

Genetic Diversity and Improvement in Leucaena

Potential for Improvement of *Leucaena* through Interspecific Hybridisation

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Abstract

The interspecific hybrid approach to leucaena improvement is reviewed. *Leucaena* species hybridise readily (76%) giving breeders the opportunity to transfer genes of interest from wild species into breeding populations and cultivated species. Techniques have been developed to help make the approach practical. Several interspecific hybrids appear promising on the basis of their growth rate and combinations of traits. Aspects requiring further research, including appropriate deployment of interspecific hybrids, are considered.

THIS paper describes the potential for improving leucaena by breeding useful hybrids between species. It reviews:

- reasons for using hybrids;
- hybrids that seem promising;
- technical advances that help make hybridisation possible;
- particular breeding strategies;
- heterosis (hybrid vigour) and its importance to interspecific leucaena hybrids.

Leucaena species hybridise far more readily than do most other herbaceous and semi-woody plant genera. Ninety-one of the 120 possible interspecific combinations among 15 leucaena species are compatible (Sorensson and Brewbaker, in press), in contrast to 5% compatibility when alfalfa species are combined (Quiros and Bauchan, 1988).

Tropical tree genera, for example *Eucalyptus*, *Erythrina* and *Acacia*, generally tolerate crossing between species. Trees may be tolerant of wide hybridisation either because of genetic redundancy in their DNA or because tree species often evolved in geographical, phenological and ethological isolation from related species, and thus have had little or no pressure to develop barriers to hybridisation.

Reasons for Hybridising *Leucaena* Species

The main reason for breeding interspecific hybrids is that it enables us to move genes from species that have useful traits to others that lack them. *Leucaena leucocephala* is widely-grown and has excellent qualities, including adaptability to a range of sites and agronomic practices, drought resistance, forage palatability/digestibility and bypass protein, high yields of leaf and wood, aggressive coppicing, wood pulping characteristics, and nitrogen fixation. However, it also has several limitations. For example, it sets a great deal of seed, exhibits seasonality of growth, is sensitive to attack by psyllids (*Heteropsylla cubana*), and yields poorly when grown at cool sites (below about 15°C) or in acid soils with high available aluminium to calcium ratio. Other negative aspects of *L. leucocephala* include slow seedling establishment, high mimosine content, intolerance to waterlogging, and the susceptibility of its postwood to termites.

At least half of the lesser-known species in the genus *Leucaena* display superior qualities that we would like to transfer to *L. leucocephala*. For example, four diploid species (*L. collinsii*, *L. diversifolia*, *L. esculenta* and *L. pallida*) are highly resistant to psyllids (Sorensson and Brewbaker 1986; Bray et al. 1990). *L. diversifolia* is also regarded as a source of genes for cool temperature tolerance (Brewbaker et al. 1988) and acid soil tolerance (Hutton 1990). *L. esculenta* and *L. pallida* have high seedling vigour (Sorensson et al. 1994).

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There are other reasons for attempting to breed interspecific hybrids in the genus *leucaena*.

- They may be adapted to a wider range of sites.
- They may be highly vigorous (heterosis) and out-yield their parents.
- The hybrids may be unique biochemically because of their genetic diversity, as expressed by compounds such as multimeric enzymes.
- Segregating interspecific hybrids exhibit an enormous range of phenotypes, which at least indicate genetic recombination, if not high genetic diversity.
- Interspecific hybrids of tropical trees generally set less seed. Seedless *leucaena* hybrids would be useful in some agricultural production schemes.
- If superior interspecific hybrids were identified, they might form the basis of a commercial seed industry.
- Interspecific hybrids could have unique combinations of the phenotypic traits desired for horticultural use in gardens and landscapes.

Promising *Leucaena* Interspecific Hybrids

The first notable interspecific hybrids to be reported in the literature were those of *L. pulverulenta* x *L. leucocephala* in Indonesia. In contrast to *L. leucocephala*, which is a self-compatible tetraploid, *L. pulverulenta* is outcrossing. About a hundred years ago, *L. pulverulenta* was introduced as a shade crop for coffee in cool upland sites where *L. leucocephala* was relatively unproductive. When occasional interspecific hybrids formed as a result of cross-pollination by bees, they were apparently identifiable by their outstanding growth rates and low seed production (advanced generation lines that segregated for seedless individuals). Although this particular hybrid is quite susceptible to psyllids, and is therefore not appropriate for planting in many parts of the wet tropics, it is potentially useful for arid parts of India (V. Gupta, pers. comm.).

In northern South America where *leucaenas* originated, cultivated species like *L. esculenta* have been moved outside their original range, providing opportunities for species to hybridise. There is now substantial evidence that interspecific hybridisation has played a significant role in forming new species, particularly with regard to the four tetraploid species that all appear allopolyploid (Hughes and Harris these Proceedings). The offspring of *L. esculenta* x *L. leucocephala* is one of these natural hybrids. It occurs sporadically in south central Mexico, and often grows to impressive dimensions (Hughes and Harris 1994). The seed sterility of this natural hybrid has been verified in hybrids artificially bred in Colombia and Hawaii (Sorensson 1987; Hutton

1988). Segregants among F1 *L. leucocephala* K8 x *L. esculenta* K138 produce gums (Brewbaker and Sorensson, 1990) with some structural similarities to gum arabic.

Two other triploid interspecific hybrids that show promise for cultivation are *L. diversifolia* (n = 26) x *L. leucocephala* and *L. pulverulenta* x *L. diversifolia* (n = 52) (Brewbaker and Sorensson, 1990). Although F1 hybrids segregate some unthrifty seedlings, most are very productive. Triploids of *L. diversifolia* x *L. leucocephala* set few seeds, have readily digestible leaf dry matter, are highly psyllid-resistant and can apparently tolerate frost (Sorensson 1987, 1993; Gutteridge and Sorensson 1992). Triploids of *L. pulverulenta* x *L. diversifolia* are essentially seedless, highly psyllid-resistant, and potentially good wood producers, but appear to have low forage quality (Sorensson 1993).

The hybrid *L. diversifolia* subsp. *diversifolia* (4n) x *L. leucocephala* has caused wide interest, and has been evaluated fairly thoroughly internationally in the Nitrogen Fixing Tree Association *Leucaena* Psyllid Trials and *Leucaena* Seed Production Trials. This self-compatible tetraploid is based on two parents that are relatively uniform, and is itself quite uniform in the F1 generation. Advanced generation progeny segregate individuals with exceptional vigour and tolerance to cool conditions. Although most of them are prolific seeders and only moderately psyllid-resistant (Wheeler and Brewbaker 1990), a recurrent inbreeding selection program could produce lines from this hybrid with excellent qualities for wood production. Moderate levels of psyllid resistance are considered to be adequate for wood production.

Perhaps the most exciting hybrid is *L. pallida* K748 x *L. leucocephala* K636 (= CPI 84581 x PI 443740). In replicated two-year-old forage trials conducted against eleven species in Hawaii, and against six species in Brisbane, Australia, this hybrid yielded more total above-ground biomass and edible dry forage than the other species (Castillo 1993; M. Austin and colleagues, unpublished). Its seedling vigour was also superior (Sorensson et al. 1994). Total seedling weight of *L. pallida* x *L. leucocephala* hybrids was more than double that of *L. leucocephala* K636 on day 84, both in pots in the absence of psyllids, and in the field with psyllids present.

This hybrid (referred to in Hawaii as KX2) is self-incompatible in the first generation (Sorensson 1989a) but segregates self-compatible individuals in later generations that can be selfed and entered into recurrent selection programs. It usually has a spreading habit with basal branching. Like *L. diversifolia* (4n) x *L. leucocephala* (= KX3), KX2

segregates wildly in later generations. An apparent association between psyllid resistance and small leaflet dimension in KX2 becomes less evident in later generations (Sorensson 1993). Continuing work in Hawaii by Brewbaker and colleagues aims to produce stable KX2 lines. Current line x tester experiments with KX2 combinations in Hawaii and Australia indicate that certain tree x tree crossings produce hybrids with high specific combining ability for forage yield, but low proportions of leaf to stem (Sorensson et al. 1994) and high condensed tannin content (Castillo 1993).

Technical Aspects of Interspecific Hybridisation

Several technical aspects of interspecific hybridisation have received serious study.

Researchers have identified three self-compatible species that could require emasculation; *L. diversifolia* (4n), *L. leucocephala* and *Leucaena* sp. 'glossy' (Sorensson 1989b). Differences in their floral anatomy (i.e. relative lengths of androecia and gynoecia) account for differences in their response to cross-pollinations in the absence of emasculation, which have been more than 90% successful for *L. diversifolia* but less so (about 50%) for *L. leucocephala* (Sorensson and Sun 1990; Sorensson 1993). Three emasculation methods have been described — soap (Hutton and Gray 1959), pre-dawn emasculation (Sorensson 1988a), and bud emasculation (Gupta and Patil 1984).

Leucaena pollen can be manipulated using newly developed techniques. Pollen desiccated over calcium chloride remains viable for 20 days when stored at -20°C , and for up to three months when stored at -75°C (Sorensson 1993). Pollen has been germinated and grown in vitro on sucrose-agar-borate slides to assess its viability (Sorensson and Nagahara 1989). Studies on radioresistance of *leucaena* pollen to gamma irradiation indicate that 30 k rads can render pollen genetically ineffective, and irradiated mentor pollen have been applied in pollen mixtures to overcome the self-sterility of *L. pallida* (Sorensson 1993).

Several aspects of flowering have been studied. Flowering seasons of *Leucaena* species have been described in northern South America (Hughes 1993), in India (Gupta 1990) and in Hawaii (Sorensson 1988a). They do not differ substantially between sites. Immature inflorescences develop at rates which are linear and vary from species to species (Sorensson 1989b). Inflorescences of most species begin meiosis when they reach 60% of their mature diameter (Sorensson 1993). We know roughly how much pollen *leucaena* species produce, and the

length of time during which styles are receptive to pollen before and after anthesis (Sorensson 1988b). Most shaded branches do not flower profusely. Flowering in *L. leucocephala* K636 can be partially synchronised by timing irrigation to manage soil water stress on Molokai, Hawaii (N. Dudley pers. comm.).

Several morphological traits of interspecific hybrids have been modelled. For example, flower colour is shown primarily to be inherited additively, and triploids exhibit dosage effects. The latter are also significant in leaf parameters — pinnule length and width, number of pinnules per leaf, pinnae pairs per leaf. With rare exception, leaf parameters are inherited on a geometric scale between parents (Sorensson 1987, 1993), apparently as a result of additive interaction between developmental regulatory genes. Leaf morphology can also be used to identify hybrids from parental-type seedlings (Sorensson 1990, 1993; Sorensson and Shelton 1992). Diameters of inflorescences, shapes and numbers of petiolar glands, and leaflet shapes of interspecific hybrids are primarily controlled by additive inheritance (Sorensson 1987, 1990).

The quantity of seed produced by cross-breeding fifteen *leucaena* species has been measured, following nearly 60 000 floret pollinations (Sorensson and Brewbaker in press). The average number of viable interspecific seeds resulting from cross-pollination of ten florets has been tabulated (Sorensson in press). A technician pollinating about 30 inflorescences of a highly compatible interspecific mating can produce about 2500 viable hybrid seeds in one day (assuming six pods per inflorescence and fifteen seeds per pod). The potential for hybrid seed production in orchards with bee pollination has been partially demonstrated by the heavy hybrid seed production of a single tree of diploid *L. diversifolia* planted amongst *L. leucocephala* (Gutteridge and Sorensson 1992).

Several aspects of seed production have been considered. The susceptibility of *leucaena* pods to seed beetles in Hawaii has been surveyed, with *L. diversifolia* (2n, 4n) being less preferred by beetles (Sorensson 1993). Visual estimation of insect infestation in *leucaena* seed has been shown to be inaccurate. An inexpensive and effective seed cleaning method, in which the seed is soaked in water and separated by floating in salt water, has been successfully tested (Sorensson 1993). Freezing seeds does not reduce seed viability (Cobbina et al. 1990) and is highly effective in killing insects infesting the seeds (N. Dudley, pers. comm.; C. Hughes, pers. comm.). In Hawaii, seeds from *Leucaena* species take from about 70 to 330 days to mature (Wheeler 1991).

Breeding Strategies Involving Interspecific Hybridisation

There are two ways of breeding interspecific hybrids — one relies on clonal reproduction to capture heterosis (hybrid vigour) and the other does not.

Several tropical trees have been greatly improved by propagating clones of their elite segregants, and large areas have already been planted to clonally propagated interspecific hybrids of *Eucalyptus* and *Casuarina*. Equally impressive gains could be made by use of clonal leucaenas, as has been partially demonstrated at Pondok Gedeh, Indonesia (Toruan-Mathias et al., these Proceedings) using a naturalised line apparently derived from a hybrid of diploid *L. diversifolia* and *L. leucocephala*. Several rooted cutting techniques show promise for large-scale use (e.g. Osman, these Proceedings).

All vigorous interspecific leucaena hybrids produce flowers, with the exception of *L. pulverulenta* x *L. lanceolata* (Sorensson 1987). Of 52 different hybrids that flowered in Hawaii, 30 produced some seed from open pollination. Triploids have proved difficult to use in standard breeding programs because their chromosomes are unstable and because they tend to set little or no seed (Hutton 1985). Diploid hybrids vary greatly in their seed production. A few vigorous diploid hybrids produced seed readily in Hawaii (*L. shannonii* x *L. collinsii*, *L. diversifolia* x *L. collinsii*, *L. shannonii* x *L. salvadorensis*, *L. lanceolata* x *L. shannonii*, *L. pulverulenta* x *L. retusa*, *L. lanceolata* x *L. macrophylla*), and it appears they could be bred further. Pollen stainability and seed production are generally well-correlated (Sorensson 1987, 1993).

Three tetraploid species, *L. diversifolia*, *L. leucocephala* and *L. pallida*, make up the primary breeding pool, and are completely compatible with each other (Sorensson and Brewbaker in press). All tetraploid hybrids produced among the four known tetraploid leucaena species have been reasonably vigorous and seed productive, although a few tetraploid lines derived from *L. diversifolia* x *L. leucocephala* in Brazil were unstable, losing between 1 and 18 chromosomes (Freitas et al. 1991). Previous breeding programs based on advanced generation progenies have emphasised mass selection, but many now concentrate on self-compatible segregants and involve inbreeding and recurrent selection. Tetraploid hybrids may be backcrossed to a parent, crossed to a third species (three-way), or even crossed with a different interspecific hybrid (four-way).

Breeders can use several breeding tactics with interspecific hybrids which set fertile seed.

- (i) Move small numbers of genes from one species to another by backcrossing and recurrent selection.
- (ii) Mass-produce hybrid seed from particular parent trees, either species or hybrids, whose hybrid progeny have high general and specific combining ability for yield or quality.
- (iii) Select elite true-breeding lines from a population of primarily outcrossing hybrids by cycles of selfing or sibbing, and bulk the inbreds as a synthetic line with reasonably broad genetic diversity.
- (iv) Repeatedly mass select hybrid populations to cull undesirable genes but otherwise maintain genetic diversity.
- (v) Move genes from diploid species into the tetraploid breeding pool via induced polyploidy or unreduced gametes. This approach was used in Hawaii to produce several tetraploid hybrids from $2n-4n$ or $4n-2n$ matings (Sorensson 1988b) and could well have been an important mechanism of allopolyploid speciation in the genus *Leucaena*.

Heterosis

Heterosis is a major reason for interest in leucaena's interspecific hybrids, as it is in other tree groups such as poplars, pines and eucalypts. Heterosis, or hybrid vigour, is the phenomenon in which offspring of crosses between populations perform better than the average of the two populations. Heterosis is thought to result from dominance effects when heterozygous loci, in favourable states, mask or suppress the negative effects of mildly unfavourable alleles (which may be tightly linked to favourable alleles and thus hard to breed out).

