for 16 leucaena species and hybrids (Fogarty 1993).

Tannins may also have a role in preventing bloat. Molecular biologists and biochemists are investigating techniques for incorporating genes for tannin synthesis into highly productive, but bloatinducing, clovers. As well, tannins are suspected of having physiological effects on the endocrine system of sheep, inducing more efficient body growth (Barry et al. 1986). There appears to be substantial evidence for both beneficial and detrimental effects of tannins on animal metabolism, but there is still not enough information to allow prediction of their mode of action.

Microbial Metabolism of Tannins

The use of leucaena as a forage was initially limited by high levels of mimosine, toxic to unadapted animals. Then Jones and Lowry (1984) found that Indonesian goats were protected from toxicity by a bacterium capable of metabolising the toxin to harmless end products. A similar solution is now being sought to correct the detrimental effects of high tannin content in some tree legumes such as *Acacia aneura* (mulga) and *Calliandra calothyrsus*. These studies may be relevant to *Leucaena* species with comparable tannin contents.

Condensed tannins form complexes with plant proteins, with digestive enzymes and with endogenous protein, and are thought to have an anti-microbial action. Previously, it was thought that this complexed form of protein was protected from attack by micro-organisms in the rumen. Now, studies in the CSIRO Division of Tropical Animal Production suggest that some bacteria are capable of cleaving the tannin-protein bond and of metabolising tannins (McSweeney, pers. comm.). Some of these bacteria have been isolated, from the rumens of sheep and goats fed Calliandra, on media containing tannins purified from Calliandra calothyrsus. More morphotypes of these kinds of bacteria came from the goats than from the sheep. The same approach could be used with leucaena tannins, although bacteria isolated from calliandra are probably significantly cross-reactive with leucaena tannins. We need to identify and characterise bacteria capable of cleaving tannin-protein complexes. Research should confirm that there will be nutritional benefits from the presence of these bacteria in the rumen.

Tannins and Psyllid Resistance

The mechanism of psyllid resistance in some *Leucaena* species is still not understood. However, it is thought to be related to the presence of secondary metabolites such as mimosine, phenolics, flavonal glucosides, tannins, saponin or volatile attractants or repellents (Darma and Sutikno 1990). Of this group, Rhodes (1985) reported that the phenylpropanoids are the most widely distributed and exist as both monomers (cinnamic acid, flavonoids and isoflavonoids) and polymers (tannins

and lignins). Many researchers suspect that tannins confer psyllid resistance because the tannins are present in higher concentrations in resistant lines of leucaena, and the astringence conferred by tannins may deter psyllids (Darma and Sutikno 1990).

Whether tannins do change insects' feeding behaviour (and if they do, how) is subject to controversy (Griffiths 1991). Theories based on a tannin-protein interaction in the gut of insects have been challenged (Martin et al. 1987). Haslam (1988) suggested that tannins may help make leaves tough and woody, so protecting foliage from insect attack, and Castillo (1993) has found psyllid resistance is related to detergent fibre content. Fogarty (1993) found some association between psyllid resistance and tannin content ($r^2 = 0.56$), but Castillo (1993) found they were not associated, although coefficients of determination were statistically significant at 0.32 and 0.34 respectively.

An alternative hypothesis is that plants with a high proportion of cell walls have low proportions of soluble cell contents (protein and soluble carbohydrates). Such plants may be unattractive to sapsucking psyllids. Unfortunately, they would be less digestible for ruminants as well. We need to understand the relationship between the chemical composition of Leucaena species and their resistance to psyllid attack. This information is essential for selection and breeding of pysllid-resistant leucaena of high nutritive value.

Conclusions and Priorities

The principal objective of research into lesser-known leucaena species is to identify plants which yield plenty of good quality forage for ruminant livestock. Good yields will come from plants which are adapted to insect pests, grazing, cold, drought and low soil fertility. There appears to be strong evidence that plants with these characteristics also contain large amounts of condensed tannins, which may decrease their nutritive value for grazing animals. This possibility must be evaluated in selection programs for new leucaena cultivars.

Acknowledgments

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Possibilities for Developing Low Mimosine Leucaena

R.A. Bray¹

Abstract

Nutritional problems with leucaena in non-ruminants are largely due to mimosine and its metabolite DHP. Many factors affect mimosine concentration in the plant, including plant part, leaf age, and growing conditions. Some species (*L. collinsii, L. diversifolia, L. esculenta, L. greggii, L. pallida, L. pulverulenta*) have low levels of mimosine, but have other agronomic drawbacks. There is little evidence of meaningful variation for mimosine concentration within species. Breeding programs using interspecific hybrids have not been successful in combining low mimosine and high yield. The use of molecular biology techniques to develop low mimosine leucaena may still have other nutritional problems, and may have less effective resistance to pests and diseases.

IN non-ruminant animals, high levels of leucaena in the diet can result in poor weight gains and infertility problems as well as side-effects such as hair loss. These effects have been observed in a wide range of species (including horses, pigs, chickens, rabbits, fish, shrimp, and humans). This has restricted the widescale use of leucaena as a feed other than in ruminant production systems. Even ruminants (cattle, goats and sheep) show toxicity in some countries. The undesirable effects of feeding leucaena have largely been attributed to mimosine, a non-protein amino-acid (B[N-(3-hydroxy-4oxypyridyl)]-cr-aminopropionic acid) that is restricted in its natural occurrence to all species in the genus Leucaena and to Mimosa pudica. The problems associated with long-term feeding of leucaena are also related to DHP (3-hydroxy-4-1(H)pyridone), a metabolite of mimosine. The toxic action of mimosine and DHP has been reviewed by Jones (1979).

