GENETIC IMPROVEMENT OF *LEUCAENA* AND *ACACIA KOA*

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ABSTRACT

Studies on *Leucaena* and *Acacia koa* tree improvement were undertaken in Hawaii from 1992-1996. These studies were described in two sections. The first section includes identification of high biomass yield varieties, estimation of DNA content, vegetative propagation, and forage yield management among *Leucaena* species and hybrids. Among interspecific hybrids of KX2 F_1 (*L. pallida* x *L. leucocephala*) and 3-way crosses (*L. pallida* x KX3 F_1 (*L. diversifolia* x *L. leucocephala*)), the best entries yielded over 16 Mg $ha^{-1}yr^{-1}$ of edible forage dry matter and over 40 Mg $ha^{-1}yr^{-1}$ for wood biomass. All these hybrids were psyllid-tolerant. Heterosis for forage yield averaged 48% (-75 to 160%) and for wood biomass averaged 85% (-99 to 223%). Among intraspecific hybrids involving six *L. leucocephala* accessions, the best entries were the crosses between K397, K565, and K608. They outyielded a widely planted K636 (*L. leucocephala*). Heterosis for biomass yield averaged 16% (-28% to 80%). A composite from the selected intraspecific hybrids was released. Nuclear DNA content varied from 1.32 to 1.74 pg/2C for diploid species and from 2.67 to 3.09 pg/2C for tetraploid species. Successful cloning method with more than 80% rooting for K1000 and K1001 was developed. More than 30% increase in forage yield

was obtained by optimizing the harvest intervals of K636 and KX2 F_1.

The second section includes studies of the identification of quality seed source for reforestation and selection of superior progenies for genetic improvement among *Acacia koa* collections. A total of 334 koa accessions were collected from the Hawaiian Islands. These accessions were evaluated for various important traits from seed to tree growth characters. Seven field trials involving 178 accessions were established at Hamakua, Hawaii and Mauanawili, Oahu. The koa populations clearly showed great variations in these traits. These variations are essentially genetic in origin and are useful in selecting progenies for tree improvement. High quality koa seed sources from the Islands were identified. The advanced progenies based on fast growth and tree form were selected for further testing. Silviculture practice study showed that koa trees in the mixed plots grew significantly slower than trees grown alone.

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SECTION ONE

GENETIC IMPROVEMENT OF *LEUCAENA*

Leucaena Bentham (*Leguminosae: Mimosoideae*) is a morphologically and genetically diverse genus of economically valuable multipurpose trees and shrubs that includes 16 or 17 validated species (Brewbaker, 1987; Hughes, 1993; Brewbaker and Sorensson, 1994). It is native to Central America, and ranges from Southern Texas (USA) to northern Peru.

The genus *Leucaena* includes the most widely used tree species for plantations in tropical regions of the world. Among the 16-17 species, *Leucaena leucocephala* (Lam.) de Wit (2n=104) is the most widely planted species and is the only fully domesticated tree in the genus. The "giant" *Leucaena* cultivars K8 and K636 released by Dr. Brewbaker at the University of Hawaii in the 1960s and early 1990s, respectively, are among the most productive and versatile multipurpose tree legumes available in tropical agriculture.

This species is also the most widely used forage crop among woody legumes (Shelton and Brewbaker, 1994). Its greatest advantages for ruminant production are the high forage yield (20 to 30 tons ha^{-1} yr^{-1} of dry forage in psyllid-free environments), the high crude protein content (20% to 35% of leaf weight), high forage digestibility (55% to 65%) and drought tolerance. As a versatile tree species in agroforestry systems, it is often used as livestock forage and fuelwood, or for reforestation and green manure for soil conservation (NAS, 1984; Brewbaker, 1987).

Multi-purpose uses and fast growth rate have contributed to the great interest that leucaenas enjoy in many tropical countries outside its native range. Besides the fast growth that allows for short rotations, leucaena trees also fix nitrogen in their root systems. *Leucaena leucocephala* plantations were reported to occupy 2 to 5 million ha. worldwide (Brewbaker and Sorensson, 1990). However, this area has not increased steadily in recent years and the great expectations held for *Leucaena* in the 1960s and 1970s have not been fully realized. This is due primarily to the narrow germplasm base available to producers and also due to damage to the trees from the psyllid insect (*Heteropsylla cubana* Crawford) that spread globally since 1984. The other limitations of *L. leucocephala* include: lack of tolerence to cold, acid soils and waterlogging, poor seedling vigor, heavy seed production causing concern about weediness, and moderate wood quality for fuelwood or construction (Brewbaker, 1987; Shelton and Brewbaker, 1994).