Some of leucaena's interspecific hybrids show outstanding heterosis. Field workers may recall plots of species that were dominated by a suspiciously large and productive tree with morphology similar, but not identical, to the parental species. In perhaps twenty such cases that I have investigated, the outstanding individual has always proved to be an interspecific hybrid. Further inspection sometimes identified additional hybrids whose performances were unremarkable. It is difficult to say how superior these hybrids really are because they shade out their neighbors. However, in a seedling vigour study (Sorensson et al. 1994), F1 hybrids of both *L. pallida* x *L. leucocephala* and *L. diversifolia* x *L. leucocephala* had grown taller than their parents, at rates of about one cm per day, by day 84.

Several biochemical or genetic models have been developed to explain heterosis. In one such model, an undesirable allele of one gene (A) is tightly linked

to a desirable allele of another gene (B). If the gene product of B uses the gene product from A as a precursor, then providing A through interspecific hybridisation may enable the potential of allele B to be fully expressed. This could remove a factor limiting yield. Correcting such factors is critical to the success of a plant breeder (Mangelsdorf 1952).

Two additional points should be noted. Firstly, in the absence of A's gene product, the allele B, though present, confers no advantage to the parent. As an example, when plants with good pest resistance are grown in a pest-free environment their pest resistance gives them no advantage, and may even carry a metabolic cost. Secondly, if heterosis is conferred by a single locus H, then it is possible to inbreed many generations and retain heterosis, assuming the breeder carefully selects for heterosis (Mangelsdorf 1952).

Another model for heterosis involves multimeric enzymes. In the simplest case with dimeric enzymes, parent 1 has an enzyme composed of identical subunits A and parent 2 has an enzyme composed of identical subunits B. Their hybrid has *three* forms of dimeric enzymes — both parental types (AA, BB) plus a unique form (AB). The situation rapidly gets more complex when enzymes are composed of three or four subunits. The hybrids benefit from the greater diversity of enzymes, as well as from unique forms of enzymes *within* plants. Populations of hybrids bred from few parent trees may validly be criticised for being 'inherently narrowly-based' genetically (Hughes 1993). Yet, *within* individual plants, there may be significant diversity of isozymes. This diversity may show up as stability across environments, and probably underlies the wide environmental adaptability of *L. leucocephala*, which is thought to have a hybrid origin with *L. pulverulenta* as the maternal parent (Hughes and Harris, these Proceedings).

Polyploidy also can increase genetic diversity within plants, and result in heterosis by making it more likely that alleles will exist in favourable heterozygous states. Tetraploid leucaenas which are multiallelic could produce a range of isozyme forms within a plant. Polyploidy also protects the genus against wide hybridisation (Sorensson and Brewbaker in press). It enhances chromosome pairing in F1 interspecific hybrids by allowing chromosomes to preferentially pair within genomes — poor chromosome pairing within genomes could have caused hybrid breakdown in F2 of three-way tetraploid hybrids (Sorensson 1989a).

If heterosis is not visible in hybrid plants grown in some environments, it may show up in exotic environments. Plant species have developed enzymatic systems which operate efficiently in the

soil-light-water regimes of their native environments, but which may be ill-adapted to exotic environments. Hybrids may do better in such environments, because of the diversity of their enzymes and gene products. *Leucaena diversifolia* (4n) x *L. leucocephala* is a vigorous hybrid: grown at an exotic upland site in Hawaii under low temperatures it performed outstandingly better than its parents (Brewbaker et al. 1988).

Research Priorities

The most important area for new research is hybrid seed production, since new hybrid germplasm can be neither tested nor adopted without seed. Parental lines could be clonally propagated, and then pilot seed production orchards could be set up at sites that are relatively free of seed pests (such as eastern Australia or northern India) to try to produce mass quantities of seed for distribution. Basic research topics might include:

- how to optimise pollen transfer by bees between trees used as males and females
- how distant seed orchards should be from foreign pollen sources to prevent pollen contamination
- how to synchronise flowering
- management of tree form and spacing to optimise and mechanise seed harvest
- how to produce several genotypes of seed from a single orchard (this would mainly investigate the effects of compatible and incompatible pollen on seed set).

Since seed orchards need vegetative propagation, more work is needed on vegetative propagation methods, particularly on those that already show promise (Osman, these Proceedings; Toruan-Mathius et al., these Proceedings). Unfortunately, it appears that rooting ability may be genotype-specific, as it is in Eucalyptus (Toruan-Mathius pers. comm.). Clones are needed in order that yields of elite genotypes can be measured accurately, traits' sensitivity to environmental conditions can be estimated, clones can be used as replicates in some experimental designs, and for mass propagation of seedless or gum-producing plants. Cloned stands of self-incompatible species or hybrids would remain seedless if planted as a monoculture (Brewbaker 1988).

Three other research areas warrant attention. First, can chromosomes or chromosome segments containing useful genes be transferred between species, for example by backcrossing? Perhaps this could be done through chromosome addition (*Leucaena* 'confertiflora' has eight more chromosomes than *L. leucocephala*).

Second, in order to decide which hybrids are appropriate for cultivation at specific sites,

researchers need to identify the qualities that make interspecific hybrids different from wild *Leucaena* species. Are hybrids truly better adapted to marginal or stressful sites than parent species? Is the heterosis or combining ability of some hybrids significant in economic terms? Do seedless hybrids channel carbon into leaves and wood that is otherwise used up in flower and seed production? Can seedless hybrids play a major role in revegetating damaged and fragile ecosystems, perhaps acting as biological nurse trees?

Third, recommendations are needed to guide the use of interspecific hybrids by small landholders. Hybrids, particularly segregating ones, may not be appropriate for small landholders who cannot easily afford to buy elite seed, and who may find it difficult to properly manage the great phenotypic variability of a segregating population. If weak and ill-adapted segregants could be culled out in a hedgerow or protein bank forage system — perhaps at the age of six months (Sorensson et al. 1994), this could leave a genetically-diverse and extremely productive plant population that would be quite beneficial to a small landholder. Hybrids could be ill-suited to other situations too, such as low plant density for wood production, or limiting soil moisture.

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References

- Bray, R.A., Julien, M.H. and Room, P.M. 1990. *Leucaena* psyllid in Australia — the current situation. In: Napompeh, B. and MacDicken, K.G. ed., *Leucaena Psyllid: Problems and Management*. Bogor, Indonesia, January 16-21, 1989. Bangkok, Thailand, F/FRED, ICRC and NFTA, 8-11.
- Brewbaker, J.L. 1988. Cloning of seedless leucaenas for plantation use. *Leucaena Research Reports* 9, 111-112.
- Brewbaker, J.L. and Sorensson, C.T. 1990. *Leucaena* Benth.: new tree crops from interspecific hybrids. In: Janick, J. and Simon, J. ed., *Advances in New Crops*. Portland, Oregon, Timber Press, 283-289.
- Brewbaker, J.L., Wheeler, R.A. and Sorensson, C.T. 1988. Psyllid-tolerant highland leucaena yields. *Leucaena Research Reports*, 9, 11-13.
- Castillo, A. 1993. Agronomic performance, psyllid (*Heteropsylla cubana* Crawford) resistance and forage quality of *L. leucocephala* (Lam.) de Wit, *L. pallida* Benth., *L. diversifolia* Schlecht. and their hybrids. M.Agr.Sc. thesis, University of Brisbane, Queensland, Australia.
- Cobbina, J., Kolawole, G.O. and Atta-Krah, A.N. 1990. *Leucaena* and *Gliricidia* seed viability and germination as influenced by storage conditions. *Leucaena Research Reports*, 11, 91-93.
- Freitas, L.H.D., Schifino Wittman, M.T. and Paim, N.R. 1991. Floral characteristics, chromosome number and meiotic behavior of hybrids between *L. leucocephala* ($2n = 104$) and tetraploid *L. diversifolia* ($2n = 104$). *Brasilian Journal of Genetics*, 14(3), 781-789.
- Gupta, V.K. 1990. Genetics and breeding of *Leucaena leucocephala* (Lam.) de Wit. In: Pathak, P.S., Deb Roy, R. and Singh, P. ed., *Multipurpose Tree Species for Agroforestry Systems*. IGFRI, Jhansi, India, 99-109.
- Gupta, V.K. and Patil, B.D. 1984. A simple technique of hand emasculation in *Leucaena leucocephala*. *Leucaena Research Reports*, 5, 29-30.
- Gutteridge, R.C. and Sorensson, C.T. 1992. Frost tolerance of a hybrid of *L. diversifolia* x *L. leucocephala* in Queensland, Australia. *Leucaena Research Reports*, 13, 3-5.
- Hughes, C.E. 1993. *Leucaena Genetic Resources: The OF1 Leucaena Seed Collections and a Synopsis of Species Characteristics*. Oxford Forestry Institute, Oxford, UK, 117 p.
- Hughes, C.E. and Harris, S.A. 1994. Characterisation and identification of a naturally occurring hybrid in *Leucaena*. *Plant Systematics and Evolution*, 192, 177-197.
- Hutton, E.M. 1985. Problems in breeding low-mimosine types in the genus *Leucaena*. *Tropical Agriculture*, 62, 329-333.
- 1988. Results from two interspecific leucaena crosses: *L. leucocephala* x *L. esculenta* and *L. leucocephala* x *L. shannonii* grown in an acid oxisol. *Leucaena Research Reports*, 9, 37-39.
- 1990. Field selection of acid-soil tolerant leucaena from *L. leucocephala* x *L. diversifolia* crosses in a tropical oxisol. *Tropical Agriculture*, 67(1), 2-8.
- Hutton, E.M. and Gray, S.G. 1959. Problems in adapting *Leucaena glauca* as a forage for the Australian tropics. *Empirical Journal of Experimental Agriculture*, 27, 187-196.
- Mangelsdorf, A.J. 1952. Gene interaction in heterosis. In: Gowen, J.W. ed., *Heterosis: a Record of Researches Directed Toward Explaining and Utilizing the Vigour of Hybrids*, 320-329.
- Quiros, C.F. and Bauchan, G.R. 1988. The Genus *Medicago* and the Origin of the *Medicago sativa* Complex. *Alfalfa and Alfalfa Improvement*, ASA/CSSA/SSSA, Madison, Wisconsin, Monograph No. 29, 107.
- Sorensson, C.T. 1987. Interspecific Hybridisation in the Genus *Leucaena* Benth. M.Sc. thesis, Dept of Agronomy and Soil Science, University of Hawaii at Manoa.
- 1988a. Pollinating and emasculating techniques for leucaena species. *Leucaena Research Reports*, 9, 127-130.
- 1988b. Utilizing unreduced gametes for production of novel hybrids of leucaena species. *Leucaena Research Reports*, 9, 75-76.
- 1989a. Status and mechanisms of self-incompatibility in leucaena species. *Plant Cell Incompatibility Newsletter*, 21, 77-85. PO Box 2000, Cortland, New York 13045.

- 1989b. Rate of inflorescence development of leucaena species. *Leucaena Research Reports*, 10, 80-83.
 - 1990. Shapes of pinnules of leucaena species and F1 interspecific hybrids. *Leucaena Research Reports*, 11, 124-128.
 - 1993. Production and Characterisation of Interspecific Hybrids of the Tropical Tree *Leucaena* (Leguminosae: Mimosoideae). PhD dissertation, University of Hawaii at Manoa.
 - - in press. Seed productivity of artificial hybridisations among fifteen species in the genus *leucaena*. In: Proceedings of the 1992 IUFRO conference on Breeding Tropical Trees, Cali, Colombia, October 9-14.
- Sorensson, C.T. and Brewbaker, J.L. 1986. Psyllid resistance of leucaena species and hybrids. *Leucaena Research Reports*, 7, 13-15.
- - in press. Interspecific compatibility among fifteen leucaena species (Leguminosae: Mimosoideae). *American Journal of Botany*.
- Sorensson, C.T. and Nagahara, D. 1989. In vitro pollen germination of leucaena species. *Leucaena Research Reports*, 10, 84-86.
- Sorensson, C.T. and Shelton, H.M. 1992. Number of pinnules per pinna used as a marker to identify seedlings of *L. diversifolia*, *L. leucocephala*, *L. pallida* and their hybrids. *Leucaena Research Reports*, 13, 91-94.
- Sorensson, C.T., Shelton, H.M. and Austin, M.T. 1994. Seedling vigour of some leucaena spp. and their hybrids. *Tropical Grasslands*, 26.
- Sorensson, C.T. and Sun, W.C. 1990. Production of interspecific hybrid seed without emasculation. *Leucaena Research Reports*, 11, 129-132.
- Wheeler, R.A. 1991. Guide to Management of *Leucaena* Seed Orchards. Winrock International-F/FRED, Bangkok, Thailand, 18 p.
- Wheeler, R.A. and Brewbaker, J.L. 1990. An evaluation of the results from the leucaena psyllid trial network. *Leucaena Research Reports*, 10, 11-16.

Systematics of *Leucaena*: Recent Findings and Implications for Breeding and Conservation

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Abstract

The status of *leucaena* systematics is reviewed. Considerable taxonomic confusion persists and is shown to be hampering the efficient utilisation, genetic improvement and conservation of *leucaena* species. Herbarium research, morphological and molecular analyses, and recent field exploration and collection are providing new insights into the evolution of the genus, the status of species, species relationships, the origin of tetraploid species and parentage of natural hybrids. Results of recent research are summarised and reveal a more complex picture of the genus than has generally been acknowledged. The full extent, scope and importance of human interference in the recent evolution of the genus is now becoming apparent. Results demonstrate that the widespread indigenous domestication of *leucaena* species for pod production in Mexico accounts for recent speciation and provides a plausible scenario for the origin of *L. leucocephala* in domestication. The multidisciplinary approach has been highly beneficial, combining field and laboratory work and including both morphology and molecules to study the genus. It has allowed significant advances to be made towards a better understanding of the genus.

LEUCAENA is a small genus of around 17 species belonging to the tribe Mimoseae. All species are native to the New World with the greatest species diversity in Mexico (13 species, 6 endemics) and northern Central America (6 species, 3 endemics) in seasonally dry, mainly tropical habitats. The genus extends north into southern Texas, USA, sporadically across the Caribbean, and into South America as far south as Peru.

Status of *leucaena* systematics

The taxonomic history of *leucaena* is complex. At one time it included another genus, *Schleinitzia*, and it was later split into three genera. However, the delimitation of species has been the greatest source of difficulty and confusion. While some species are narrowly distributed, uniform and clearly distinct, the complex patterns of morphological variation in some of the widely distributed polymorphic species

and species groups, notably the *L. diversifolia*, *L. shannonii*, *L. esculenta* and *L. macrophylla* groups, have led to difficulties in the circumscription of species. Choice between specific and subspecific rank has also been a source of debate and confusion. These problems are aggravated by the considerable human interference known to have affected *leucaena* through its indigenous domestication in Mexico, and by known polyploidy and interspecific hybridisation.

Bentham (1842) first established the genus *leucaena*, initially with four species, and later with nine (Bentham 1875). Botanical exploration over the next 50 years resulted in the description of a proliferation of supposed new species and culminated in the revisions of Standley (1922) who recognised 15 species, and Britton and Rose (1928) who added 24 species, bringing the total to 39. A long dormant period followed with only minor additions and limited synthesis prompted by the compilation of the Floras of Peru (Macbride 1943), Guatemala (Standley and Steyermark 1946), Panama (Schery 1950) and Novo-Galicia (McVaugh 1987). In total, some 61 different species or subspecies names have been ascribed to *leucaena*.

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The ensuing taxonomic confusion was radically simplified by Brewbaker and Ito (1980), Brewbaker (1987) and Sorensson and Brewbaker (1994) who questioned the validity of the numerous binomials and accepted first 10, and subsequently 16, species as legitimate, establishing a temporary working taxonomy for the genus. In a brief synoptic paper on the genus for Mexico, Zarate (1984a) mentioned several new taxa and these have now been published validly in a new revision of *leucaena* for Mexico (Zárate 1994). Despite several recent minor taxonomic clarifications (Hughes 1988, 1991; Zárate 1987a,b; Pan 1988), no complete taxonomic revision has been published since that of Britton and Rose (1928).

In cultivated taxa and their wild relatives, the efficient use, improvement and conservation of biological diversity must be guided by biology of the taxa concerned (Falk and Holsinger 1991), understanding (i) the phylogeny (ii) the amount and distribution of genetic variation (iii) the effective design of suitable sampling strategies (iv) effective conservation methods.