For many years, the existence of mimosine/DHP toxicity in ruminants in only some areas of the world was puzzling. The explanation lies in the presence or absence of bacteria that metabolise DHP (Jones and Lowry 1984). A means of overcoming the problem has been provided by Jones (1985), in isolating the rumen bacteria that metabolise mimosine and DHP. Inoculation of a small pro-

portion of a herd of animals with these bacteria provides an elegant, effective, long-term solution to DHP toxicity.

There remains the desirability of low-mimosine leucaena for non-ruminants. In some countries, leucaena leaf meal is a potentially valuable export product. However, in the open market it is disadvantaged relative to its main competitor (alfalfa meal) because of its mimosine content. Possible treatments of leucaena leaf (e.g. heating, fermenting, treatment with ferric sulphate) have been suggested as means of reducing the mimosine levels. However, as Lowry et al. (1983) have pointed out, these generally have the effect of converting mimosine to DHP, which probably does not solve the toxicity problem at all. Chelation has not generally proved to be beneficial. If low mimosine leucaena is a priority, the only practical way of achieving it is by breeding and/or selection.

Estimating Mimosine Concentration

Analysis of mimosine concentration itself is not a problem. There are several useful quantitative analytical methods for determining mimosine concentration, with different methods being applicable for dried and fresh material. The calorimetric method of Megarrity (1978) was widely used, but newer methods of analysis use HPLC technology because of its improved accuracy and its ability to measure mimosine and the two isomers of DHP separately (Lowry et al. 1985).

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Leaves have been the most popular plant fraction for the actual plant sampling. Many different methods have been used, ranging from leaves of a particular age or position to bulking leaves from whole plants. However, no matter which technique is tried, it is difficult to reduce the Coefficient of Variation (CV) of a single sampling to less than about 18%. (The implication of a CV of 20% is that about 8 observations per treatment are needed to establish an lsd of 20% of the mean). Thus several samplings at carefully specified plant stages are needed to establish differences between plants. In the past, I tended to use a sample of the 'second most recently expanded leaves', but now find that taking a combined sample of the first five expanded leaves is somewhat more precise (CV 12% vs 17%). In seedling populations, Megarrity (pers. comm.) found a correlation of 0.92 between the mimosine concentration of the heaviest leaf and the total mimosine in the plant. It is uncertain how good the relationship would be in older plants. Perhaps we should analyse samples that reflect the plant as utilised rather than just a small part of it?

Ontogenetic and Environmental Factors That Affect Mimosine Concentration

Many workers have shown that mimosine concentration varies depending on the part of the plant sampled, its growth stage, and its growth rate. The results of Adeneye (1991) are representative of studies on mimosine concentration of different plant parts: cotyledons 12.3%) young leaves 5.1%) old leaves 2.6%, young seeds 6.2%, mature seeds 3.2%. An unusual feature of this data was the finding that the green and brown seedcoats (testas) and empty brown pods contained no mimosine.

Leaf age

There is a clear gradient of mimosine concentration as leaves age. Tangendjaja et al. (1986) showed a decrease from 4.5% in one-week-old leaves to less than 0.2% in ten-week-old leaves. Typical figures from Townsville observations on cv Cunningham were leaf 2, 6.1%; leaf 4, 5.3%; leaf 6, 4.5%; and leaf 8, 3.4%. In this experiment we found no interaction between mimosine concentration and leaf age (as judged by position on the plant) for a range of cultivars. This finding contrasts with the conclusion of Endrinal and Mendoza (1979) who found that the mimosine concentration in mature leaves of tall varieties was less than that in short or intermediate varieties.

Growth stage

In general, seedlings have low mimosine. I have found that seedlings up to 12 weeks of age, even when actively growing, rarely have mimosine concentrations above 2%. Figure 1 shows data from a glasshouse experiment in Townsville where both seedling growth and mimosine concentration were measured over a three-month period. The factors affecting mimosine concentration of seedlings are unknown, but could well be related to nitrogen status and onset of successful nodulation.

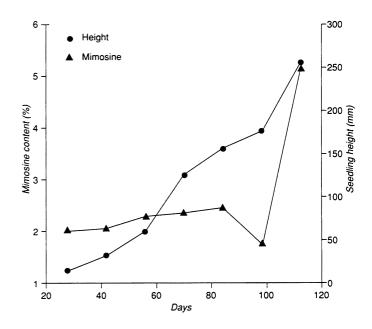


Figure 1. The relationship between growth and mimosine content in leucaena seedlings.

Correlations between mimosine concentrations of seedlings and adult plants are not high. Brewbaker and Hylin (1965) found a correlation of 0.57, but this was in a population of species with very different mimosine concentrations. In a population of 90 F1 hybrids between *L. leucocephala* and *L. pulverulenta*, Bray (1984) found no correlation (r = -0.03) between the means of two seedling mimosine determinations and a field sampling. Given the generally low mimosine status of seedlings, this means that sampling should be delayed until the plants are more mature.

Season

The season and time of year can have a major effect on mimosine concentration. In Townsville, typical values for mimosine concentration of cv Cunningham leaves are 5.5% in summer (hot, wet season) and 3.5% in winter (cooler, dry season). Similar fluctuations have been observed by others (e.g. Gupta et al. 1992; Arora and Joshi 1986). While these concentrations are clearly related to growth rates (better growth, higher mimosine), the actual levels of mimosine are not entirely predictable.

Response to stress

Environmental stresses, such as water shortage, can significantly raise mimosine levels (Bray and Hoekstra 1985). In this work, plants subjected to moisture stress showed an almost immediate elevation of mimosine concentration, and after 14 days had approximately twice the mimosine concentration of well-watered control plants. Moisture stress increased mimosine concentration dramatically, both in newly expanded and in older leaves. This is not unexpected, as many plant species accumulate apparently non-essential compounds in response to moisture stress. Mimosine concentration in stressed leaves remained relatively constant with age, in contrast to leaves of control plants.