The other species in the genus exhibit a rich diversity of characteristics and indicate great opportunity to domesticate the lesser known species and develop improved *Leucaena* germplasm to address the limitations of *L. leucocephala* (Brewbaker and Sorensson, 1994). These species are a valuable source of genes for resistance to diseases, insects and abiotic stress, and also show great potential for domestication (Brewbaker and Sorensson, 1994).

Opportunities exist to exploit the lesser known species directly or to develop new cultivars through intra- and inter-specific hybridization and recurrent selection from hybrids which incorporate the beneficial qualities of two or more parents.

Little research has been devoted to the development of molecular genetic information about species of this genus, in spite of its importance in agroforestry and worldwide efforts in collecting and evaluating germplasm. Estimates of DNA content are needed, as they constitute important information for genome structure analysis, genetic mapping of qualitative and quantitative trait loci, and especially for devising strategies to isolate and clone genes of interest. Such estimates are important also for understanding the evolution of plant polyploids from putative diploid ancestors, an important phenomenon in the genus.

The proposal of this study is to improve *Leucaena* biomass yield and psyllid resistance through interspecific hybridization/cloning, progeny testing and recurrent selection, and to quantify DNA contents of the genus by using flow cytometry.

CHAPTER ONE

LITERATURE REVIEW

The genus *Leucaena*, a versatile tree species, is widely used as livestock forage, fuelwood, reforestation trees, and green manure for soil conservation (NAS, 1984; Brewbaker, 1987). It is also used as a minor food plant for consumption of unripe seeds, pods, and other plant parts in its native area of Latin America, as well as in parts of SE Asia (Whitaker and Cutler, 1966; Zarate, 1984; Brewbaker and Sorensson, 1994). The first species in the genus used outside its native range was the "common" *L. leucocephala ssp. leucocephala*. It reached the Philippines in 1500s from early Spanish sailors, from whence it was distributed throughout tropics in the 19th century (Brewbaker and Hutton, 1979; Sun, 1992). Only after the UH "Hawaiian Giant" varieties were released to the world in the 1960s, the genus *Leucaena* Bentham became widely popularized and used in agroforestry in most developing countries (NAS, 1984).

Because of its economic importance in most tropical developing countries and its limitations involved, efforts have been make to improve varieties through germplasm evaluation, hybridization and selection, notably for high

psyllid resistance, acid and cold tolerance, and high biomass yield (Brewbaker, 1987; Hutton, 1990; Austin, 1995).

Leucaena genetic improvement

The genus *Leucaena* has been the research subject of many institutes since recognition of its importance in tropical agroforestry, especially after the release of "Hawaiian Giant" (*L. leucocephala ssp. glabrata*) in 1960s (Brewbaker, 1987; Brewbaker and Sorensson, 1994). Major world collections of *Leucaena* germplasm have been conducted by the University of Hawaii (UH), the Oxford Forestry Institute (OFI), and the Commonwealth Scientific and Industrial Research Organization of Australia (CSIRO) from 1960s to late 1980s with a total of about 2000 collections (Brewbaker and Sorensson, 1994). Many research activities have been focused on evaluating adoptability, production, and utilization of the genus, improving variety through hybridization and selection for high psyllid resistance, acid soil and cold tolerence,and high biomass yield (Brewbaker *et al.*, 1972; Brewbaker, 1987; Hutton, 1990; Austin, 1995). They also include several genetic studies of the inheritance of traits in *Leucaena* species (Gray, 1967a; Gonzalez, 1966; Pan, 1985; Gupta, 1990).

The genes of the genus Leucaena

Genetic studies of *Leucaena* by several authors revealed that most traits were quantitatively controlled and some were simply controlled by few genes. Gonzalez (1966) reported the quantitative character of mimosine, a non-essential amino acid found in all *Leucaena* species. Gray (1967a) described that erect growth habit was dominant over bushy habit and absence of strong basal branching was dominant over its presence in *L. leucocephala* (2n=104). Pan (1985) reported that several morphological characters (i.e. leaf size) of *Leucaena* were quantitatively inherited and that F1 hybrids were intermediate to the parents. Single gene loci were ascribed to pubescent branches and leaves, strong flower odor, pendulous inflorescence and straight stem, each of which were dominant over glabrous, no odor, upright inflorescence and branching or shrubby habit, respectively. Progeny crosses revealed that 4 S alleles controlled self-incompatibility in diploid *L. diversifolia* and isozyme studies identified 4 peroxidase gene loci (Pan, 1985). Sorensson (1989) reported that the gene *Luteus* was a lethal recessive. Gupta (1990) reported that early flowering was dominant over late, absence of flowering in summer over its presence. Sun (1992) confirmed the segragation of three aconitase genes and two isocitrate dehydrogenase genes in crosses of *L. lanceolata*.