Successful long-term taxon management depends on knowledge of the genetics and demography of the taxon, enabling the design of biologically sound management strategies. Such data are increasingly important in the development of integrated conservation strategies, combining population and taxon management with in-situ and ex-situ collections.

Leucaena systematics remain very confused. There is no good guide for identifying species, and accurate species distribution maps have only recently been compiled (Hughes 1993). Several new species have been discovered and await formal description while several undescribed species have been used in breeding and improvement. The origins of the known polyploid species are still unconfirmed, and the accuracy and status of several published names is still under debate. As an ever wider spectrum of species and hybrids is brought into use and breeding, it will become more difficult to identify species properly. Lack of a clear taxonomic framework hampers other research, germplasm acquisition, tree improvement and genetic conservation programs so they inevitably follow sub-optimal strategies. The taxonomy of the genus is urgently in need of revision.

The OFI *leucaena* research program: background and objectives

Early work on *leucaena* at the Oxford Forestry Institute (OFI) concentrated on assembling seed collections of some of the lesser-known and potentially valuable species from Central America

(Hughes 1986, 1988, 1991). Collections were later expanded to include the complete spectrum of *leucaena* species (Hughes 1993).

Since 1990, research has focused on the systematics of the genus with the overall objective of completely revising the taxonomy of *leucaena*, including species descriptions, keys to the identification of species, botanical illustrations, distribution maps, a clear understanding of species relationships, interspecific hybridisation, the origin of the known polyploid species and the phylogeny of the genus.

Here we present the main results of the first four years of the OFI *leucaena* systematics research, and discuss their implications for *leucaena* utilisation, improvement and conservation. Detailed results are presented and discussed elsewhere (Hughes 1991, 1993; Harris et al. 1994a,b,c; Hughes and Harris, 1994). Research is continuing to complete a taxonomic revision.

Methods

Herbarium and botanical database

More than 2700 botanical specimens from 20 Mexican, U.S. and European herbaria have been examined and logged onto a botanical database, BRAHMS (Filer 1993). This has allowed accurate species distribution maps to be produced for the first time (Hughes 1993). Accurate distribution maps not only underpin the taxonomy but are the basis of efficient utilisation, breeding and conservation. The maps have made it possible to make preliminary assessment of environmental tolerances for different species (Hughes 1993), to design efficient and accurate sampling strategies for germplasm collections, and to plan collection expeditions and in situ genetic conservation (Hellin and Hughes 1994).

Exploration and field collection

Botanical material has been collected during field exploration throughout *leucaena*'s range. Several new taxa have been discovered as well as numerous extensions to species distributions. All known taxa have been explored and observed in the field, and complete flowering and fruiting material gathered, forming a working collection for taxonomic description and morphological analysis. In addition, we have obtained seed, dried leaf material for DNA extraction, fixed flowers, wood and bark samples, bruchid seed predators and photographs, and gained information on ethnobotany and conservation status. This unique collection has also formed the foundation for molecular analysis of the genus.

Field trials and morphology

Morphology has been re-assessed, both using the large collection of botanical specimens assembled at Oxford, and on a living collection established by CONSEFORH in Honduras in 1989. Previous work on leucaena systematics had relied on a limited range of often unreliable taxonomic characters such as leaf size, shape and number of pinnae and leaflets, pod size and pubescence. Work is in progress to identify a wider range of reliable diagnostic characters for the genus. Bark, inflorescences, extrafloral nectaries, seeds, seedlings, pollen and flowers are providing good characters for analysis.

Molecular analysis

Over the past decade, molecular genetics has started to make a significant contribution to general understanding of (i) phylogenetic relationships; (ii) the amount and distribution of genetic variation; (iii) hybrid parentage; (iv) the genetic stability of in situ and ex situ collections. However, the vast majority of such studies has been conducted in herbaceous crops such as cereals and grain legumes, with very few studies of tree crops and even fewer of tropical trees.

Molecular analysis of leucaena has been carried out using a suite of techniques and different molecules. Chloroplast DNA (cpDNA) and nuclear ribosomal DNA (rDNA) have been used to examine species relationships, the origins of known tetraploid species (Harris et al. 1994a,c) and natural hybrid parentage (Hughes and Harris 1994). Isozymes and seed storage proteins have been used to look at species relationships and the distribution of genetic variation within and between species and populations (Harris et al. 1994b; Chamberlain 1993). Intact total DNA is readily extracted from either fresh or dried leaves of all leucaena species. Harvesting of leaves into plastic bags and drying with silica gel, according to the method of Chase and Hills (1991), has been successfully used to obtain DNA from leucaena trees in the field, overcoming the need for seed (Hughes and Harris 1994). Chloroplast DNA, rDNA, isozymes and seed storage proteins show amounts of variation that enable patterns of genetic variation to be analyzed at all levels, from the overall structure of the genus to the identification of putative hybrids and the structure of individual populations. Analysis of cpDNA and rDNA data has allowed a suite of species-specific molecular markers to be identified for the majority of species. Chloroplast DNA has been found to be maternally inherited.

Results

1. Structure of the genus and status of species and subspecies

Considerable progress has been made towards a better knowledge of the structure and evolution of the genus, relationships between species (Fig. 1), and the status of species and subspecies. The importance of indigenous domestication and the occurrence of interspecific hybridisation in leucaena are better understood. A provisional delimitation of valid species and subspecies and their synonyms is presented in Table 1. Some of the principal results are summarised below and discussed for each of the main species groups.

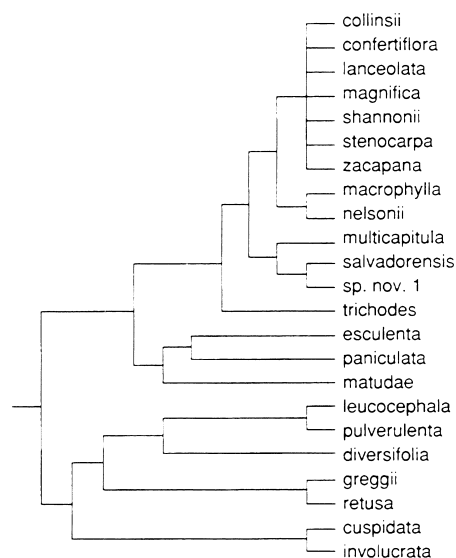


Figure 1 Chloroplast DNA phylogeny of the genus *Leucaena*, generated via a parsimony approach using character data derived from the analysis of 14 restriction enzymes and 8 mung bean chloroplast DNA probes that covered approximately 85% of the chloroplast genome.

Large-leaflet leucaena complex

The large-leaflet morphological complex of leucaena comprises four species *L. lanceolata*, *L. macrophylla*, *L. multicapitula* and *L. trichodes*. There has been some debate over how the members of this complex are related, and how many species and subspecies it includes (Zárate 1984a; Brewbaker 1987), but morphology and cpDNA evidence confirm the four species above.

Brewbaker (1987) placed *L. multicapitula* into synonymy with *L. trichodes*, although this was reversed by Sorensson and Brewbaker (1994). There is good morphological and molecular evidence that

Table 1. Leucaena taxonomy, species names, synonymy and chromosome numbers (2n).

Recognised species and authorities	Recognised subspecies	Synonyms	Chromosome number (2n) notes
<i>L. collinsii</i> B&R	<i>collinsii</i>	<i>L. esculenta</i> (Mot. et Sesse ex ADC) Benth. <i>ssp collinsii</i> S. Zàrate	52, 56
	<i>zacapana</i> C.E. Hughes		? Previously confused with <i>L. diversifolia</i> .
<i>L. confertiflora</i> Zàrate ined.	Two subspecies recognised by Zàrate (1994)		c. 112
<i>L. cuspidata</i> Standley	Possibly two subspecies.		?
<i>L. diversifolia</i> (Schlecht.) Benth.	<i>diversifolia</i>	<i>L. laxifolia</i> Urban <i>L. trichandra</i> (Zucc.) Benth.	104
	<i>stenocarpa</i> (Urban) S. Zàrate	<i>L. stenocarpa</i> Urban <i>L. standleyi</i> B&R <i>L. revoluta</i> B&R <i>L. guatemalensis</i> B&R <i>L. molinae</i> Standley and Williams	52 A widespread polymorphic taxon
<i>L. esculenta</i> (Moc. & Sesse ex ADC) Benth.	<i>esculenta</i>	<i>L. confusa</i> B&R <i>L. doylei</i> B&R	52
	<i>matudae</i> Zàrate		?
	<i>paniculata</i> (B&R) S. Zàrate	<i>L. dugesiana</i> B&R <i>L. oaxacana</i> B&R <i>L. paniculata</i> B&R <i>L. pallida</i> B&R	104 Confusion remains over the correct name. <i>L. pallida</i> has been widely used
	<i>pueblana</i> (B&R) C.E. Hughes comb. ined.		? Unpublished combination for Tehuacan Valley endemic.
<i>L. greggii</i> S. Watson		<i>Rhyncoleucaena greggii</i> (S. Watson) B&R	56
<i>L. involucrata</i> S. Zirate			? Discovered in 1991 by OFI in Sonora, Mexico; allied to the <i>esculenta</i> complex
<i>L. lanceolata</i> S. Watson	Zàrate recognises two subspecies <i>lanceolata</i> and <i>sousae</i> but not clearly defined	<i>L. microcarpa</i> Rose <i>L. brandegeei</i> B&R <i>L. pubescens</i> B&R <i>L. rekoii</i> B&R <i>L. sonorensis</i> B&R <i>L. cruziana</i> B&R <i>L. palmeri</i> B&R <i>L. purpusii</i> B&R <i>L. sinaloensis</i> B&R <i>L. nitens</i> M.A. Jones	52 A widespread and variable species

Table 1. *Leucaena* taxonomy, species names, synonymy and chromosome numbers (2n). (continued)

Recognised species and authorities	Recognised subspecies	Synonyms	Chromosome number (2n) notes
<i>L. leucocephala</i> (Lam.) de Wit	<i>leucocephala</i>	<i>L. glauca</i> (Willd.) Benth.	104 = the shrubby or Hawaiian varieties
	<i>glabrata</i> (Rose) S. Zàrate	<i>L. glabrata</i> Rose	104 = the giant or Salvador type
<i>L. macrophylla</i> Benth.	<i>macrophylla</i>	<i>L. macrocarpa</i> Rose <i>L. houghii</i> B&R	52 Doubtfully distinct from <i>L. trichodes</i>
	<i>nelsonii</i> (B&R) S. Zàrate	<i>L. nelsonii</i> B&R	?
<i>L. multicapitula</i> Schery			52 Doubtfully distinct at species level from <i>L. trichodes</i> and <i>L. macrophylla</i>
<i>L. pulverulenta</i> (Schlecht.) Benth.		<i>Acacia pulverulenta</i> Schlecht.	56
<i>L. retusa</i> Benth.		<i>Caudoleucaena retusa</i> (Benth.) B&R	56
<i>L. salvadorensis</i> Standley ex. B&R		<i>L. shannonii</i> J.D. Smith subsp. <i>salvadorensis</i> (Standley) S. Zàrate	56 Previously confused with <i>L. shannonii</i> and with the Salvador type of <i>L. leucocephala</i> (subsp. <i>glabrata</i>)
<i>L. shannonii</i> J.D. Smith	<i>shannonii</i>		52, 56
	<i>magnifica</i> C.E. Hughes		?
<i>L. trichodes</i> (Jacq.) Benth.		<i>L. canescens</i> Benth.	52
		<i>L. bolivarensis</i> Britton & Killip	Part of the <i>L. macrophylla</i> / <i>L. multicapitula</i> complex
		<i>L. colombiana</i> Britton & Killip	
<i>L. sp nov 1</i>			? Unnamed; discovered by CONSEFORH in 1990 in Honduras; in the <i>L. shannonii</i> complex

it is a distinct species. Zarate (1984a) recognised two subspecies within both *L. lanceolata* (with material from southern Mexico assigned to subsp. *sousae*) and *L. macrophylla* (with lowland coastal material from Guerrero and Oaxaca attributable to subsp. *nelsonii*). The cpDNA results show two plastome types in *L. macrophylla* corresponding to the subspecies and supports their recognition (Harris et al. 1994a). Further evidence for the distinction of two subspecies within *L. macrophylla* comes from morphology, geography and because they grow radically differently in trials (Stewart et al. 1991). Patterns of morphological variation within *L. lanceolata* are more complex and appear to follow a clinal pattern from NW to SE, which suggests continuous variation rather than two recognisable subspecies. The more complex pattern of variation within *L. lanceolata* is supported by the cpDNA data.

The *shannonii* complex

The *shannonii* complex comprises three species — *L. salvadorensis*, *L. shannonii* (with two subspecies *shannonii* and *magnifica*) and a new, as yet undescribed, species from northern Honduras designated *L. sp nov 1*. We do not know how the members of this complex are related and study has been hampered by the confusion surrounding the names of some of the taxa.

For example, *L. salvadorensis* (Hellin and Hughes 1994) had been placed into synonymy with *L. leucocephala* subsp. *glabrata* (Brewbaker 1980) or alternatively called a subspecies of *L. shannonii* (Zarate 1987b). However, detailed studies of morphology, growth characteristics and distribution show that *L. salvadorensis* might be treated as a species in its own right (Hughes 1988; Hellin and Hughes 1994). The cpDNA, seed storage protein and isozyme data clearly separate it from both *L. shannonii* and *L. leucocephala* (Harris et al. 1994a; Chamberlain 1993). There is now general consensus that *L. salvadorensis* should be treated as a valid species.

The discovery in 1991 of a new species, *Leucaena sp nov 1*, by the CONSEFORH project in northern Honduras, is another sign of the incomplete state of leucaena systematics. This fine tree is clearly distinct and appears to be most closely related to *L. salvadorensis*, based on its morphology and molecular evidence (Fig. 1) (Harris et al. 1994a; Chamberlain 1993).

Hughes (1991) described *L. shannonii* subsp. *magnifica* based on a range of morphological differences from typical *L. shannonii*. It is a narrowly restricted endemic from southeastern Guatemala. Chloroplast DNA and isozyme variation support the recognition of two subspecies within *L. shannonii*, and suggest

that there is very little within-population genetic variation for subsp. *magnifica* compared to other species in the *shannonii* complex (Chamberlain 1993).

The *diversifolia* complex

L. diversifolia sensu lato is the most widespread species in the genus, occurring through the highlands of south-central Mexico and Central America as far south as Nicaragua. Apart from *L. leucocephala*, it is the most widely known and planted species outside its native range. *L. diversifolia* was studied by Pan (1985) who confirmed the distinction of diploid and tetraploid cytotypes and designated them as separate subspecies *trichandra* and *diversifolia* respectively (Pan 1988). The diploid subspecies was described as *L. diversifolia* subsp. *stenocarpa* by Zárate (1984a, 1994). The tetraploid subspecies *diversifolia* was found at that time to be restricted to a small area in central Veracruz in Mexico and was hypothesised to be an autopolyploid, i.e. derived from diploid *L. diversifolia*. The *L. diversifolia* complex *sensu* Pan (1985) also includes *L. pulverulenta* and *L. esculenta* subsp. *paniculata* (= *L. pallida sensu* Brewbaker 1987). The nomenclature of this tetraploid remains controversial (Brewbaker 1987; Zárate 1984a, 1994). We discuss this taxon further in relation to the *esculenta* group.

From recent exploration, it appears that the tetraploid subspecies *diversifolia* is more widespread than believed by Pan (1985). It occurs from Hidalgo, south along a narrow belt on the wet slopes of the Sierra Madre Oriental facing the Gulf, then through Veracruz, northern Oaxaca and Chiapas in Mexico, into northern Guatemala in Huehuetenango (Hughes 1993). Populations from northern Oaxaca were described by Zárate (1984a, 1994) as *L. brachycarpa*, but are apparently indistinguishable morphologically from *L. diversifolia* subsp. *diversifolia*. However, recently collected material from these areas has not yet been verified as tetraploid. In the analysis of cpDNA, leucaena accessions identified as *L. diversifolia s.l.* were found in two separate and robustly supported plastome clades (Fig. 1). The majority of the populations examined fell into groups according to their morphological subspecies. The cpDNA phylogeny casts doubt on Pan's hypothesis that tetraploid *L. diversifolia* is an autotetraploid derivative of diploid *L. diversifolia*. Instead it suggests that it is a segmental allotetraploid, with *L. pulverulenta* as the most likely maternal progenitor of the tetraploid (Harris et al. 1994a). This fits well with present-day distributions (*L. pulverulenta* and tetraploid *L. diversifolia* are found sympatrically in parts of central Veracruz around Misantla) and with the close morphological affinities of these two species as recognised by Zárate (1984a).