Genetic Variation for Mimosine Concentration

There is ample evidence for variation in mimosine concentration in different species of Leucaena (e.g. Brewbaker and Hylin 1965, Brewbaker and Kaye 1981, Chandrasekharan and Govindaswamy 1985, Hauad and Foroughbakh 1991, Hutton 1985). Although the results from different authors differ widely in absolute terms, relativities seem to be fairly well maintained, and it is possible to group species in general terms into those with high, medium and low levels of mimosine. Given that Leucaena leucocephala has medium levels of mimosine, the lowmimosine group probably contains L. collinsii, L. diversifolia, L. escuienta, L. greggii, L. pallida and L. pulverulenta. There is a positive relationship between mimosine concentration and leaflet size, with smaller leaved species generally having less mimosine. However, they all contain significant quantities of mimosine.

One way of obtaining 'low-mimosine leucaena' would be to utilise these 'low-mimosine' species directly. However, as agronomic properties must also be considered, *L. diversifolia* and *L. pallida* are probably the most promising in this regard from within the above group.

Is there variation for mimosine concentration *within* species? Data from the CSIRO collection at Lansdown (Table 1) illustrate both the means and ranges in the different species. These samples were all taken at a single time, with no attempt to standardise growth factors other than using 'second expanded leaves' for sampling. It is not known how

much of the apparent variation within species is genetic. There are several reports of variation in mimosine concentration within *L. leucocephala* (*e.g.* Hutton and Gray 1959, Mendoza 1983), but the long-term reality of these differences is uncertain. The finding by Gonzalez et al. (1967) of relatively low mimosine concentrations in several weedy Colombian accessions of *L. leucocephala* suggests that some accessions need further evaluation.

Table 1. Variation in mimosine concentration between and within *Leucaena* species at Lansdown.

Species	No. of lines Mimosine concent screened (%)		
	sereened	Range	Mean
L. pulverulenta	24	0.80-3.58	2.22
L. pallida	11	0.93-4.63	2.58
L. diversifolia	30	1.56-5.74	3.28
L. leucocephala,	345	2.61-9.40	5.30
L. macrophylla ¹	26	4.05-15.86	9.14

¹ Covers all large-leaflet species

Because of sampling variability, determining genetic differences within populations is difficult. Based on extensive sampling over 28 site/harvest combinations, Bray et al. (1988) showed significant differences in mimosine concentration among 10 accessions of L. leucocephala, but the range of variation was only relatively small (5.6% to 4.1%). I have not been able to detect variation in mimosine concentration among 40 replicated cloned plants of cv. Cunningham, sampled over three harvests. The cross-pollinated species, especially L. diversifolia (ssp. diversifolia) and L. pallida may offer more scope for selection, but I am unaware of any work in this field. The superior agronomic performance (except for psyllid resistance) and feeding value of L. leucocephala means that any decision to exploit another species needs careful consideration.

Breeding for Low Mimosine

There have been several suggestions that it should be possible to breed for low mimosine based on variation within *L. leucocephala* (e.g. Hutton and Gray 1959; Mendoza 1983) but there have been no apparent successes, probably because of the very limited variation available. Given that only very limited progress will be possible through selection within *L. leucocephala*, or through direct usage of other species, what other possibilities are there? The only breeding work for low mimosine that I am aware of, and that has been carried through to field trials, has used interspecific hybrids between L. leucocephala and L. pulverulenta. This has taken two paths: the direct exploitation of the F1 interspecific hybrids (Bray 1984) and a breeding program involving the selection of fertile progeny after backcrossing the interspecific hybrids to L. leucocephala (Bray et al 1984, Hutton 1985). The F1's were very vigorous, and outvielded standard L. leucocephala cultivars across a range of sites (Bray 1984, Bray et al. 1988) as well as having lower mimosine. Of course, seed production of these hybrids presents a continuing challenge. Seed orchards were set up with scions of L. pulverulenta on L. leucocephala rootstocks used as female parents, and pollinated by L. leucocephala as males. Unfortunately, crosspollination was not complete, and many selfed seeds of L. pulverulenta resulted. Other parental clones of L. pulverulenta may have been a better choice.

Hutton's breeding program did produce lines with lower mimosine, but these (after two backcrosses to L. leucocephala) did not yield well enough, compared to the standard cultivars, to be worthy of commercialisation. Although the mimosine concentration of the bred lines was about 70% of cv Cunningham, so was the dry matter yield. The low-mimosine lines still had a mimosine concentration of about 4% when actively growing. In this material, there was evidence of a positive genetic correlation between mimosine concentration and growth rate, illustrating the difficulty of the task of combining low mimosine with good growth. Further backcrossing to L. leucocephala was accompanied by a reduction in the number of vigorous low mimosine segregates (Hutton 1985).

Brewbaker's current breeding program at the University of Hawaii is aimed primarily at psyllid resistance, and uses interspecific hybrids (*L. leucocephala* x *L. diversifolia* and *L. leucocephala* x *L. pallida*). These hybrids may have the potential to produce low-mimosine progeny, as both *L. diversifolia* and *L. pallida* tend to be in the low-mimosine species group. Hybrid populations may contain some desirable segregates. Hutton's long-term breeding program for acid-tolerance, using hybrids between *L. leucocephala* and *L. diversifolia*, could also have the potential for providing low-mimosine segregates.

Other Possibilities for Developing Low-Mimosine Leucaena

Knowledge of the biosynthetic pathway of mimosine (and its regulation) is very incomplete (Romeo 1989). However, it is known that the precursors of mimosine include lysine, and also probably pipecolic and hydroxypipecolic acid (Hylin 1964). It is probable that there are as many as ten enzymatic steps from lysine to mimosine.