Genetic improvement of the genus

Genetic improvement of the genus has long been sought by many tree breeders in several institutes worldwide. Early studies on *Leucaena* improvement in Hawaii, USA and Australia were concentrated on locating variability in *L. leucocephala* (Brewbaker and Hutton, 1979). These studies resulted in the formal release of K8 and K636 from UH and "Cunningham" from CSIRO (Brewbaker, 1975; Hutton and Beattie, 1976; Brewbaker and Sorensson, 1994), and the informal release off many other cultivars (e.g., K28, K29, K67, K72, K156, K743).

Improvement of *Leucaena* through using natural interspecific hybrids has been used by foresters in Indonesia. Dutch foresters successfully selected and used the natural triploid of *L. pulverulenta* and *L. leucocephala* as a shade tree for other crops by means of vegetative propagation in the early twentith century (Lammers, 1940; NAS, 1984). Detailed evaluation of these triploids between several *L. pulverulenta* and *L. leucocephala* lines made by artificial pollination was conducted in Hawaii and in Australia and it proved to be a potential dual purpose plant for forage and fuelwood production (Gonzalez *et al.*, 1967; Bray, 1984; Bray *et al.*, 1988). The triploids yielded up to 50% more edible material and up to 100% more non-edible material (Bray, 1984). Due to the difficulties of large scale vegetative propagation and triploid seed production, wide utilization of these triploids has been limited.

Because of the growing recognition of the narrow genetic base of *L. leucocephala*, recent efforts have been focused on evaluating lesser-known species and infusing genes from these species through interspecific hybridization (Brewbaker and Sorensson, 1990). Wide range interspecific hybridization among species in the genus was attempted at University of Hawaii for unique growth habits, ecological adaptions, wood and fodder quality, pest resistance and high productivity. Hybrids showed great promise for many uses including gum and fodder production, furniture, construction and polewood materials, and fuelwood, pulpwood, roundwood, charcoal, parquet and craftwood (Brewbaker and Sorensson, 1990).

Three interspecific crosses of three tetraploid species (*L. diversifolia, L. leucocephala,* and *L. pallida*) in all combinations and advanced selections therefrom were very promising (Brewbaker and Sorensson, 1990). The cross of *L. leucocephala* x *L. pallida* showed high resistance to psyllids and very high forage yield under psyllid infestation (Brewbaker and Sorensson, 1990; Austin, 1995). Other interesting hybrids were the triploids between diploids of *L. diversifolia* (2x), *L. esculenta* and *L. pulverulenta* and tetraploids of *L. diversifolia* (4x) and *L. leucocephala* (Brewbaker and Sorensson, 1990). Interspecific hybrids between diploid *L. diversifolia* and tetraploid *L. leucocephala* and its recurrent selections therefrom also

gave promising results for acid soil tolerance in Brazil and Malaysia (Hutton, 1990; Hutton and Chen, 1993).

Seeds of these hybrids were available only for experimentation, due to the high cost of production through hand pollination. Three methods were proposed for large-scale production of these valuable interspecific hybrids seeds, including vegetative propagation, using self-incompatible parents (e.g. *L. diversifolia (2x), L. pallida*) and producing self-incompatibile *L. leucocephala* hybrids (Brewbaker and Sorensson, 1990).

Leucaena pallida (2n=104) collections showed resistance (or tolerance) to the insect psyllid, variations in growth habits, and high interspecific compatibility with *L. leucocephala* (Sorensson and Brewbaker, 1986; 1994; Sun, personal obs.). *L. leucocephala ssp. glabrata* collections showed different growth performance (Wheeler and Brewbaker, 1990; Austin, 1995) and also were polymorphic in allozyme expression (Sun, 1992; Harris *et al.*, 1994b). Only few hybrids of *L. leucocephala* and *L. pallida* have been advanced in breeding.