The nomenclature of the *L. diversifolia* group is complex and unresolved. Tetraploid *L. diversifolia* is probably of allopolyploid origin and is more closely related to *L. pulverulenta* than to the diploid, so it seems illogical to maintain these two taxa as subspecies; they should be treated as separate species.

Both morphological and molecular analysis show that *L. diversifolia* s.l. is one of the most variable taxa in the genus. Molecular analysis of twenty populations indicated that the two cytotypes can be readily distinguished, and that both contain high levels of genetic diversity, the diploid being more variable than the tetraploid. There is tremendous morphological variation across the disjunct range of diploid *L. diversifolia* so it is possible that the species may need to be split taxonomically. This is being postponed until detailed morphometric studies are complete.

Many of the introductions of *L. diversifolia* outside the Neotropics have been of the Hawaiian accession K 156, a self-compatible tetraploid derived from seed collected in Veracruz (Brewbaker 1987). This accession is likely to have a narrow genetic base, but there is tremendous variation available within *L. diversifolia*, so a much broader genetic base could be examined. Breeders might incorporate improved adaptability, productivity, product quality and psyllid tolerance into *L. diversifolia*, either for direct planting or for use as a parent in the production of hybrids. Seed source will probably be of critical importance in *L. diversifolia*, justifying detailed provenance trials for this species.

The *esculenta* group

The *esculenta* group, comprising three taxa, *L. esculenta* subsp. *esculenta*, *L. esculenta* subsp. *paniculata* and *L. esculenta* subsp. *matudae*, has been investigated most actively by Zárate (1984a) who proposed this designation of subspecies. Zárate (1994) described a fourth taxon *L. involucrata* belonging to this group. Pan (1985) proposed that the tetraploid *L. esculenta* subsp. *paniculata* was an amphidiploid of *L. esculenta* subsp. *esculenta* and diploid *L. diversifolia*. The cpDNA data would support a hybrid origin with subsp. *esculenta* as the maternal parent (Harris et al. 1994a). As mentioned above, the nomenclature of this taxon is under debate (Pan 1985; Brewbaker 1987; Zarate 1984a). Considerable morphological and molecular variation has been demonstrated within subsp. *paniculata*. Recent field work suggests that this may be partly accounted for by the occurrence of a distinct taxon, endemic to the Tehuacan Valley in Puebla and Oaxaca which is clearly referable to the basionym *L. pueblana*.

Zarate (1984a) identified a distinct taxon endemic to the Balsas Depression in Guerrero and designated it a subspecies of *L. esculenta* subsp. *matudae*. This name has now been formally published (Zárate 1994) but apparently leucaena researchers have not widely accepted it as valid (Brewbaker 1987). The taxon's morphology and molecular analysis link it to the *esculenta* group. However, subsp. *matudae* is readily separated from typical *esculenta* by a suite of diagnostic morphological and molecular characters and by its unique growth form in trials (Stewart et al. 1991), and may be more appropriately treated as a distinct species.

The last taxon, *L. involucrata*, was discovered in remote mountains in north-central Sonora. Although allied to the *esculenta* group, this taxon's location is more than 1500 km north of the nearest known occurrence of other members of the group. The finding adds to the confusion and debate about this group, and the problem of assigning rank to taxa. One reason for the extreme complexity of the *esculenta* group may be the particular attention paid to the group during its indigenous domestication. Clearly, these taxa have been transported, cultivated and even selected over several thousand years throughout south-central Mexico.

Leucaena cuspidata and *L. confertiflora*

These are two of the least known leucaena taxa and consequently their taxonomy and relationships are very poorly understood. Brewbaker (1987) has questioned the validity of *L. cuspidata* as a separate species. Our work suggests that both *L. cuspidata* and *L. confertiflora* are valid species, not only crucially important to the understanding of the evolution of the genus but also of considerable interest in leucaena research and use.

Leucaena cuspidata is a very distinctive species with large woody pods, glossy cuspidate leaflets and unusual floral bracts. It occupies a basal position on the cpDNA phylogeny (Fig. 1) (Harris et al. 1994a) and is clearly a valid species. It is distributed in the Mexican states of Hidalgo, Queritaro and San Luis Potosi.

Leucaena confertiflora was first discovered in 1974 in the village of San Pedro Chapulco in Puebla, Mexico by Sergio Zárate and Bob Reid. At that time Zarate (1984a) emphasised its affinities to *L. cuspidata*, treating it as a subspecies of *cuspidata*. *L. confertiflora* has now been formally described, and clearly represents a valid species in its own right with many interesting attributes. It is a self-compatible tetraploid (Sorensson and Brewbaker 1994) but further work will be required to find out its origin and affinities. Preliminary cpDNA evidence does not support a close association between *L. confertiflora* and *L. cuspidata*.

Leucaena greggii and *L. retusa*

Although Britton and Rose (1928) placed *L. greggii* and *L. retusa* in two segregate monotypic genera (*Rhyncoleucaena* and *Caudoleucaena* respectively) all subsequent authors have treated them as part of the genus *Leucaena* (Zárata 1984a; Brewbaker 1987; Hughes 1993). Morphologically *L. greggii* and *L. retusa* are separated from the rest of the genus by their yellow flowers held on long peduncles; long caudate, exserted or short pointed floral bracts; thickened woody pods with longitudinal or oblique transverse seed alignment; and small erect peg-shaped extrafloral nectaries (Hughes 1993). To emphasise their separation from the rest of the genus, these species are geographically and ecologically isolated in northern Mexico and Texas (Hughes 1993), where they withstand regular frost and snow with minimum temperatures down to -15°C (Glumac et al. 1987). *L. retusa* fails to nodulate when inoculated with strains of *Rhizobia* which effectively nodulate other species of *Leucaena* (Halliday and Somasegaran 1983), and both species show very poor growth and survival in field trials compared to other *Leucaena* species (Brewbaker 1987; Stewart et al. 1991).

Evidence from cpDNA implies that these two species are closely related, and supports the view that they are distinct from other members of the genus and that the group is primitive. Other data suggest that *L. cuspidata*, another northerly distributed taxon, is also primitive. Overall, the evidence suggests that leucaena originated at northerly latitudes and migrated south, radiating furthest in south-central Mexico.

Leucaena leucocephala

Although *L. leucocephala* is used throughout the tropics, its origin and natural distribution remain unknown. Brewbaker and his colleagues (Brewbaker 1983; Pan 1985; Pan and Brewbaker 1988) speculated that *L. leucocephala* was an amphidiploid between two sympatric leucaena species such as diploid *L. diversifolia* and *L. shannonii*. However, they presented no firm evidence of possible parentage. So far, no natural populations of the species have been located anywhere in Mexico or Central America. As a result, this species' germplasm has been collected from cultivated material, and only limited genetic variation has been located. We need a clear understanding of the origin of *L. leucocephala* to understand why it lacks genetic variation.

Two subspecies are recognised. Subsp. *leucocephala* is a shrubby taxon that occurs mainly in the Yucatan peninsula and the Isthmus of Tehuantepec, while subsp. *glabrata* is a more arborescent

taxon corresponding to the giant, 'Peru' or 'Salvador' types (Zárata 1987a). The two subspecies are readily distinguished using morphology or isozyme multi-enzyme phenotypes. The natural distribution of this important tetraploid species has been obscured by human interference. Recent exploration and molecular analysis are providing new insights into the origin of *L. leucocephala*. Patterns of infraspecific genetic variation within the species are being found, but the picture is still not complete (Harris et al. 1994a,b,c).

A third variant has been discovered in the highlands of Huehuetenango, Guatemala, in the upper valley of the Rio Cuilco, where it is cultivated by local people. It has small pods and glabrous leaves and is also distinct in its isozyme phenotype (Harris et al. 1994b). As far as we know this variant has not been collected or used in *Leucaena* breeding efforts.

Evidence from the chloroplast genome shows that *L. leucocephala* is closely associated with *L. pulverulenta* along with tetraploid *L. diversifolia* (Harris et al. 1994a) (Fig. 1). This evidence seems to show that *L. pulverulenta* is the maternal parent of *L. leucocephala*. That would mean *L. leucocephala* originated in, or near, the distribution of *L. pulverulenta* on the eastern coast of Mexico. The paternal parent is unknown and cannot be identified from cpDNA (which is known to be maternally inherited). Initial analysis of ribosomal nuclear DNA indicates that the paternal parent would lack a site for the restriction enzyme *Stu-I* within its rDNA. Further work, using rDNA, randomly amplified polymorphic DNA (RAPD) or sequencing, are needed to pin-point the paternal parent. It has been shown that hybrids formed following human interference when *Leucaena* species were being domesticated in Mexico. This has led Harris et al. (1994c) and Hughes and Harris (1994) to speculate that *L. leucocephala* may have originated in domestication.

Leucaena species are cultivated and used as minor food plants to produce pods in many parts of Mexico (Standley 1922; Whitaker and Cutler 1966; Zárata 1984b; McVaugh 1987; Casas 1992). We are now discovering how many species are used indigenously, and over what geographic spread and time span. We can deduce the resulting degree of domestication and its importance to the evolution of the genus (Harris et al. 1994c; Hughes and Harris 1994). At least 13 species of *Leucaena* are currently used for human food in different parts of Mexico and northern Guatemala. Archaeological evidence shows that unripe pods and seed have been harvested and used for several thousand years in the Tehuacan Valley and other areas (Smith 1967). Species such as *L. esculenta* and *L. leucocephala* have been transported over long distances and are widely cultivated

and marketed throughout central Mexico. The transport, use and cultivation of a wide range of *Leucaena* species continues to the present day.

This long history of use and importance of *Leucaena* species in indigenous communities means that local people have intimate knowledge of different species in terms of pod characteristics, season of pod production and flavour. Local residents in Oaxaca cull the sterile *L. leucocephala* x *L. esculenta* hybrids, calling them 'Guaje macho', or 'male' leucaena (Hughes and Harris 1994). In many parts of Oaxaca and Puebla, farmers cultivate three or more species to obtain pods year-round, with a variety of qualities. The pods are sold in markets throughout southern and central Mexico.

Given this rich and detailed ethnobotanical background it is quite plausible that *L. leucocephala* arose in domestication during the last few thousand years following cultivation of one or both of the parental species somewhere in eastern coastal Mexico. The Huastec region of eastern coastal Mexico in north-central Veracruz is one of the areas where early agriculture first developed (MacNeish 1965). An artificial origin would account for the lack of known natural populations and the limited genetic variation found in *L. leucocephala*, although two separate origins would have to be postulated to account for the two known subspecies. At present, *L. leucocephala* is extensively distributed in cultivation throughout Mexico, probably because of its favourable pod production characteristics. It yields abundant pods more or less continuously all year, and with high seed set per pod. Pods are easy to harvest and both pod and seed are sweet compared to those of the more bitter *L. esculenta* group. Such a tree, arising in cultivation, would have been immediately noticed and seized upon for wider cultivation and use. The continued attempts to cultivate it for pod production today, at or beyond its site limits in the colder and drier parts of central Mexico, are witness to this drive and interest.

Genetic variation within *L. leucocephala* is likely to be very limited and there is little value in further testing of varieties within that species. Below, we discuss possible future natural or semi-natural hybridisation leading to the formation of new species.

2. Interspecific Hybrids

Sorensson and Brewbaker (1994) have thoroughly investigated the possibility of artificially crossing species with *Leucaena*. They have shown that there are few genetic barriers to initial interspecific hybridisation within the genus. The production of artificial interspecific hybrids has been the main

focus of leucaena breeding efforts to date (Brewbaker and Sorensson 1990; Sorensson 1992 and these Proceedings). However, the occurrence of natural leucaena hybrids has received only sporadic attention, despite its obvious importance concerning the possible hybrid origins of the known tetraploid species.

Interspecific hybridisation is accepted as an important way of generating evolutionary novelty in the plant kingdom (Anderson 1949; Stebbins 1959; State 1975; Grant 1981). Recent data have confirmed that stabilisation of hybrids can lead to:

- (a) the origin of new homoploid hybrid derivative taxa (Gallez and Gottlieb 1982; Rieseberg et al. 1990);
- (b) the introgressive origin of new intraspecific taxa (Rieseberg et al. 1990; Abbott et al. 1992);
- (c) the origin of new allopolyploid (amphidiploid) species (Soltis and Soltis 1990; Ashton and Abbott 1992).

There are many unconfirmed reports of natural hybrids in the genus. From Mexico and Central America, these reports include *L. leucocephala* x *L. esculenta*, *L. diversifolia* x *L. leucocephala*, *L. pulverulenta* x *L. leucocephala* (Sorensson and Brewbaker 1994) and *L. pulverulenta* x *L. diversifolia* (Zárate 1982). In addition, hybrid origins have been proposed for *L. esculenta* subsp. *paniculata* (= *L. pallida*) (Pan 1985), *L. leucocephala* and *L. diversifolia* (Harris et al. 1994a). Zárate (1984a) suggested a hybrid origin for *L. lanceolata* subsp. *sousae*.

One of these hybrids has been thoroughly investigated. Hughes and Harris (1994) were able to show that hybrids of *L. leucocephala* subsp. *glabrata* x *L. esculenta* subsp. *esculenta* occurred in six states in south-central Mexico. While investigating this hybrid we discovered several important aspects of leucaena evolution and implications for future use of *Leucaena* species. The hybrid's identity was confirmed beyond reasonable doubt from a combination of geographical, morphological and molecular evidence (Hughes and Harris 1994). Molecular evidence showed that *L. leucocephala* was the female parent in all the hybrids tested.

This hybrid has now been found over a wide area of south-central Mexico in the states of Chiapas, Oaxaca, Guerrero, Morelos, Puebla and Hidalgo. In all cases the plants occur in disturbed areas in towns or villages where the parental species are cultivated for pod production. This suggests that the hybrid is the result of human interference where species have been brought into artificial sympatry through cultivation, and thus may be described as semi-natural. It is largely or completely sterile and all trees must therefore have arisen as separate F₁

hybrids. The widespread occurrence of this sterile F₁ hybrid raises the possibility that speciation could occur through chromosome doubling and the formation of a fertile allohexaploid, reproductively isolated from each of the parents (Ashton and Abbott 1992).

We have also observed and collected a further six putative hybrid combinations, some of which are common and widespread while others are rare and restricted in Mexico and Central America. Work is in progress to confirm their identities. In all cases, these additional hybrids are also the result of human interference, such as transport and cultivation for pod production, which has brought together species that would not naturally occur in the same place.

Although there are few published reports of confirmed natural hybrids, it is becoming clear that hybridisation has been a major mechanism for the generation of new species in *Leucaena*, largely (if not totally) induced by human interference. *Leucaena* species are increasingly being spread and used in cultivation and breeding. They are being planted in close proximity in mixed species evaluation trials, in arboreta and in agroforestry plantings, both in Central America and elsewhere. In Mexico and Central America, *L. leucocephala* is cultivated so widely that it is unusual to find *Leucaena* species with no *leucocephala* trees within the natural populations. Inevitably the resulting spontaneous hybridisation, and the evolution of new taxa, will present both opportunities and hazards.

New hybrids could turn out to be useful in tree planting programs, but the seed collected in such areas will not have guaranteed genetic integrity, creating further confusion and sub-optimal seed material for planting. Aggressive new taxa could arise which are potentially bad weeds (Abbott 1992). In the case of natural populations, introduction of alien species could result in genetic pollution of native germplasm. It is of fundamental importance that we should be able to identify the hybrids which arise. To do so, we will need clear documentation of species introductions, information on morphology and species-specific molecular markers.