The use of induced mutations may at first glance seem attractive, but R.D. Brock (pers. comm.) has calculated that, assuming diploid inheritance, some 25 000 M2 progeny would need to be screened to have a 90% chance of finding a mutation in the mimosine pathway. Given the polyploid nature of the species, it is quite likely that the actual number that would need to be screened would be greater by several orders of magnitude. In addition, the mutation would probably have to be close to the mimosine end of the pathway to avoid upsetting synthesis of other vital plant compounds. A better approach might be to start with one of the diploid species, which may offer a better chance of recovering a suitable mutation. However, these are all outcrossing and not particularly promising agronomically.

Molecular biology offers potentially exciting possibilities. One method of attack might be to identify the key enzyme systems in the last few steps of mimosine synthesis, and the genetic code that controls them. Gene shears technology might then be used to stop the gene action. However, this sort of approach would require detailed knowledge of the biosynthesis of mimosine, considerable inputs from protein biochemists, and the existence of a transformation system for leucaena (of which I am unaware). Clearly, this would be a long-term project (possibly ten years?), but may offer the only hope of no mimosine leucaena. Another molecular approach would be to use segregating populations to develop molecular markers for the mimosine gene(s). However, if we already had clearly segregating populations, would we really need to rely on molecular biology? Still another approach might be to try to transfer the DHP-degrading gene from the rumen bacteria Synergistes jonesii into L. *leucocephala*.

Possible Undesirable or Unexpected Effects of Low Mimosine

Low-mimosine progenies derived from *L. pallida*, *L. diversifolia* and *L. pulverulenta* will almost certainly have higher levels of tannin and possibly lower digestibility and lower leaf:stem ratio than existing cultivars of *L. leucocephala*. All these factors could reduce the nutritive value. Using a high proportion of older leaves in leucaena meal would give lower mimosine levels, but would also mean lower protein (Tangendjaja et al. 1986). This may or may not be acceptable depending on what factor is limiting the use of leucaena. In monogastric animals, many other factors as well as mimosine concentration may reduce the value of leucaena as a feed, including tannins, lignin and saponins. For example, removing mimosine does not make leucaena a good feed for poultry (D'Mello and Acamovic, 1989). Low-mimosine leucaena will still not be the perfect feed.

In our breeding program described above, there was a genetic correlation of 0.6 between plant weight and mimosine concentration, suggesting that selecting for low mimosine may well be interfering with other vital growth factors. Whether selection that specifically reduces mimosine would affect growth is not known. The low-mimosine accessions of Gonzalez et al. (1967) were all unproductive agromically, and it may prove difficult to combine high leaf production with very low mimosine concentration.

Leucaena as a genus is fairly free of pests and diseases, and this could be due in part to the presence of mimosine, which is toxic to some common legume pathogens (Ebuenga et al. 1979, cited by Romeo 1989) and insects. I have observed that glasshouse-grown low-mimosine selections appeared to be more susceptible to scale insects than the normal varieties. If this is a general phenomenon, care needs to be taken not to create a whole new set of problems for the plant by removing a possible defence mechanism.

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An Update on the Status of the Leucaena Psyllid in Southeast Asia

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Abstract

This paper provides a brief update on major research findings since 1989 regarding the leucaena psyllid, *Heteropsylla cubana*, a serious pest of *Leucaena leucocephala*. New findings cited here include temperature- and humidity-specific life table studies of *H. cubana*, laboratory comparisons of two *H. cubana* parasitoids, information on fungal pathogens of the pest, summaries of its economic impact in Southeast Asia and Australia, and updates on its spread into Africa. Suggestions are made for research priorities for managing pest problems for *L. leucocephala* and for other multipurpose tree species. Surveys are recommended for potential pests of these trees, both in native and exotic ranges, to avoid bad infestations in future. It is suggested that agroforestry technology packages aim for species diversification.

IN the past ten years, the leucaena psyllid, Heteropsylfa cubana Crawford (Homoptera: Psyllidae), has spread westward from its native range in tropical America to almost encircle the globe. As the primary pest of the widely planted multipurpose tree Leucaena leucocephala (Lam.) de Wit (Leguminosae), H. cubana has had an immediate, costly, and highly visible impact. The infestation has initiated two major international meetings (Withington et al. 1987; Napompeth and MacDicken 1990a) and a great deal of research. Napompeth (1990) and MacDicken (1987) have reviewed the topic, and Heydon and Affonso (1991) have reviewed its economic impacts, with a new review in preparation by Geiger et al. This paper briefly summarises selected research on the topic since the conference in 1989, and suggests implications for further research and development of Leucaena and other multipurpose tree species (MPTS).

Update on the Impact of the Leucaena Psyllid

Since 1989, *H. cubana* has continued its rapid spread westward, infesting the entire Indian peninsula in 1989 (Singh and Bhandari 1989), Mauritius and Reunion in 1991 (Hollis 1992), Tanzania and the Kenya coast in early 1992, reaching Uganda, Burundi, and Sierra Leone (unconfirmed) later that year (G. Hill, IIBC 1993, pers. comm.), and most recently Khartoum, Sudan (W. Ciesla, pers. comm.).

Psyllid damage varies widely from location to location. More severe damage is generally observed in areas with pronounced dry seasons, although leucaena psyllid populations may fall to very low levels during extended hot, dry periods (Napompeth 1990; Sanchez 1990; Villacarlos et al. 1990). Data on the role of seasonality are conflicting. From 1989 to 1991 in East Java, which has distinct seasons, insecticide-check studies found the psyllid caused 28% forage and fuelwood losses, reducing plant height more than biomass (Darma et al. 1992). In wetter areas of West Java, fuelwood production losses averaged 56% during the dry season and 41% in the rainy season (Intari et al. 1992a). In Australia, Room et al. (1993) measured psyllid damage at seven sites with varying microenvironments, and found a 3-75% loss in dry weight production (site average 36%), on the least productive and second most

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productive sites, respectively. These figures may be overstated, since the effect of grazing on both insect and tree was not measured.