Discussion, Conclusions and Future Work

The natural populations of the genus *Leucaena* have now been more thoroughly explored, collected and investigated than those of almost any other tropical woody genus. The information provides a basis for our rapidly improving knowledge of the systematics of the genus. New taxa are still coming to light in

the more isolated and under-collected parts of Mexico and Central America, probably with more still to be found. The work has shown that the genus is more complex, in terms of number of taxa, than was previously acknowledged, and has highlighted the importance of continuing human interference and interspecific hybridisation in the rapid recent evolution of new species.

Traditionally, forest genetic resources have been assessed by examining a combination of morphological and agronomic traits, but these mainly exhibit continuous variation and their effectiveness has been questioned by several authors (Gottlieb 1977; Brown 1979). On the other hand, the application of biochemical and molecular techniques has provided a set of powerful tools for studying genetic diversity within and among species and plant populations. These tools have generated useful data during the first phase of molecular analysis of the genus. For example, analysis of proteins and DNA provides important information.

If we examine all the known species in the genus we should find a range of nuclear DNA species-specific markers so that we can unambiguously identify hybrids and determine the origin of the tetraploid species. We are currently searching for RAPD species-specific markers and testing for their occurrence in known hybrid material. Promising data have been obtained.

The true potential of the genus as a source of agroforestry trees is only now becoming apparent. New and valuable species and genetic diversity have been found, and locations for on-going germplasm collection pin-pointed. A sound base for genetic conservation is also being built - for the first time it is possible to assess what to conserve and where conservation is needed.

Recent research on leucaena systematics confirms that sound taxonomy is essential for the wise use, improvement and conservation of leucaena genetic resources. A new taxonomic revision is being prepared to tackle the problems of species identification that underlie all leucaena research and use.

To be accepted and useful, the revision must not only be accurate systematically, but must prompt general consensus and be user-friendly. Consensus is essential to avoid continued confusion and on-going debate over species names. Stable nomenclature is needed to support all other research on the genus. If we present the results of systematic research before completing the new taxonomic revision, there should be time for further discussion and debate. Alongside a formal taxonomic revision, a user-friendly guide to the identification of species is essential, so that it can be widely distributed and used by non-botanists.

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References

- Abbott, R.J. 1992. Plant invasions, interspecific hybridisation and the evolution of new taxa. *Trends in Ecology and Evolution*, 7, 401-405.
- Abbott, R.-J., Ashton, P.A. and Forbes, D.G. 1992. Introgressive origin of the radiate groundsel, *Senecio vulgaris* L. var. *hibernicus* Syme. *Heredity*, 68, 425-435.
- Anderson, E. 1949. *Introgressive Hybridisation*. New York, John Wiley.
- Ashton, P.A. and Abbott, R.J. 1992. Multiple origins and genetic diversity in the newly arisen allopolyploid species *Senecio cambrensis* Rosser (Compositae). *Heredity*, 68, 25-32.
- Benthams, G. 1842. Notes on Mimoseae, with a short synopsis of species. *Journal Botany (Hooker)*, 4, 416-417.
- 1875. Revision of the suborder Mimoseae. *Transactions of the Linnean Society of London*, 30, 335-668.
- Brewbaker, J.L. 1980. What is giant *Leucaena*? *Leucaena Research Reports*, 1, 43-44.
- 1983. Systematics, self-incompatibility, breeding systems and genetic improvement of *Leucaena* species. In: *Leucaena Research in the Asia-Pacific Region, Workshop Proceedings*. Singapore, 17-22.
- - 1987. Species in the genus *Leucaena*. *Leucaena Research Reports*, 7, 6-20.
- Brewbaker, J.L. and Ito, G.M. 1980. Taxonomic studies of the genus *Leucaena*. *Leucaena Research Reports*, 1, 41-42.
- Brewbaker, J.L. and Sorensson, C.T. 1990. *Leucaena*: new tree crops from interspecific hybrids. In: Janick, J. and Simon, J., ed., *Advances in New Crops*. Oregon. Timber Press.
- Britton, N.L. and Rose, J.N. 1928. Mimosaceae. *North American Flora*, 23, 121-131.
- Brown, A.D.H. 1979. Enzyme polymorphisms in plant populations. *Theoretical Population Biology*, 15, 1-42.
- Casas, F. A. 1992. *Etnobotánica y procesos de domesticación en Leucaena esculenta (Mot & Sesse ex DC)* Benth. MSc thesis, UNAM, Mexico City, Mexico.
- Chamberlain, J.C. 1993. *The Taxonomy and Population Structure of Leucaena Benth. and Gliricidia H.B.K.* DPhil thesis, Cambridge, University of Cambridge, 210 pp.
- Chase, M.W. and Hills, H.H. 1991. Silica gel: an ideal material for field preservation of leaf samples for DNA studies. *Taxon*, 40, 215-220.
- Falk, D.A. and Holsinger, K.E. 1991. *Genetics and Conservation of Rare Plants*. Oxford, Oxford University Press.
- Filer, D.L. 1993. *BRAHMS: Botanical Research and Herbarium Management System, Reference Manual, Version 3.1*. Oxford, University of Oxford.
- Gallez, G.P. and Gottlieb, L.D. 1982. Genetic evidence for the hybrid origin of the diploid plant *Stephanomeria diegensis*. *Evolution*, 36, 1158-1167.
- Glumac, E.L., Felker, P. and Reyes, I. 1987. A comparison of cold tolerance and biomass production in *Leucaena leucocephala*, *L. pulverulenta* and *L. retusa*. *Forest Ecology and Management*, 18, 251-271.
- Gottlieb, L.D. 1977. Electrophoretic evidence and plant systematics. *Annals Missouri Botanical Garden*, 64, 161-180.
- Grant, V. 1981. *Plant Speciation*. New York, Columbia University Press.
- Halliday, J. and Somasegaran, P. 1983. Nodulation, nitrogen fixation and *Rhizobium* strain affinities in the genus *Leucaena*. In: *Leucaena Research in the Asia-Pacific Region. Workshop Proceedings*, Singapore. Ottawa, IDRC, 27-32.
- Harris, S.A., Hughes, C.E., Ingram, R. and Abbot, R. J. 1994a. A phylogenetic analysis of *Leucaena* (Leguminosae: Mimosoideae). *Plant Systematics and Evolution*, 191, 1-26.
- Harris, S.A., Hughes, C.E., Abbott, R. J. and Ingram, R. 1994b. Genetic variation in *Leucaena leucocephala* (Lam.) de Wit. (Leguminosae: Mimosoideae). *Silvae Genetica*, 43, 159-167.
- Harris, S.A., Chamberlain, J.L. and Hughes, C.E. 1994c. New insights into the evolution of *Leucaena* Benthams (Leguminosae: Mimosoideae). In: Pickersgill, B. and Polhill, R.M., ed., *Advances in Legume Systematics*. Kew, Royal Botanic Gardens, in press.
- Hellin, J.J. and Hughes, C.E. 1994. *Leucaena saivadorensis*: utilization and conservation in Central America. *Misc Publ. Siguatepeque, Honduras, CONSEFORH*.
- Hughes, C.E. 1986. A new *Leucaena* species from Guatemala. *Leucaena Research Reports*, 7, 110-113.
- 1988. *Leucaena salvadorensis*: status, distribution and genetic conservation. *Leucaena Research Reports*, 9, 99-105.
- 1991. Two new subspecies of *Leucaena* (Leguminosae Mimosoideae) from Guatemala. *Kew Bulletin*, 46, 547-557.
- 1993. *Leucaena Genetic Resources: the OFI seed collections and a synopsis of species characteristics*. Oxford, Oxford Forestry Institute, 117 pp.

- Hughes, C.E. and Harris, S.A. 1994. Identification and characterisation of a naturally occurring hybrid in *Leucaena* (Leguminosae: Mimosoideae). *Plant Systematics and Evolution*, 192, 177-197.
- Macbride, J.F. 1943. Flora of Peru. Family Leguminosae. Botanical Series Field Museum of Natural History, 8, 98-100.
- MacNeish, R.S. 1965. The origins of American Agriculture. *Antiquity*, 39, 87-94.
- McVaugh, R. 1987. Flora Novo - Galiciana. A descriptive account of the vascular plants of western Mexico. Volume 5, Leguminosae. Ann Arbor, University of Michigan Press, 786 pp.
- Pan, F.J. 1985. Systematics and Genetics of the *Leucaena diversifolia* complex. PhD thesis, University of Hawaii.
- 1988. Comparison of diploid and tetraploid *Leucaena diversifolia*. *Quarterly Journal of Chinese Forestry*, 21, 89-98.
- Pan, F.J. and Brewbaker, J.L. 1988. Cytological studies in the genus *Leucaena*. *Cytologia*, 53, 393-399.
- Rieseberg, L.H., Beckstrom-Sternberg, S. and Doan, K. 1990. *Helianthus annuus* subsp. *texanus* has chloroplast DNA and nuclear ribosomal genes of *Helianthus debilis* subsp. *cucumerifolius*. *Proceedings National Academy of Sciences USA*, 87, 593-597.
- Schery. 1950. Flora of Panama. *Leucaena*. *Annals of the Missouri Botanical Garden*, 37, 302-304.
- Smith, C.E. 1967. Plant Remains. In: Byers, D.S., ed., *The Prehistory of the Tehuacan Valley*. Vol. 1. Environment and Subsistence. Austin, USA, University of Texas Press.
- Soltis, D.E. and Soltis, P.S. 1990. *Isozymes in Plant Biology*. London, Chapman and Hall.
- Sorensson, C.T. 1992. Strategies for small scale *Leucaena* hybrid seed production. *Proceedings IUFRO Conference S.2.02.08*, Cali, Colombia, November 1992, in press.
- Sorensson, C.T. and Brewbaker, J.L. 1994. Interspecific compatibility among 15 *Leucaena* species (Leguminosae: Mimosoideae) via artificial hybridizations. *American Journal of Botany*. 81(2), 240-247.
- State, C.A. 1975. *Hybridization and the Flora of the British Isles*. London, Academic Press.
- Standley, P.C. 1922. Trees and shrubs of Mexico. *Contributions to the U.S. National Herbarium*, 23, 366-369.
- Standley, P.C. and Steyermark, J.A. 1946. *Flora of Guatemala, Part V*. *Fieldiana Botany*, 24, 46-48.
- Stebbins, G.L. 1959. The role of hybridization in evolution. *Proceedings American Philosophical Society*, 103, 231-251.
- Stewart, J.L., Dunsdon, A.J. and Hughes, C.E. 1991. Early biomass production of *Leucaena* species in Comayagua, Honduras. *Leucaena Research Reports*, 12, 101-104.
- Whitaker, T.W. and Cutler, H.C. 1966. Food plants in a Mexican market. *Economic Botany*, 20, 6-16.
- Zárate, S.P. 1982. Las especies de *Leucaena* Benth. de Oaxaca con notas sobre la sistematica del genero para Mexico. MSc thesis, UNAM, Mexico.
- 1984a. Taxonomic revision of *Leucaena* Benth. from Mexico. *Bulletin of the International Group for the Study of Mimosoideae*, 12, 24-34.
- 1984b. Domestication incipiente del 'Guaje Zacatzin'. *Resumenes 9 Congreso Mexicano de Botanica*, Mexico City, Sociedad Botanica Mexicana.
- 1987a. Taxonomic identity of *Leucaena leucocephala* (Lam.) de Wit with a new combination. *Phytologia*, 63, 304-306.
- 1987b. Clarification of the taxonomy of *Leucaena salvadorensis* Standley ex Britton & Rose. *Annals Missouri Botanical Garden*, 74, 449.
- 1994. Revision del genero leucaena en Mexico. *Anales Inst. Biol. Univ. Auton. Mexico, Ser. Bot.* 65(2), 83-162.

Leucaena Germplasm Collections, Genetic Conservation and Seed Increase

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Abstract

The status of leucaena germplasm collections is reviewed, showing that leucaena has been more thoroughly explored and collected than most tropical woody genera. Despite comprehensive germplasm collecting, genetic conservation has been neglected and the status of the genetic resources of leucaena species in situ is shown to be very degraded. The paper describes ex situ genetic conservation programs and reveals that only a small fraction of the genetic variation available in the genus is currently conserved. Finally, seed production for planting is discussed, demonstrating the need for expanded efforts in seed increase and deployment of improved genetic material.

Exploration of *Leucaena* Genetic Resources

Historical spread of *Leucaena* germplasm

The genetic resources of leucaena have been under scrutiny for at least the last two millennia as a source of useful plants. Archaeological evidence suggests that Aztec, Mixtec, Maya, Toltec and Zapotec peoples used leucaena seeds and pods as human food up to two or three thousand years ago in parts of south-central Mexico (Smith 1967; Zárate 1984a; Casas 1992). Thus leucaena seed was already being collected and transported within Mexico in pre-Colombian times and there is evidence to suggest that the distributions of widely used species such as *L. esculenta* and *L. leucocephala* were greatly extended by this process of indigenous domestication. Seed of a wide range of *Leucaena* species continues to be transported within Mexico today by local people who are still cultivating trees for pod production (Whitaker and Cutler 1966; Zárate 1984b; Casas 1992; Harris et al. in press; Hughes and Harris these Proceedings).

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Spread of leucaena around the globe to the Old World occurred as early as the sixteenth century when Spanish colonists introduced one species, *L. leucocephala* subsp. *leucocephala* to the Philippines. It is not clear whether this introduction was deliberate or accidental (Brewbaker 1987; Zárate 1987). The same species was also in cultivation in European botanic gardens in the first part of the eighteenth century or earlier. This subspecies of *L. leucocephala*, which corresponds to the shrubby or common variety, continued its spread throughout the tropics and is now extensively naturalised and weedy in many tropical countries (Hughes and Styles 1989; Cronk and Fuller 1995). Recent isozyme studies indicate that this subspecies is represented by a single self-pollinated variety outside of its native range in the Americas.

Several other species of *Leucaena* were introduced into Old World cultivation as shade trees over coffee or cacao about 100 years ago. For instance, *L. pulverulenta* and *L. diversifolia* were introduced by Dutch foresters to Indonesia at the end of the nineteenth century (Djikman 1950), and *L. diversifolia* was introduced to West Africa in Cameroon and Ivory Coast, and probably also to Jamaica in the Caribbean.

Modern germplasm collections

When leucaena became an important forage plant in the 1960s, systematic germplasm collections

started. Since that time, three major germplasm collections of *Leucaena* species have been assembled by the University of Hawaii (USA), CSIRO (Australia) and OFI (UK). These collections are summarised in Table 1 and described in detail below. As well as these three international collections there are many national leucaena germplasm collections, which vary in size and level of activity, and we briefly discuss these also.

University of Hawaii

In 1962, the University of Hawaii (UH) started assembling the first germplasm collection of leucaena, initially based on miscellaneous seed acquired world-wide from the various non-native sources where leucaena was cultivated. Early collections were dominated by the one species of greatest interest at that time, *L. leucocephala*, and by seed from non-native sources. Organised efforts to explore and collect seed from the natural populations of leucaena began in 1967 when Brewbaker led a UH team to Mexico and other countries in Latin America. For the first time this collection gathered a range of *Leucaena* species other than *L. leucocephala*. Germplasm collections by UH researchers, led by Brewbaker, continued intermittently over the next 20 years. Major expeditions in 1977, 1978, 1985 and 1988 sampled natural populations throughout Latin America (Brewbaker and Sorensson 1994). By 1988 a large collection - almost 1000 accessions, representing most taxa - had been assembled and grown in Hawaii. It was still dominated by *L. leucocephala* which accounted for 541 out of 967 accessions.

Seedlots are documented with basic passport data held on a spread sheet database using the 'K' number series running chronologically from K1 to K967. Seed from the Hawaii collection has been distributed for testing and includes the well-known and widely planted K8 and K636 varieties of *L. leucocephala* and K156 variety of *L. diversifolia*. This collection also forms the core of several other large germplasm collections (approx. 90% of the United States Department of Agriculture (USDA) collection and a significant proportion of the National Seed Storage Laboratory (NSSL), International Livestock Centre for Africa, Ethiopia (ILCA) and Indian Grassland and Fodder Research Institute (IGFRI) collections). Identities are verified from living collections in Hawaii.