The use of damage estimates is further complicated because the degree of damage probably depends on both tree regeneration and sizes of psyllid populations. The pest may have larger impact during low growth periods if there is little alternative fodder (Room et al. 1993).

A long-term trend has been observed. Damage is generally heavy in about the first two years of infestation, then gradually weakens in duration and severity. In Indonesia, the Philippines, Thailand and Australia, the first year of infestation caused an estimated US\$525 million in damages, with US\$316 million in Indonesia alone (Oka 1990; Heydon and Affonso 1991). On one large Central Java coffee estate, 11% of trees died in 1986, the first year of psyllid infestations. Only 5.25% of trees were killed in 1987, and the percentage has decreased steadily since then (Mangoendihardjo et al. 1992). Similarly, in the Philippines, psyllid damage to forage production peaked at around 80% in the second year of infestation, but has declined gradually ever since (Villacarlos et al. 1990). By late 1993, leaf meal production had 'normalised' in the Philippines (F. Sanchez, pers. comm.), and plantings on Mindanao have continued to expand. Plantations in wetter areas of Indonesia are green again. Reports attribute the recovery to ambitious biological control efforts (Mangoendihardjo and Wagiman, 1990; I.N. Oka, pers. comm.).

Update on Leucaena Psyllid Biology and Ecology

Since the 1989 conference in Bogor, 50 or more papers have been published on H. cubana, though only a few are discussed here.

In their recent revision of the genus *Heteropsylla*, Muddiman et al. (1992) identified the true host plants for *H. cubana* as *Leucaena leucocephala* (Lam.) de Wit, *L. trichodes* (Jacq.) Benth., *L. pulverulenta* (Schlecht.) Benth., *L. diversifolia* (Schlecht) Benth., and *L. salvadorensis* Standley ex Britt. & Rose. The revision provided keys to both adult and late instar nymphs.

Microbial pathogens of *H. cubana* have been further characterised, particularly by Villacarlos and Robin (1989; 1990), and four new species of Entomopthorales are currently being described. Patil and coworkers compared two Hymenopterous parasitoids of *H. cubana, Psyllaephagus yaseeni* Noyes (Encyrtidae) and *Tamarixia leucaenae* Boucek (Eulophidae) in laboratory tests (Patil et al. 1992a; Patil et al. 1992c). They concluded that *P. yaseeni* is potentially better for biocontrol releases. Patil et al. (1992b) also conducted temperature- and humidity-controlled life table experiments with *H. cubana*, and reported that humidity has little effect on psyllid mortality or development as long as plants are kept well watered. Temperature, however, showed major effects, with an upper developmental temperature threshold apparently existing in the range $30-35^{\circ}C$.

Psyllid-resistant *Leucaena* species are discussed in detail elsewhere in these Proceedings. Within the species *L. leucocephala*, var. K636 is psyllid-tolerant while maintaining good growth and form. Hybrid trials have shown much promise, but logistics for seed distribution are more difficult.

Update on Control Strategies

In comparative studies of the two major biological control agents for H. cubana, the coccinellid predator Curinus coeruleus Mulsant appeared more useful than P. yaseeni (Intari et al. 1992b). However, their comparative effectiveness has not yet been rigorously quantified. Mass releases of C. coeruleus are reported to have been responsible for a significant reduction in psyllid damage in wetter areas of Indonesia. Forty C. coeruleus individuals per tree (180 growing shoots) were required to minimise psyllid damage (Wagiman et al. 1989). In drier areas, C. coeruleus has proved difficult to establish (Oka, 1990; Sanchez, 1990; C. Doungsa-ard, pers. comm.), while P. vaseeni establishes and disperses quite readily, having reached both Malaysia and the Philippines without human intervention (Guan-Soon Lim, MARDI, 1993, pers comm.; F. Sanchez, UPLB, 1993, pers. comm.). Tamarixia leucaenae has not yet been successfully introduced for biocontrol. Chemical controls are not recommended except for nursery stock (Napompeth and MacDicken 1990b).

Research Priorities for Leucaena Pest Management

The search for potentially damaging pests of other *Leucaena* species should be of high priority (see below). In addition, field or on-farm experiments could explore ways of maintaining natural enemies, such as *P. yaseeni* and *C. coeruleus* populations, in leucaena plantations. For example, interplanting leucaena with plant species preferred by the predators' alternate prey, such as scales or mealybugs, could provide the predators with a food source through the dry season when psyllids are scarce. On-farm experiments could also test simple,

farmer-based methods for culturing or inoculating entomopathogenic fungi in leucaena plantations.

More temperature/humidity-specific life table studies are needed to pinpoint maximum and minimum temperature thresholds for development. Studies relating phloem composition to psyllid growth might yield useful information on the nature of resistance. Finally, a systems approach to analysing leucaena psyllid outbreaks is required, using models based on measured parameters, and validated against actual data.

Research Priorities for Pest Management in Other Multipurpose Tree Species

The story of the leucaena psyllid should be a cautionary tale, warning against the introduction of a single species on a massive scale, as occurred with *L. leucocephala*. Surveys conducted in the Philippines following the psyllid attack showed that farmers lost significant amounts of income, and lost confidence in external recommendations (Sanchez 1990). As a result, progress towards larger goals (e.g. reforestation) has also been set back.

Infestations can be particularly destructive when pests follow their host plants to exotic environments, free from the natural enemy complex of their native areas. In the case of the leucaena psyllid, damage in Indonesia and the Philippines amounted to more than half a billion US dollars within the first year alone. Most other multipurpose tree species (MPTS) are also introduced species, and therefore similarly vulnerable to attack. The genus Heteropsylla alone contains psyllids that feed on Acacia angustissima, A. villosa, A. farnesiana, A. glomerosa, Albizia adinocephala, A. saman, Calliandra houstoniana, and Prosopis juliflora, among others (Muddiman et al. 1992). Although the potential for outbreaks is largely unknown, most agencies promoting agroforestry technologies devote little or no attention to potential pest problems on the trees they introduce.

Poor farmers greatly dislike risk. If a productive new technology is to be truly beneficial, then not only must it carry as little inherent risk as possible, but also it must allow farmers to hedge their own bets knowledgeably. For example, farmers could grow tree varieties in preliminary on-farm trials. Clearly, agroforestry researchers should investigate all existing and potential pest risks as thoroughly as possible. Agroforestry extension officers should also introduce new agroforestry systems as sets of options, offering farmers a range of species and varieties to try out and adopt, instead of introducing single species in isolation. To assess pest risks researchers need some of the following information:

- (i) the tree's known pests in its native range and their natural enemies, in a variety of environments - especially in areas with distinct seasons, where the potential for outbreaks may be higher.
- (ii) the pests' potential to be involved in outbreaks in their exotic range, based on
 - (a) taxonomy of the insect (similarity to known outbreak species)
 - (b) known history of severe outbreaks in native and introduced range
 - (c) biological potential for outbreaks, judging by population parameters and other laboratory-determined quantities
 - (d) characterisation of native natural enemy complexes.

Since such information is international in scope, major international agroforestry or biological control research organisations should take the lead in such a preventative program, by taking the following steps:

- augment permanent staff dealing with pests and diseases of MPTS
- conduct thorough literature searches on pests of MPTS in various regions
- institute a series of regional surveys of these trees for pest insects, in a variety of environments and seasons
- screen the data for potentially serious pests
- if feasible, conduct basic laboratory tests on likely pests to further clarify their potential
- create a database to record the findings, preferably integrated with existing databases on MPTS. Make information easily available to researchers and extension workers worldwide
- discontinue single-species promotion. Instead, offer technology packages with scope for diversified systems, farmer experimentation, and the possibility of unforeseen calamities such as the leucaena psyllid.

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Diseases and Pests of Leucaena

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Abstract

This paper reports recent developments concerning pests and diseases of leucaena. The results of a pilot assessment of diseases in native populations in Central America and Mexico are presented, together with a list of important diseases that are already established. The latter include Camptomeris leaf spot, gummosis, and various root and stem rots. Future introductions of germplasm from leucaena's centre of origin may be at risk from rust fungi. Future research priorities are identified, and include: collection, publication and dissemination of all pest records; multi-locational screening for pests; more intensive study of pests of known importance; pest identification manuals; and seed testing in order to establish quarantine guidelines. The paper also stresses the need for high quality and timely advice on pests if plant damage is to be minimised.

This paper outlines new developments in the field of leucaena diseases and pests and provides an assessment of research priorities. Unless otherwise specified, the term 'pests' in this paper will generally refer to both insect pests and pathogens (i.e. diseasecausing organisms such as fungi, bacteria and viruses).

For those growing or working with leucaena, pest problems are a relatively new concern. The earliest record for Camptomeris leaf spot (CLS) dates from Puerto Rico in 1919 (Hughes 1952). However, only since the early 1980s have CLS and other significant diseases and insect pests been recorded, coincident with the increase in planting of leucaena.

Several recent reviews have collated published data on the diseases of leucaena, the most comprehensive being that of Lenné (1991). To our knowledge, there have been no similar reviews of leucaena's insect pests, although Pratap and Sujan Singh (1987) and other forest entomology texts do make some reference to such. An annotated bibliography of nitrogen-fixing trees is being published by the Natural Resources Institute (NRI) in 1994. Apart from the obvious exception of the leucaena psyllid, no major insect pest is currently known. We cannot afford to be complacent, however — we need to be aware of potential pest problems rather than simply waiting to react to epidemics (the 'fire brigade' approach).

Known and Potential Pests of Leucaena

The important diseases known to affect leucaena are shown in Table 1. As most of these derive from published records relating to *Leucaena leucocephala*, there is a great need to identify pests and diseases that may affect other species, especially as the genetic base of plantings is being widened.

Preliminary work on diseases in natural populations of *Leucaena* spp. was conducted as part of a broader project (funded by the Forestry Research Programme of the Overseas Development Administration, U.K.) which included work on *Gliricidia sepium* and *Calliandra* spp. in Central America and Mexico. Working closely with CONSEFORH in Honduras during a one year period, the research included three extensive surveys in Honduras, Guatemala, Belize and Mexico. The results, and an indication of pest problems assessed to be of quarantine importance, are shown in Table 2.

Only two groups of diseases warrant moderate significance for quarantine purposes, and of these the rusts are the most important (Table 3). Anthracnose is known to attack leucaena pods in the Philippines and may be of more importance than previously thought. Pod rots (another potentially important but little studied group of pathogens) have been reported as causing extensive damage in Mexico (C. Hughes, pers. comm.) and *Fusarium* spp. may be associated with these attacks. Most of the disease records related to leucaena (i.e. to *L*.