CSIRO

Between the mid 1950s and the mid 1970s CSIRO assembled a largely opportunistic collection of 200 accessions, mainly *L. leucocephala*, from non-native

sources. Extensive targeted collections were made, principally in Mexico in the late 1970s and early 1980s by R.Reid (working with S. Zárate) mainly of species other than *L. leucocephala*. Passport data for the collections is documented under the CPI (Commonwealth Plant Introduction) numbering system, and stored by the Australian Tropical Forages Genetic Resource Centre in a UNIX-based data management system, designed for the purpose.

Oxford Forestry Institute

In the mid 1980s, OFI started to assemble a new leucaena seed collection, for two reasons. Firstly, several little known and potentially valuable species had been discovered in Guatemala and Honduras, apparently having been overlooked by previous collection expeditions. Secondly, OFI realised that several of these species were already severely degraded and under threat of substantial genetic erosion or even extinction (Hughes 1986, 1988; Hellin and Hughes 1994). The main objective of the OFI collection program was to assemble all *Leucaena* species, but to concentrate on the lesser-known species. From 1984 to 1992, a series of annual expeditions to Mexico and Central America made collections in collaboration with the Forest Authorities and tree seed banks in the regions. These collections are now complete and are documented by Hughes (1993). Identities are verified from botanical voucher specimens deposited in major herbaria in Mexico, USA and Europe.

National Collections

National collections or collections at regional centres such as ILCA or Centro Internacional de Agricultura Tropical (CIAT), are largely derived from the three large international collections listed above. As well, several contain small numbers of unique accessions from their local areas. A good example is the diploid *L. diversifolia* collection RSB01 from Indonesia (Oka 1990). Within Mexico and Central America several agencies maintain seed collections of local species based on bulk collections from natural populations. The Forest Tree Seed Banks in Guatemala, Honduras and Nicaragua are notable in this respect. They regularly collect bulk seed from natural stands of a wide range of native *Leucaena* species, for direct use in tree planting projects.

Seed collection strategies

The international leucaena germplasm collections have used different sampling strategies because they have radically differing objectives. Methods of collecting agricultural crop germplasm are not the same

as methods of collecting germplasm for forest tree improvement.

The UH and CSIRO collections were made essentially as for any crop germplasm. The collection expeditions were short, they sampled widely across localities and included small but variable numbers of parent trees. The resulting seedlots were termed 'accessions'. An accession can thus be progeny from a single parent tree or from several trees, but rarely includes more than ten parents. Seed from individual trees cannot be identified, except for self-fertile tetraploid species. This approach makes little attempt to select particular trees within natural populations, and does not assemble representative population samples, but it allows cost-effective sampling of as many sites as possible within the time available. It ensures coverage of as broad a range of environments as possible by sampling in relation to soil changes and microclimate. Unreplicated arboretum-style trials are used to initially screen the many accessions collected. Further evaluation and breeding then relies on seed increase from superior accessions.

In contrast, forest tree improvement programs (OFI collections) are largely directed to out-crossing species. They follow highly structured sampling strategies and collect seed from natural populations. During the longer seed collection expeditions, large numbers of parent trees are sampled across large populations, avoiding neighbouring trees. Bulk collections termed 'provenances' are assembled. These collections usually include a minimum of 25 parent trees and often up to 50-60, and are expected to be genetically diverse and amenable to subsequent selection and breeding. The seed source can be identified and provenance variation can be used as a starting point in any improvement program. In many cases, individual tree seedlots are also collected at the same time. Such an approach, with maintenance of family identity, allows family-based half-sib progeny trials (out-crossing species) and seed orchards to be established. It aims to maintain a much stricter level of control over pedigree and a much broader genetic base within provenances.

Status of *Leucaena* germplasm collections

Table 1 summarises the numbers of accessions in the main germplasm collections of leucaena (a simplified taxonomy has been used to summarise germplasm, with no subspecies included). The many hybrid accessions in the UH collection are not in this list, but hybrids in the NSSL and ILCA collections are placed in the spp. category. In the OFI collection, the first numeral indicates the number of provenances held and the second numeral the number of individual tree or half-sib family accessions.

Across all the collections there is clearly significant duplication, roughly estimated here as more than 25%. This means that the total number of original or unique accessions is almost certain to be considerably less than 3000. Internal duplication is essentially zero in all collections. In the table, duplication refers to cases where the same seedlot is represented in two or more collections, which happens when material is exchanged and distributed. (Figures are broad estimates, not based on detailed investigations.) For example, the USDA collection is largely derived from the UH collection, so duplication is probably over 90% between these two. The UH and CSIRO collections also have some seedlots in common. Duplication can also arise when different organisations independently collect material from the same site and even the same population. We do not know how often this happens nor how important it is.

The new OFI collections have diminished the domination by *L. leucocephala*, but collections from this species still make up about 50% of the total in Table 1. Many of the *L. leucocephala* accessions are known to be genetically uniform and therefore almost redundant, because of the marked lack of genetic variation that has been found within the species, and particularly within the shrubby subspecies *leucocephala*. Since the advent of the psyllid, many of the early collections of *L. leucocephala* have become obsolete, especially now that modern collections include a wider range of species and more germplasm from natural populations. In contrast, the much higher level of genetic variation known to exist in species such as *L. diversifolia* and others is only sparsely represented in the collections (Table 1). A few species such as *L. confertiflora* and *L. cuspidata* are represented by only a handful of accessions.

There are limited quantities of seed for many of the accessions, in all but the OFI collection. For example, more than 500 of the 967 accessions held by the University of Hawaii have only 100 or fewer seeds in stock. Although CSIRO aims to maintain 200 g of seed available for all its accessions, it has succeeded only for self-fertile types. A similar situation applies to the USDA and some other collections. The NSSL collections may be regarded as being in permanent long-term storage and are 'not accessible'. This means that most of the accessions listed in Table 1 need a major program of regeneration to make them available for widespread use and testing. Larger seed quantities are available for the remainder of the accessions and for the majority of the OFI collection.

Considerable confusion surrounds the identity of material in many collections, partly because there are several numbering systems. The three major

Table 1. Major *Leucaena* germplasm collections showing numbers of accessions.

Species	UH	USDA ⁴	OFI ¹	CSIRO	NSSL ⁵	ILCA	IGFRI ³	TOTAL
<i>collinsii</i>	37	19	5(113)	10	-	4	11	194
<i>confertiflora</i>	2	-	5(18)	4	-	-	-	24
<i>cuspidata</i>	-	1	5(20)	2	-	1	-	24
<i>diversifolia</i>	118	53	19(168)	27	1	21	29	41?
<i>esculenta</i>	55	23	3(48)	13	2	3	10	154
<i>greggii</i>	32	7	3(50)	9	-	1	30	129
<i>lanceolata</i>	47	35	7(83)	41	1	3	35	245
<i>leucocephala</i>	541	493	19(78)	590	125	102	268	2197
<i>macrophylla</i>	17	6	4(30)	19	-	2	12	86
<i>multicapitula</i>	3	-	2(20)	-	-	-	2	25
<i>pallida</i> ²	20	12	6(50)	15	-	10	1	108
<i>pulverulenta</i>	18	14	3(33)	45	4	6	10	130
<i>retusa</i>	18	4	1(10)	13	-	2	12	60
<i>salvadorensis</i>	3	1	6(123)	-	-	1	10	138
<i>shannonii</i>	33	23	6(130)	12	1	4	55	258
<i>trichodes</i>	23	4	2(50)	15	-	1	11	104
spp.	-	14	3(92)	-	5	13	-	124
total	967	709	1116	815	140	174	496	4417
% duplication	?10	?10	0	?30	?100	?100	?100	?>25

UH = University of Hawaii; USDA = United States Department of Agriculture; OFI = Oxford Forestry Institute; CSIRO = Commonwealth Scientific and Industrial Research Organisation; NSSL = National Seed Storage Laboratory, USA; ILCA = International Livestock Centre for Africa, Ethiopia; IGFRI = Indian Grassland and Fodder Research Institute, India

¹ OFI: first numeral indicates number of provenances, second numeral number of individual tree or half-sib family accessions

² *L. pallida* is sometimes referred to as *L. esculenta* subsp. *paniculata*

³ From Gupta (1990)

⁴ G.A. White, USDA, pers. comm. 1992

⁵ S.A. Eberhart, NSSL, pers. comm. 1988. Long-term, essentially permanent storage, 'not accessible'.

independent international germplasm collections each have their own numbering systems, and several national and other collections also have their own numbering systems, even though their material is partially derived from the three major collections or from each other. To confuse identification even more, no-one has accurately assessed levels of duplication across collections, nor levels of redundancy in the collections due to low seed quantities or uniformity of genetic material. Clearly, individual collections need to be rationalised and the major collections need to be catalogued, with cross-referencing of identities and duplicate collections, to produce a World Germplasm Catalogue for leucaena.

Practical aspects of leucaena seed collection and storage

In general, *Leucaena* species produce large quantities of viable seed at an early age. Most species are less prolific, less precocious and more seasonal than *L. leucocephala* in their seed production. A few species, such as *L. salvadorensis*, may not produce significant quantities of seed until 3-4 years of age. Seed

collection and extraction methods are straightforward. For most species, it is critical to time seed collection to coincide with peak ripeness. Seed predators can cause major losses in seed collected from the natural populations and seed beetles are also a problem in other areas, such as in Hawaii. Some areas, such as Australia, apparently have no seed predation problem. Effective methods for separating beetle-infested seed from good seed have been developed using flotation in salt water (Sorensson 1993) and gravity table methods (Hughes 1993).

Leucaena seed retains high viability for several decades when stored under normal conditions of 8-10% moisture content at +4°C. Seed viability in long-term storage at lower temperatures (-20°C) has not been tested but, given the hard seed coat of most leucaena species, seed can probably be stored safely for long periods. Seed stored under poor conditions of high moisture content and temperature can rapidly lose viability. While most organisations with large seed collections of *Leucaena* species have access to adequate seed storage facilities, cold storage facilities are still inadequate in some countries.

Leucaena Genetic Conservation

Status of *Leucaena* genetic resources

Leucaena species are distributed mainly in the seasonally dry tropical and upland forests of Mexico and Central America. More than 98% of the tropical dry forest type in this region has already disappeared (Nations and Kramer 1983) and less than 0.08% lies in any form of biological reserve (Janzen 1986). Tree cover is thus reduced to fragmented remnants, scattered trees in fence lines, around houses and in inaccessible areas such as steep gullies or cliffs. Even these last remnants of forest are being continually degraded by further clearance of land for agriculture, dry season fires and extensive overgrazing in some areas.

Without exception, *Leucaena* species have suffered some level of genetic degradation. This means that all the germplasm collections outlined above have been assembled from disturbed populations. Few pristine populations of *Leucaena* species remain. *Leucaena* species are susceptible to grazing and many have been progressively reduced because of the huge increase in grazing pressure since goats and cattle were introduced to Mexico by the Spanish in the sixteenth century. This is particularly apparent for the mid elevation and highland species of the Mexican mesetas including *L. retusa*, *L. greggii*, *L. cuspidata*, *L. pallida* and *L. confertiflora*. These species are now often restricted to steep gullies and other areas that grazing animals cannot reach.

For a few species, particularly those with restricted distributions, degradation has now reached critical levels. Active measures are needed to conserve the genetic resources of these species. For example, *L. shannonii* subsp. *magnifica*, a taxon restricted to a small area of south-east Guatemala, is now apparently reduced to fewer than 400 individuals (Hughes 1986, 1991). Similarly, *L. salvadorensis*, a species native to restricted areas in eastern El Salvador, southern Honduras and northern Nicaragua, has been reduced to small, scattered and fragmented stands (Hughes 1988; Hellin and Hughes 1994). Seed collections of *L. salvadorensis* carried out in these areas have been forced to cover large areas to include sufficient parent trees; for example, inclusion of sixty parent trees required collection over more than 100 km² in northern Nicaragua in 1991.

Humans have also interfered with the genetic resources of *Leucaena* species by the processes involved in indigenous domestication. Many species of *Leucaena* are now protected and managed, their seed is harvested and, in large parts of south-central Mexico, cultivated. Levels of genetic diversity in these populations have not been widely investigated

but might be expected to be lower than in corresponding natural populations. Casas (1992) presented evidence that pod morphology showed reduced phenotypic diversity in cultivated populations of *L. esculenta* and interprets this as a sign of past selection by local people for favourable pod characteristics.

Ex situ genetic conservation

Ex situ genetic conservation refers to conservation of species outside the natural ecosystem in which they originally evolved. Traditionally it involves long-term seed storage, and different types of living collections including arboreta, botanic gardens, conservation stands and seed production areas. At present there are few programs specifically directed towards ex situ genetic conservation of *Leucaena* species, so at present the genetic variation in the genus is not well conserved in any form of ex situ conservation.

All but one of the large seed collections of *leucaena* are aimed at short or medium term storage. These 'working collections' are kept under normal seed storage conditions of low humidity and temperature where viability remains high for 30 or more years. The only substantial collection in long-term storage, at very low temperatures, is held by the USDA National Seed Storage Laboratory, NSSL, at Fort Collins, USA (see Table 1). This collection includes 140 accessions, of which 125 are of *L. leucocephala*, all of which are held jointly by the UH and USDA regional working collections. Thus only a small fraction of the genetic diversity in the genus is represented in long-term storage and most species are not included at all. Further, the material in long-term storage bears no relation to the conservation status of species in their native ranges; the most threatened taxa are not represented. Smaller collections of *leucaena* are held in other long-term stores such as those of the National Bureau of Plant Genetic Resources, New Delhi, India and the CATIE long-term seed store in Costa Rica. The problems of regeneration of long-term *leucaena* seed collections have not been tackled so far.

As with most of the seed collections, the ex situ living collections of *Leucaena* species are not specifically directed at long-term genetic conservation. There are many arboretum-style collections, in Australia, Brazil, Colombia, Ethiopia, Hawaii, Honduras, India, Indonesia, Taiwan and other places. In every case these are working collections or unreplicated evaluation trials, not conservation collections. All are relatively insecure because they lack resources and support for maintenance and are susceptible to sporadic disasters such as fire,

hurricane or urban development. The amount of genetic diversity that can be conserved is limited again by lack of resources such as land; in general species are represented by few trees per plot and few accessions or provenances. Finally, the genetic integrity of seed collected from mixed-species living collections cannot be guaranteed, due to high levels of interspecific compatibility and hybridisation.

There are a few examples of ex situ plantings in the form of conservation stands or seed production areas or seed orchards where larger amounts of genetic variation can be included in isolated blocks guaranteeing production of pure seed. A good example of this type of approach is the ex situ seed orchard program being undertaken in Honduras by the CONSEFORH project for *L. salvadorensis* (Ponce, these Proceedings).

Thus at present the genetic variation in *Leucaena* species is not well conserved in ex situ seed collections or living collections.

In situ genetic conservation

In situ genetic conservation refers to conservation of species within the ecosystem in which they originally evolved. Traditionally it has been confined to protected areas such as national parks and nature reserves. As indicated above, only a small fraction of the total dry tropical forest of Mexico and Central America is protected in any form of biological reserve. Most species of *Leucaena* are not present in any of the small and scattered reserves that do exist. There are a few exceptions. For example, *L. lanceolata* occurs in the Chamela reserve in Jalisco, Mexico, and *L. retusa* is found in the Big Bend National Park in Texas, USA. Biological reserves cannot therefore be expected to make more than a very minor contribution to conservation of *Leucaena* genetic resources even at species level, let alone conservation of intraspecific genetic diversity.