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Condition	Pathogen	Country	Notes
Camptomeris leaf spot	Camptomeris leucaenae	Central and South America (widespread); Mexico; Caribbean; Taiwan; Philippines; India	Reduces forage yield and quality. Resistance identified (eg. Lenné 1980). Major problem where annual rainfall >2000 mm.
Gummosis	Not identified. Dispute concerning involvement of <i>Fusarium</i> .	India; Sri Lanka; Hawaii; Taiwan	Described as most serious disease of <i>Leucaena</i> <i>leucocephala</i> in India and Sri Lanka
Ganoderma root rot	Ganoderma lucidurn; G. applanatum; G. tornatum	India; Papua New Guinea	Ganoderma causes widespread damage throughout the tropics on a wide variety of woody hosts.
Root and Stem rot	Pseudolagarobasidium leguminicola	Taiwan	Sporadically important, related to soil type.
Stem canker	Pirex subvinosus (anamorph — perfect stage — Hydnum subvinosum)	India; Australia	Considered a serious threat to cultivation of <i>L</i> . <i>leucocephala</i> in recorded countries.
Blight canker	Fungal complex: Calonectria rigidiuscula (Fusarium decemcellulare) + F. roseum	Taiwan	Sporadically important, related to soil type.
Bacterial pod rot	Pseudomonas fluorescens Biotype II	Belize; Brazil; Colombia; Mexico; Honduras; Guatemala; Panama	Cvs. Cunningham and Peru particularly susceptible under humid conditions in Colombia.
Fusarium pod rot and seedling disease	Fusarium spp.	Colombia; Brazil; Philippines; Taiwan; Papua New Guinea; Malaysia	Losses of 10-13% reported from nurseries. Seed reduction due to pod rot not quantified.
Fusarium root rot	F. oxysporum; F. moniliforme var. subglutinans; F. solani	Sri Lanka; India; Mauritius	Increasing problem in fodder plantations and shade plantings in Sri Lanka.

Table 1. Important diseases of leucaena recorded in the literature¹ (adapted from Lenné 1991).

¹ Note that the majority of published records are from *Leucaena* leucocephala with isolated examples from L. *diversifolia*, L. *esculenta*, L. *lanceolata*, L. *pulverulenta* and L. *shonnonii*

leucocephala) have involved fungi known to have broad host ranges. With the exception of CLS, there is little evidence of diseases that have moved from native populations. However, several rust fungi have been recorded from native populations, as well as one from Taiwan (Table 3). Although none are known to cause serious damage, they may cause more impact under different growth conditions, as happened with the rust *Ravenelia hermosa* on *L. salvadorensis* in trials in Honduras (Table 2).

Relevant Advances in Pest Management

Foliar symptoms associated with nutrient disorders in *L. leucocephala* may be confused with disease or

insect pest attack, but a recent publication helps distinguish the differences through an excellent series of colour photographs (Smith et al. 1992). This publication also provides a good model for the disease identification manuals suggested below.

Significant advances in plant pathology have been made in the last decade, especially concerning the diagnosis and characterisation of pathogens through biotechnology. Although almost all this work has been related to annual crops, the outcomes have included an array of powerful new tools with potential application to leucaena diseases such as ganoderma and fusarium rots. For example, NRI has commissioned work by the International Mycological Institute in the UK to examine the biological

Species	Disease	Country	Importance	Quarantine	
L. collinsii	Camptomeris leaf spot — CLS (Camptomeris leucaenae)	Guatemala	Moderate damage seen at one site in this study. (CLS is only known to cause major losses in areas with annual rainfall >2000 mm).	Minor significance. Select healthy planting material. Seed transmission unlikely but could be on debris.	
	Pot damage (anthracnose) (<i>Colletotrichum</i> sp.)	Honduras	Minor damage seen, but damage could be more serious and distribution wider at other times of the year. Disease known from India and Philippines.	Moderate significance. Care should be taken in selection of seeds and sifting debris. New record for this host.	
L. lanceolata (probably)	Little leaf (possible MLO)	Honduras	On broad-leaved species in CONSEFORH trial - one specimen only. Further study may be necessary.	Possible significance. Need to be alert to similar symptoms on other <i>Leucaena</i> species.	
<i>L. leucocephala</i> (includes K series accessions from Hawaii used in trials in Guatemala)	Rust (unidentified)	Honduras	Blight and dieback.	Possible significance.	
	Rust (Ravenelia hermosa)	Honduras	Roadside trees. Minor damage observed.	Rusts need closer examination — a group of potentially important pathogens.	
	Camptomeris leaf spot	Guatemala; Honduras	Noted in Guatemala on plants grown from Hawaiian seed.	Minor significance.	
	Bacterial pod rot (Pseudomonas fluorescens)	Guatemala; Honduras; Mexico	Minor damage on isolated trees. Pod rot has been recorded on other species.	Minor significance. Widespread bacterial species.	
	Mildew	Honduras	Minor damage. Recorded from Solomon Islands.	Minor significance.	
L. salvadorensis	Rust (Ravenelia hermosa)	Honduras	First observed attacking pods in CONSEFORH trial, but also in other trials and natural populations. Associated pod rot included attack by <i>Fusarium</i> sp. (det. H.C. Evans, IIBC)	Moderate significance.	

Table 2. Significance of notable diseases seen on *Leucaena* spp. in native populations in Central America and Mexico (from Boa and Lenné 1993).

Species	Disease	Country	Importance	Quarantine
	Mottle (possibly a virus)	Honduras	Unconfirmed virus attack seen in a trial. One of only two records of virus/MLO type symptoms on Leucaena.	Possible significance. If confirmed as a viral disease would require further study.
L. shannonii	Rust (Ravenelia hermosa)	Guatemala	Only one record from natural population; wider distribution seen from herbarium specimens.	Moderate significance. Minor damage - linked to dieback?
	Dieback (cause unknown)	Guatemala	Indirect evidence that dieback is more general problem. Could be linked to rust. Needs further study.	Possible significance. Pathogen link needs to be demonstrated.
Leucaena sp.	Bacterial pod rot	Mexico	Seen in market.	Minor significance.

Table 2. Significance of notable diseases seen on Leucaena spp. in native populations in Central America and Mexico (from Boa and Len& 1993). (continued)

Table 3. Rusts on leucaena recorded in the literature and from surveys of natural populations and herbarium records (from Len& 1991; Boa and Lenné 1993; and unpublished records).

Host'	Rust*	Country	Notes
Leucaena sp.	Dicheirinia spinulosa	Mexico	Lenné 1991
1	Ravenelia hermosa	USA	Lenné 1991
	R. leucaenae	Mexico	Lenné 1991
L. collinsii	To be identified	Mexico	Herbarium specimen (one accession)
L. diversifolia	R. leucaenae	Mexico	Lenné 1991
L. esculenta	R. leucaenae	Mexico	Lenné 1991
L. lanceolata	R. hermosa	Mexico	Lenné 1991
	To be identified ³	Mexico	Herbarium specimens (17)
L. leucocephala	R. expansa	Guatemala	Lenné 1991
	R. hermosa	Honduras; India	Boa and Lenné 1993;
	Uredo leucaenaglaucae	Taiwan	Lenné 1991 Lenné 1991
	Uredo sp.	Jamaica	Lenné 1991
	To be identified	Honduras; Nicaragua	Herbarium specimens (2)
L. macrophylla	To be identified	Mexico	Herbarium specimen (1)
L. pulverulenta	R. leucaenae	USA	Lenné 1991
L. puiveraienia	To be identified	Mexico	Herbarium specimens (2)
L. salvadorensis	R. hermosa	Honduras	Boa and Lenné& 1993
2. 50070000701000	To be identified	Honduras	Herbarium specimens (2)
L. shannonii	R. hermosa	Guatemala	Boa and Lenné 1993
	To be identified	Honduras; Mexico; Nicaragua	Herbarium specimens (9)

¹ Does not include subspecies ² *Ravenelia* specimens are difficult (impossible?) to identify where teliospores are absent ³ Where only one rust species has been previously identified on a host species the 'unidentified' specimens are probably the same rust.

characterisation of strains of *Ganoderma* found on oil palms, using isozyme studies and DNA techniques to determine the relatedness of isolates from different sources. The results from this still continuing study promise improved techniques for studying *Ganoderma* isolates from other hosts, such as leucaena.

The Need to Understand Pests

Despite the good general awareness of leucaena diseases, and an increasing knowledge of the leucaena psyllid, we still lack sufficient information to provide effective pest management. For example, gummosis is possibly the most important disease of leucaena in India, yet its cause is still disputed (Lenné 1991). Similarly, little is known of ganoderma root rot, another serious disease.

The widespread and severe damage caused by the psyllid has heightened awareness of the importance of understanding leucaena's pests. The response to the psyllid was hampered by a lack of basic information on its biology and behaviour, and the time taken to 'catch up' on this knowledge allowed the pest to spread. Apart from basic knowledge, dealing with any pest requires that control options are assessed carefully through the evaluation of scientifically designed trials. The latter inevitably take time and require a certain resilience in obtaining the funds for research and completing the trials while eager demands are being made for a 'magic bullet' form of pest control. Unfortunately, encouraging unsubstantiated claims for specific pest management regimes may result in far more damage than doing nothing. To counter this, we hope that LEUCNET will facilitate support for more fundamental and applied work related to diseases of both known and potential importance. We must move from having a general awareness of leucaena's pests to a more specific understanding of key diseases such as gummosis, ganoderma root rot and the rusts.

Only when this more detailed information is available can the issue of quarantine — a generally neglected area for multipurpose tree species — be addressed. Effective quarantine, for all species including leucaena, allows for the safe movement of germplasm without placing excessive restrictions on new introductions. While quarantine controls are rarely applied to tree seed in developing countries, the lack of adequate data may lead to unwarranted exclusions on the grounds that no knowledge equals bad knowledge. For example, in Australia leucaena introductions have been permitted from Hawaii but not from the native populations. Yet our recent assessment of disease risks (Table 3) has shown that there are more disease problems reported from Hawaii than from the native ranges. We hope that this kind of information will allow wider and safer introductions in future.

Information on seed-based transmission of pathogens is also needed. No work on this has yet been done, although a scientific study has been proposed. We hope that LEUCNET will help in getting this study started.

There is a significant need to provide regular research input into the issues related to leucaena pests (again in the wider context of multipurpose trees). As scientists involved with development, we are concerned with increasing the efficiency of agriculture and related activities to do with livestock and forestry. This often means suggesting new ways of doing things, different ways of growing existing species or introducing new species. Clearly, the confidence of participating farmers is a key to success in these schemes. Pest problems that arise during innovative projects can severely reduce farmer confidence, even though the long-term effects may be negligible. For example, we can cite one specific case where a minor disease problem in *Gliricidia sepium* in Honduras convinced the farmer not to adopt that planting scheme. More generally, many farmers must have had their confidence in L. leucocephala shaken by the appearance of the psyllid.

In general, therefore, the key to successful control of any pest is prevention rather than cure. Only in nurseries can chemical control methods achieve any lasting reduction in pest damage. Effective pest management is impossible without adequate information on the nature, characteristics, behaviour and interactions of pest organisms and the host. We also need timely and accurate information on where and when pest problems occur, based on the abovementioned in-depth studies (many of which could be carried out in-country with both national and international supervision).

Future Research Priorities

From the above discussion, we have prepared a list of research priorities in this field (listed in approximate order of importance).

- Collection, publication and dissemination of all available disease and pest records is essential.
- Multi-locational screening for disease and pests.
- Manuals for identification should be developed: as diagnosis is the key to pest control, visual assistance through illustrated manuals will help to alert growers and others to pest problems.
- More intensive study is required of those diseases and pests already established as of current or potential importance. This study should include

(i) quantification of importance, determination of seed association (seed testing) and research on management; (ii) relationships between different pathogens (eg. rusts, scab) that have broad host ranges among legumes (including crossinoculation and pathogenic variation studies). This also requires the identification of senior national and international scientists to lead this work and provide supervision for in-country students to study specific problems.

- Preparation of guidelines for disease management, including quarantine, should be developed based on the data collected. Seed testing is an essential precursor of any decision to establish quarantine regulations.
- Closer working relationships among pest scientists and foresters/agroforesters working with leucaena should be fostered. A list of national and international scientists specialising in pest management of leucaena or related tree species should be made widely available through LEUCNET.

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