Farmer-based conservation

Now that the limitations of ex situ conservation and traditional in situ biological reserves for crop genetic resources have been realised, farmer-based conservation is seen as making an important potential contribution to the overall conservation effort (Brush 1991; Cohen et al. 1991). Farmer-based conservation may be considered as a form of in situ conservation through use. Although only advocated so far for crop plants, it appears to have considerable potential for agroforestry trees such as *Leucaena* species (Hughes 1993; Hellin and Hughes 1994). The fact that farmers have been actively conserving a wide range of *Leucaena* species over the centuries by protecting, managing and cultivating trees, indicates the potential of farmer-based conservation for

leucaena. For many species, such as *L. diversifolia*, *L. esculenta*, *L. pallida* and *L. salvadorensis*, the relative abundance of trees in the present day is entirely due to farmer protection and cultivation. Conservation through use can operate over large areas and conserve large amounts of genetic variation. It minimises the effects of sporadic disasters such as fires and requires only limited investment of resources because the process becomes self-sustaining. As mentioned above, indigenous domestication may have led to some reduction in genetic variation but little compared to the drastic losses of genetic variation in species of *Leucaena* from natural vegetation and current ex situ conservation. A strategy for farmer-based conservation of one species, *L. salvadorensis*, was elaborated by Hellin and Hughes (1994) and appears to have the potential to be applied to most, if not all, *Leucaena* species. Active support by donors to tree planting organisations, especially non-government organisations, in Mexico and Central America could help to promote wider use of local leucaena species in situ with substantial and cost-effective conservation benefits.

Genetic conservation strategy

Current reliance on short or medium term seed storage and active breeding or arboretum collections for genetic conservation needs to change in favour of measures specifically directed to genetic conservation. Farmer-based conservation in agroforestry systems provides a viable alternative in situ conservation method for leucaena. In situ and ex situ conservation are compatible and complementary and need to be used in tandem to provide a reliable genetic conservation strategy for leucaena. Expanded ex situ conservation programs, that incorporate more genetic variation into long-term seed storage and viable conservation stands, are needed to overcome the limitations of current ex situ efforts.

Seed Increase for Deployment

Tree seed supply is a critical factor in the success of agroforestry and other tree planting programs (Turnbull 1983). Abundant seed has been an important factor contributing to the spread and widespread adoption of *L. leucocephala*. For *L. leucocephala*, early and ample seed production through self-pollination, giving true-bred material, meant that seed was spread extremely easily from farmer to farmer. Seed of most other *Leucaena* species, most of which are self-sterile, is not available in bulk or semi-bulk quantities at present and problems of producing pure seed are much greater for out-crossing species. Now that interest is focused on other species and hybrids, demand for their seed is likely to rise, but limited quantities of seed are

available. As a result, adoption of less-known species is not likely to be widespread.

Seed collections for routine planting, at least of self-incompatible species, should not be made from trials or arboreta. Research in Hawaii has shown that there are few quantitative genetic barriers to interspecific hybridisation in *Leucaena* (Sorensson and Brewbaker 1994). This means that when species are brought into close proximity in cultivation, as happens in trials and arboretum collections, there will be many opportunities for the spontaneous production of hybrids. Therefore the genetic integrity of seed collected from such areas cannot be guaranteed, except for the self-pollinated species and hybrids. Further, the small plots in trials or arboreta contain too few parent trees to provide an adequate genetic base for routine seed production activities.

The natural populations in Mexico and Central America provide the only immediately available source of bulk seed supplies for most species. Forest tree seed banks in Nicaragua, Honduras and Guatemala regularly supply bulk seed of a range of native *Leucaena* species. In the medium term, local seed self-sufficiency is a pre-requisite for success in non-industrial forestry. Careful thought must be given to the distribution of new species, hybrids and improved or select genetic material. It is notable that despite considerable effort to breed improved material of *leucaena*, only a handful of select self-compatible varieties of *L. leucocephala* and the bred Cunningham variety have been successfully deployed for widespread use, because these varieties produce large amounts of seed in small plots. For proven out-crossing species and interspecific hybrids, there is a real and immediate need for seed sources which are soundly based, well adapted and from known provenances.

Seed increase of the self-fertile polyploid species such as *L. leucocephala* and *L. diversifolia* subsp. *diversifolia* presents no major obstacles. However, for out-crossing species, broadly-based composite seed orchards need to be established to obtain good seed production. A small seed orchard of 100 trees was established in Australia with considerable success, using a mixture of *L. pallida* accessions to produce composite seed. However, it was difficult to produce seed of the single superior diploid *L. diversifolia* line CP146568, due to scant seed production. Seed orchards of *L. salvadorensis* have been established in Honduras (Ponce, these Proceedings) and Nicaragua following the Breeding Seedling Orchard design, BSO (Gibson 1993). In this, up to 50 half-sib families are planted at close spacing in a replicated design, with early assessment and progressive selective thinning to wide spacing

for seed production. So far these trees have produced little seed.

The problem of seed increase was clearly acknowledged within the *leucaena* psyllid trial (LPT) program, coordinated by the University of Hawaii. In 1989, a follow-up *leucaena* seed production (LSP) program established seed production areas for a range of psyllid tolerant species and hybrids in five countries, namely India, Indonesia, Philippines, Taiwan and Thailand. Most of the seed produced from the LSP program has been from the self-fertile polyploids such as the K636 variety of *L. leucocephala*. In the LSP, two orchards were established at each site, usually with cross sterile taxa. Seed was supplied for more than 500 trees, including a minimum of 10 accessions, and it was recommended that orchards be established at some distance from the LPT trials. Success of the LSP was mixed and seed beetle infestations limited production of viable seed at some sites. The particular problems associated with production of hybrid seed need special attention and have been discussed by Sorensson (1992). Seed production of sterile triploids utilising cloned self-incompatible female parents was discussed as a viable commercial option by Brewbaker and Sorensson (1990).

Conclusions and Priorities for Research and Development

Germplasm collections

The genetic resources of *Leucaena* species have now been more thoroughly explored and collected than almost any other tropical woody genus. There are three major international germplasm collections whose evaluation and conservation should be of much higher priority than new collection programs.

There is considerable confusion surrounding the different collections. Their status and different numbering systems need to be clarified through the compilation of a cross-referenced world germplasm catalogue covering all collections.

Current germplasm collections need to be reorganised to avoid or clarify duplication and reduce large holdings of redundant material, mainly of *L. leucocephala*.

Genetic conservation

Programs to conserve the genetic resources of *leucaena* need to be significantly expanded. A genetic conservation strategy needs to be developed for all *Leucaena* species. It should employ complementary in situ and ex situ methods.

Indigenous farmers have successfully conserved many species. Farmer-based conservation offers

greater potential than traditional in situ conservation in biological reserves. It could be greatly expanded to cover most, if not all species of *Leucaena*.

There will be significant benefits for conservation if breeding and conservation are made separate objectives of ex situ living collections and seed storage. Genetic conservation needs to be considered in its own right, not as a by-product of evaluation and breeding.

Seed production for planting programs

Seed increase and the planting out of new species and hybrids need to be greatly expanded. Appropriate systems need to be designed and adopted to produce hybrid seed and seed of out-crossing species. Out-crossing species are the norm in forest trees, so there is considerable forestry experience in seed orchard design which needs to be adapted to leucaena seed production.

Seed increase of self-pollinating species and hybrids (always polyploid) should seek to employ multi-line technologies to broaden the genetic base in growers' fields.

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References

- Brewbaker, J.L. 1987. *Leucaena*: a multipurpose tree genus for tropical agroforestry. In: Steppeler, H.A. and Nair, P.K., ed., *Agroforestry: A Decade of Development*. ICRAF, Nairobi, Kenya.
- Brewbaker, J.L. and Sorensson, C.T. 1990. New tree crops from interspecific *Leucaena* hybrids. In: Janick, J. and Simon, J.E., ed., *Advances in New Crops*. Timber Press, Portland, Oregon, USA, 283-289.
- 1994. Domestication of lesser-known species of the genus *Leucaena*. In: Leakey, R. and Newton, A., ed., *Tropical Trees—the Potential for Domestication*. Institute of Terrestrial Ecology, Edinburgh, Scotland.
- Brush, S.B. 1991. A farmer-based approach to conserving crop germplasm. *Economic Botany*, 45, 153-165.
- Casas, F.A. 1992. *Etnobotanica y procesos de domestication en Leucaena esculenta* (Mot. & Sessk ex A.D.C.) Benth., MSc Thesis, UNAM, Mexico City, Mexico.
- Cohen, J.I., Alcorn, J.B. and Potter, C.S. 1991. Utilization and conservation of genetic resources: international projects for sustainable agriculture. *Economic Botany*, 45, 190-199.
- Cronk, Q.C.B. and Fuller, J.L. 1995. *Plant Invaders: The threat to Natural Ecosystems*. Chapman and Hall.
- Djikman, M.J. 1950. *Leucaena*—a promising soil-erosion control plant. *Economic Botany*, 4, 337-349.
- Gibson, G.L. 1993. Genetic conservation of native hardwoods in the degraded dry forests of Honduras. In: *Resolving Tropical Forest Resource Concerns through Tree Improvement, Gene Conservation and Domestication of New Species*. Proceedings IUFRO Conference, Cali, Colombia, October 1992.
- Gupta, V. 1990. Genetics and breeding of *L. leucocephala*. Unpublished, IGFR, Jhansi, India.
- Harris, S.A., Chamberlain, J.L. and Hughes, C.E. New insights into the evolution of *Leucaena* Benth. (Leguminosae: Mimosoideae). In: Pickersgill, B. and Polhill, R.M., ed., *Advances in Legume Systematics*, Royal Botanic Gardens, Kew. In press.
- Hellin, J.J. and Hughes, C.E. 1994. *Leucaena salvadorensis*: utilization and conservation in Central America. Miscellaneous Publication, CONSEFORH, Siguatepeque, Honduras.
- Hughes, C.E. 1986. A new *Leucaena* from Guatemala. *Leucaena Research Reports*, 7, 110-113.
- 1988. *Leucaena salvadorensis* status, distribution and genetic conservation. *Leucaena Research Reports*, 9, 99-105.
- 1991. Two new subspecies of *Leucaena* (Leguminosae: Mimosoideae) from Guatemala. *Kew Bulletin*, 46, 547-557.
- 1993. *Leucaena* Genetic Resources: The OF1 Seed Collections and a Synopsis of Species Characteristics. Oxford Forestry Institute, Oxford, UK.
- Hughes, C.E. and Styles, B.T. 1989. Risks and benefits of woody legume introductions. In: Stirton, C.H. and Zarruchi, J.L., ed., *Advances in Legume Biology*. Monographs in Systematic Botany, Missouri Botanical Garden, 29, 505-53 1.
- Janzen, D.H. 1986. Guanacaste National Park: Tropical Ecological and Cultural Restoration. Editorial, Universidad Estatal a Distancia, San Jose, Costa Rica.
- Nations, J.D. and Kramer, D.I. 1983. Central American tropical forests: positive steps for survival. *Ambio*, 12, 232-238.
- Oka, I.N. 1990. Progress and future activities of the *Leucaena* psyllid research program in Indonesia. In: Napompeth, B. and MacDicken, K.G., ed., *Leucaena Psyllid: Problems and Management*, Winrock International.
- Smith, C.E. 1967. Plant Remains. In: Byers, D.S., ed., *The Prehistory of the Tehuacan Valley*, Vol. 1. Environment and Subsistence. Austin, USA, University of Texas Press.
- Sorensson, C.T. 1993. Production and characterisation of interspecific hybrids of the tropical tree leucaena (Leguminosae: Mimosoideae). PhD thesis, University of Hawaii, USA.
- 1994. Seed productivity of artificial hybridizations between fifteen species in the genus leucaena. In: *Resolving Tropical Forest Resource Concerns through Tree Improvement, Gene Conservation and Domestication of New Species*. Proceedings IUFRO Conference, Cali, Colombia, October 1992, 359-365.

- Sorensson, C.T. and Brewbaker, J.L. 1994. Interspecific compatibility among 15 *Leucaena* species (Leguminosae: Mimosoideae) via artificial hybridization. *American Journal of Botany*, 81(2), 240-247.
- Turnbull, J.W. 1983. Tree seed supply — a critical factor for the success of agroforestry projects. In: Burley, J. and von Carlowitz, P., ed., *Multipurpose Tree Germplasm*, ICRAF, Nairobi, Kenya.
- Whitaker, T.W. and Cutler, H.C. 1966. Food plants in a Mexican market, *Economic Botany*, 20, 6-16.
- Zárate, S.P. 1984a. Taxonomic revision of *Leucaena* Benth. from Mexico. *Bulletin of the International Group for the Study of Mimosoideae*, 12, 24-34.
- 1984b. Domesticacion incipiente del 'Guaje Zacatin'. - *Resúmenes 9º Congreso Mexicano de Botanica*, Mexico City, Sociedad Botanica Mexicana.
- 1987. Taxonomic identity of *Leucaena leucocephala* (Lam.) de Wit with a new combination. *Phytología*, 63, 304-306.

LEUCNET: Exploiting Opportunities for Improvement in Leucaena

J.L. Brewbaker¹

Abstract

LEUCNET, the Leucaena Network, is proposed as a core group of scientists and institutions working to improve the productivity and utility of *Leucaena* species. This paper discusses networking and strategic research activities, suggesting that LEUCNET should perform international collaborative trials with careful statistical analysis; should collect solid benchmarked data as the basis for cooperative research; should encourage the planting, demonstration and conservation of superior leucaena germplasm; should use state-of-the-art technologies in computer spreadsheets, electronics and genetics; should emphasise communication and rapid exchange of information; and should aim for efficient use of funds in support of tropical agroforestry.

LEUCNET is a convenient abbreviation of 'The Leucaena Network', a proposed core group of scientists and institutions sharing a common interest in improving the productivity and utility of *Leucaena* species. This definition is adapted from the Winrock publication describing forestry networks (Adams and Dixon 1986). LEUCNET's primary objective is to encourage the planting of improved leucaena trees, based on well-designed experimental trials by scientists. This is not an easy task and cannot simply be left to growers. By forming a network and cooperating towards a common goal, researchers may pool data obtained in limited experiments so they can make area-wide recommendations to growers.

Communication

Between 1980 and 1992, the series of Leucaena Research Reports (LRR) was a major vehicle for communication among cooperating 'leucaenologists'. Through the generous contribution of the government of Taiwan, LRR was printed and distributed to members of the Nitrogen Fixing Tree Association (NFTA) - more than 1400 people in 1992. Since 1980, LRR published more than 500

papers in 13 volumes from authors in more than 50 countries. Abstracts were added in 1992, to encourage translations. In 1992, administrative cut-backs at NFTA prevented further publication of LRR. I strongly urge this conference to consider the incorporation of LRR into LEUCNET, and seek appropriate sponsors. If LEUCNET were to join INTERNET and similar electronic networks, communication could be accelerated with costs diminished.

Biotechnology and plant breeding networks

To make best use of the limited funds for leucaena research, LEUCNET must harness the new genetic technology. Clonal technologies will permit universal distribution of environmentally attractive seedless triploid hybrids (Sorensson and Brewbaker 1994). Genome mapping of conventional markers and isozymes will be supplemented by molecular markers such as RAPDs (randomly amplified polymorphic DNA). These may be suitable for genetic tagging of major genes that influence such traits as cold and acid tolerance. Isozymic phenotypes have shown that the common Leucaena (*L. leucocephala*) is a single genotype introduced to many countries, emphasising the fragile base on which much leucaena research has formerly been based (Sun 1992). Antisense DNA research promises to block mimosine synthesis and create leucaenas of value as food for nonruminant

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animals (including humans) and fish. Above all, the great ease of exploiting hybridisation promises almost unlimited genetic diversity for leucaena improvement in agroforestry systems (Brewbaker 1993c; Sorensson and Brewbaker 1994).

The data in Figure 1 illustrate the large genetic advances made in corn breeding in USA, based on regional, replicated trials. Similar yield increments should be possible in *Leucaena* species through improved genetics and management. The remarkable genetic advance with corn (3.5% per year) has been based on germplasm with wide adaptability and low genotype-environment (G x E) interaction. Such information is available only through networked, cooperative trials. The bad years of drought and flood reduce corn's gains to 2.9% per year (Fig. 1).

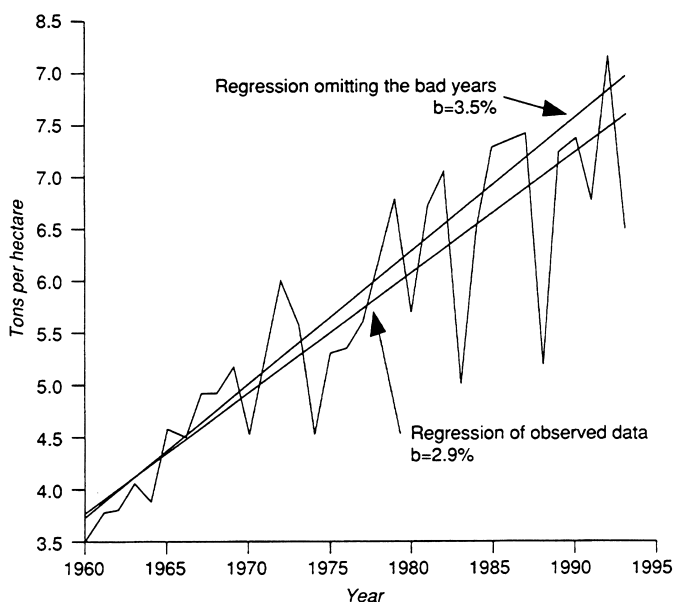


Figure 1. Corn yields in USA 1960-1993.

If comparable gains in forestry yields are possible, they may partially counteract the current loss of tropical forests at a rate of 15 million ha per year, or about 10% in a decade (or 100% in a century). Apart from a few conserved areas, there will be no tropical forests left in 100 years time unless the human race acts responsibly. Asia and the Pacific now have less than 300 million ha of forest left, a loss of more than half since 1940. Only in the Pacific islands and Australasia are continuing losses negligible.

Work on a genus like leucaena has broader goals and a much narrower financial base than work on corn. In leucaena, we seek everything from improved fuelwood yields to high quality fodder, parquet flooring and tempeh, focused largely on the less-developed countries. By networking (Adams and

Dixon 1986), scientists and institutions can optimise their efficiency in using limited resources to achieve their common goals. Preliminary unreplicated provenance trials of multipurpose tree species, and strategic or benchmarked research, can lay a valuable foundation for collaborative networked trials (Brewbaker 1986b).

Benchmarking

An experiment in a carefully chosen site serves as a *benchmark* that predicts what will happen on another but similar site. Impressive site-matching research has been done with Australian trees for use abroad. Effective agrotechnology transfer has often resulted from carefully benchmarked research of this type. Concern about genotype-environment interactions (Brewbaker 1984) may encourage site-specific evaluations, but only as a follow-up to the benchmark studies.

Many questions about leucaena can be answered at one or two locations by one or two scientists, acting as a benchmark for extrapolation to other locations. Classic examples are the hybridisation interfertility of *Leucaena* species (Sorensson and Brewbaker 1994), or the reaction to psyllid attack (Glover 1988). The altitudinal range of an accession is important benchmark advice from the plant collector, since *Leucaena* species are clearly distinguished by variations in cold tolerance. Benchmark data can include the variations in germplasm when stressed by soil and environmental factors; the digestibilities of leucaena species and lines in livestock; and genotype responses to pathogens and pests. There is always the proviso that racial variation in pathogen or pest may alter area-wide recommendations. Ultimately, models and algorithms may be used to predict area-wide performance.

Leucaena species must be viewed increasingly as valuable crops that need environments improved with fertilizers, weed management, exclusion of herbivores and people, irrigation and 'tender loving care'. Growers should be able to use benchmarked data from trials at our experiment stations, including yield estimates from experimental algorithms. Before we establish network trials, therefore, a thorough evaluation of the opportunities of benchmark trials must be made.

Types and designs of experiments

Research involves not only the collection of data but also astute statistical analysis. New tools are available, notably the spreadsheet programs QuattroPro, Excel, Lotus123 and others (Brewbaker 1993a, 1993b, 1994). New techniques, such as cluster

or principal component analysis, are being used to map molecular markers on chromosomes, characterise systematic relationships and follow tree growth in diverse environments. Members of LEUCNET should aim for impeccable experimental statistics that fully exploit these methods (Matheson 1990).

It is easy to overdesign and complicate tree and fodder experiments with leucaena. A simple experiment that precedes a multi-location experiment, say by one year, can save important time and funds. Questions that can be answered in advance include the best months for seeding and transplanting, the best methods of weed and local pest control, the best entries to include for replication, the best spacing, and the nature and severity of border effects.

When surveying leucaena research, a useful simplification is to categorise experiments into one of two classes: either Observational Trials (OT) or Yield Trials (YT). OT are customarily unreplicated trials that are analysed as completely randomised designs (CRD) using sampling error. YT are replicated and often multi-location trials. They are commonly randomised complete block (RCB) designs with sample data taken to generate both interaction and sampling error variances.

Observational Trials (OT), also called 'observational nurseries', are versatile experiments that may be of many types. They are often conducted to 'set the stage' for YT but at minimal time and expense. OT are performed primarily to convince the investigator of the worth of a hypothesis under conditions not requiring replication, for example it is a tree or a shrub; it does or does not resist psyllids. OT are often unreplicated trials of randomised entries at a single location with small plots, grown for a short duration. Sampling data, for example between trees within plots, provide error variances that show these to be research experiments, not demonstrations. For example, a typical OT experiment comparing five species with four accessions each, in plots where ten data trees are sampled, provides 180 degrees of freedom for sampling error, or three times that number if the OT is repeated in three locations. This therefore need not be considered a simple experiment, as it has adequate statistical tests.

All new accessions, breeding lines, ideas for management and for treatment should first be tested in unreplicated OT. One of the most wasteful experiences of networks has been the conducting of replicated trials of untested germplasm or treatments at many locations. Funding and common sense must not allow this to happen with leucaena.

Yield trials (YT) are commonly designed to rigorously evaluate genotype by environment (G x E) interactions. For most quantitative genetic models

it is essential to have individual tree data to provide both sampling and interaction errors (Brewbaker 1994). RCBs are the standard experimental designs, and repetition in time or space creates combined experiments, normally a form of split-plot design. Lattices are preferable if there are many entries, say 21 or more, but missing values must be rare. We are still considerably ignorant about leucaena, and must not focus solely on internationally networked yield trials. Repetition and replication of trials is expensive, time consuming and often wasteful, especially when benchmarking can adequately answer many questions.

The augmented randomised complete block (ARCB) design is ideally suited to both YT and OT experiments, permitting analysis of variance based on experimental error variances (from replicate interaction). As an example, Van Den Beldt (cited by Brewbaker 1993a, Ex.12c) planted a YT to examine spacing of leucaena, using 12 different spacing regimes ranging from 2500 to 80 000 trees/ha. Four spacings were replicated four times, and the other eight inserted as augments, at two per replicate (Fig. 2). The unreplicated treatments add significant authority to the conclusion that 2.5 year old leucaena trees are shorter when planted in denser stands, with a regression coefficient of 0.61 m reduction per 10 000 trees (Fig. 2). However, the trial was about half the size (and cost) of a fully replicated experiment.

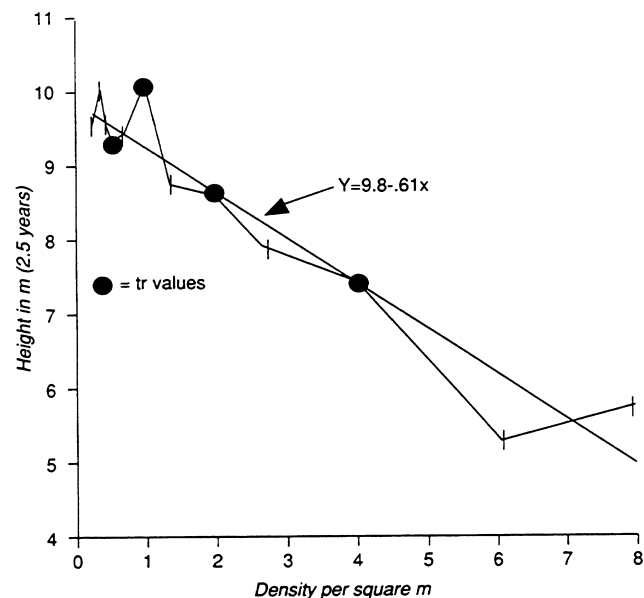


Figure 2. Heights of leucaena (x 8) at increasing population densities.

Principal components analysis (PCA) is an old technique now available for computers in simple forms of cluster analysis, and combined with analysis of variance in designs such as fractional

factorials. PCA can be extremely powerful in multi-location trials (e.g. Federer and Murty 1987).

A typical networked leucaena yield trial might be a fodder or green manure trial with 3-5 harvests annually. Plots can be very small (e.g. two 3 m rows), and locations and replications can be limited to two or three each, with 20-40 entries in two-year RCBs or lattice designs. Data are best analysed as a strip-block, recognising the probability of systematic variations in yields of consecutive harvests. Typically, the Mixed Model should be applied, with locations and replicates as random variables and with varieties and harvests as fixed variables. A data set might have the following sources of variance (following Brewbaker 1993a, Ex 9b): locations, replicates in locations, harvests, varieties, interactions between harvests x locations, varieties x locations, harvests x varieties, harvests x varieties x locations, and three different estimates of error.

Individual tree data (samples) would add a fourth estimate of error here, and could be extremely valuable in RCBs as a basis for variance component analysis to determine a more cost-effective choice of numbers of replicates and samples for future trials. The great importance of applying errors based on the Random Model should be emphasised, with F tests based on variance ratios of the main effects against interaction effects. In our experience in Hawaii, if the Fixed Model is used, conclusions are inevitably different and often misleading when extrapolated. Since the primary goal of benchmarked or networked trials is to extrapolate the information gained to other times and locations, it is imperative that the Random Model be applied. Spreadsheets are excellent for all such experiments because of their elegant simplicity and cost-effectiveness.

Networked experiments in Hawaii

Germplasm collections of the genus *Leucaena* were started in 1961 in Hawaii, and the 967 accessions were grown at least once to maturity in OT plots. Plot sizes varied, for example single-tree progeny, selfed, 10 trees; single-tree progeny, half-sib, 16 trees; composites, more than 20 trees. Our first two major plantings, 1963-1 and 1963-2, included 20 trees each of 86 accessions of 4 species in a single replicate each. Height and dbh (diameter at height of 1.3 m) data were taken annually for six years at Waimanalo (1963-1), and fodder yields and associated quality data were taken from two harvests on Kauai (1963-2).

From this experiment-station trial emerged the superior arboreal accessions of *L. leucocephala* designated K8, K28, K29, K67 and K72. All were

of the 'giant' type, uniform selfed lines, that are now dispersed and grown rather widely. Most other lines were exempted from YT, but seeds of all were retained.

More than 50 leucaena trials have been conducted with this broad germplasm since the early 1960s in the Hawaii network, and many results have been published in LRR (Brewbaker 1987). Most trials gave us experience that helped us plan or provide better materials for later trials. A primary factor in their success has been convenience of field access by scientists on a weekly basis over these three decades. Two major series of trials extending outside the State of Hawaii involved network evaluations of wood yields (Brewbaker 1986a, MacDicken and Brewbaker 1988) and of psyllid resistance in forage trials (Glover 1988).

Lessons learned in Hawaii

- Leucaenas are like crops, not like pine trees; field trials can be completed in 2 years.
- Small annual plantings are greatly preferable to large, occasional trials.
- Designs created in the lab rarely work in the field.
- Never replicate a new accession or a treatment not already tested.
- Choose carefully the months to seed and to transplant.
- Weeds must be controlled because young leucaenas do not compete well with them.
- Young leucaenas need sun and moisture.
- Young leucaenas thrive on high pH (> 5.5) and high phosphate.
- Missing trees or plots are to be expected, but up to 25% missing does not affect yield.
- Borders are essential in yield trials.
- Fodder and wood yields are highly correlated within species.
- One-year and four-year wood yields are highly correlated within species.
- Replicates and trees per plot for selfing families can be about half those for outcrossing families.
- Leucaenas thrive on high densities (> 10 000/ha); yields drop at lower densities.
- Scientist involvement is essential from time of planting.
- Benchmarked experiments save time and money.
- Augmenting saves time and money.

Leucaena, an extraordinary genus

Most foresters do not think of *Leucaena* species as respectable trees. Few have had the opportunity to observe trials with 10 m growth in 30 months (Fig. 2). Few have harvested 8-year old trees of K636, as we did in January 1994, that averaged 16 m

in height and 34 cm in diameter (ranging to 46 cm), with a density of 0.56 and a richly coloured heartwood free of defects. These are definitely respectable trees. Nonetheless, to my knowledge there are no commercial lumber, pulp, or woodfuel plantations of *leucaena* in the world at present. This is mainly because of the perception that all *leucaenas* look like the scrubby common cultivar found worldwide, full of seeds and hardly tree-like.

A primary intent of this workshop was to discuss the wide range of germplasm now available for research in this genus, and particularly the lesser-known species (Brewbaker and Sorensson 1994). The collections of provenances recently assembled by the Oxford Forest Institute, and the array of interspecific hybrids, offer something for everyone. These new species and hybrids must be brought to the attention of foresters worldwide, and there is no substitute for demonstration plantings. This is an extraordinary genus of tropical multipurpose legume trees, and there is no reason for modesty in our description or expectation of them (Anon. 1984).

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References

- Adams, N. and Dixon, R.K., ed. 1986. Forestry Networks. Proceedings of Network Workshop, F/FRED, Winrock International, Petit Jean, Arkansas, 112 p.
- Anon. 1984. *Leucaena*: Promising Forage and Tree Crop for the Tropics, 2nd edition. National Academy Press, Washington, DC.
- Brewbaker, J.L. 1984. Genotype-environment interactions and agrotechnology transfer. In: Hawaii Institute of Tropical Agricultural Research Extension Service. A Multidisciplinary Approach to Agrotechnology Transfer. Hawaii Institute of Tropical Agricultural Research Extension Service 16, Honolulu, Hawaii.
- 1986a. Performance of Australian acacias in Hawaiian nitrogen-fixing tree trials. In: Turnbull, J.W., ed., Australian Acacias in Developing Countries. ACIAR Proceedings, Canberra, Australia, 16, 180-184.
- 1986b. Multipurpose tree species provenance trials. In: Adams, N. and Dixon, R.K., ed., Forestry Networks. Winrock International, Arkansas, 15-24.
- 1987. *Leucaena*: a genus of multipurpose trees for tropical agroforestry. In: Stepler, H.A. and Nair, P.K.R., ed., Agroforestry; A Decade of Development. ICRAF, Nairobi, Kenya, 289-323.
- 1993a. Experimental Design on a Spreadsheet. Brewbaker, Kailua, HI, 185 p. (Includes DOS-based disk.)
- 1993b. Biometry on a Spreadsheet. Brewbaker, Kailua, HI, 133 p. (Includes DOS-based disk.)
- 1993c. Tree improvement for agroforestry systems. In: Callaway, M.B. and Francis, C.A., ed., Crop Improvement for Sustainable Agriculture. University of Nebraska Press, 132-156.
- 1994. Quantitative Genetics on a Spreadsheet. Brewbaker, Kailua, HI. (in press).
- Brewbaker, J.L. and Sorensson, C.T. 1994. Domestication of the lesser-known species of the genus *Leucaena*. In: Leakey, R.R.B. and Newton, A.C., ed., Tropical Trees: The Potential for Domestication. London, HMSO (in press).
- Federer, W.T. and Murty, B.R. 1987. Uses, limitations and requirements of multivariate analysis in intercropping experiments. In: MacNeil, I.D. and Humphrey, G.J., ed., Biostatistics. D. Reidel, Amsterdam, 264-283.
- Glover, N. 1988. Evaluation of *Leucaena* species for psyllid resistance. *Leucaena Research Reports*, 9, 5-18.
- MacDicken, K. and Brewbaker, J.L. 1988. Growth rates of five tropical nitrogen fixing fuelwood species. *Journal of Tropical Forest Science*, 1, 85-93.
- Matheson, A.C. 1990. Designing experiments for MPT genotype evaluations. In: Glover, N. and Adams, N., ed., Tree Improvement of Multipurpose Species. Winrock MPT Network Technical Series, 2, 67-100.
- Sorensson, C.T. and Brewbaker, J.L. 1994. Interspecific compatibility among 15 *Leucaena* species (Mimosoideae: Leguminosae) via artificial hybridisations. *American Journal of Botany*, 81, 240-247.
- Sun, W.G. 1992. Isozyme polymorphism in the leguminous genus *Leucaena*. MS Thesis, University of Hawaii, 137 p.