Progeny testing has long been employed by plant breeders for evaluating parents in breeding populations, providing source for selection of next generation, and providing material for estimation of genetic parameters (Loo-Dinkins, 1992). Progeny testing of intraspecific hybrids among *L. leucocephala* collections were tried, and average heterosis

over better parent was not found for forage and fuelwood yield (Gupta, 1986). The maximum individual hybrid vigor was observed in some crosses with 85% for forage yield and 86% for fuelwood yield as compared to the better parent (Gupta, 1986). Average heterosis was also low for plant height and date of flowering (Gray, 1967a). However, the F_1 hybrid of *L. leucocephala* between K584 and K636 showed extraordinarily uniform, fast growth performance compared with its parents in Waimanalo, HI, USA (Sun, personal obs.). In this present study, intraspecific hybrid combination with different *L. leucocephala* collections were made to produce progeny for selection and genetic parameter estimation. An option for capturing heterosis is to mix tree seeds, using this F_2 as a broad genetic base composites for distribution (Brewbaker, pers. commun.).

Recurrent selection is frequently used by crop breeders, but seldom employed by tree breeders due to long waiting period for a tree to grow from seed to seed. Although *Leucaena* is a tree crop, most species can be managed to produce seeds within 2 years. Diallel studies of stem height and stem number indicated high general combining abilities in four *L. leucocephala* varieties of contrasting growth habit (Gray, 1967b). Variance component analysis of F_2 and F_3 families of intervarietal cross in *L. leucocephala* revealed the presence of additive genetic components and the absence of non-additive genetic components (Gray, 1967c).

Recurrent selection of Gray's lines proved to be successful for forage and wood production with 29% and 49% yield increment, respectively, comparing with its better parent (Hutton and Beattie, 1976). Recurrent selection from the KX2 of *L. leucocephala x L. pallida* and the KX3 of *L. leucocephala* x *L. diversifolia* (4x) was attempted to produce high biomass yield and highly psyllid resistance with great success (Austin, 1995). It is also quite possible that similar gains could be made through recurrent selection from intraspecific hybrids in *L. leucocephala*.

Hybridization/cloning in tree improvement

Improvement of tree crops through hybridization has been sought by some forest breeders (Zobel and Talbert, 1984). Some distinct advantages of hybrids in tree breeding programs are the creation of new gene combinations, or new plants that may not occur in nature, and application of cloning techniques to capture additive and nonadditive variance in F_1 (Zobel and Talbert, 1984; Steller and Ceulemans, 1993). However, artificial hybridization has played only a modest role in applied forest tree improvement (Steller and Ceulemans, 1993). One noticeable success has been the pitch x loblolly pine hybrid in Korea (Hyun, 1976). There are many reasons including both biological and operational origins (Wright, 1976; Zobel and Talbert, 1984). It often requires great efforts to produce hybrid tree seed,

with no guarantee that the added cost is compensated by increased performance of the hybrids. Major efforts were made in the past to produce hybrid plants by means of vegetative propagation, rather than production of the desired hybrid seed.

It has been generally recognized that vegetative propagation will play a decisive role in the further application of hybridization in forestry (Zobel and Talbert, 1984; Zobel, 1992). Cloning not only permits maximal homogenity in the plantation but captures additive and nonadditive variances. It may also permit the exploitation of new, favorable genome combinations while at the same time maintaining uniformity in the plant stock (Steller and Ceulemans, 1993). Great gains are possible if the potential to mass-produce desired materials with greater uniformity is realized (Libby and Rauter, 1984). Examples are rooted cuttings of eucalyptus in South America, especially in Brazil, pine rooted cuttings in Australia, New Zealand and Chile, and spruce and cedar cuttings in northern European countries, Canada and northern United States (Zobel, 1992). The breeding techniques of hybridization and cloning involve the production of interesting hybrids, vegetative propagation of the hybrids, yield testing of the clones, and planting of selected vegetatively propagated clones.

The combined hybridization and cloning approach is expected to enhance forest tree improvement in the future

(Steller and Ceulemans, 1993). However, the procedures require quite a large investment and infrastructure to maintain and propagate hybrid clones. It will be desirable if tree species could produce hybrid seeds, e.g., by using self-incompatibility characters (Brewbaker and Sorensson, 1990).

Molecular analysis of the genus

Investigation and development of molecular genetic information about species of this genus has been very limited. A few studies include isozyme polymorphisms (Schifino-Wittmann *et al.*, 1990; Sun, 1992; Harris *et al.*, 1994a), phylogentic analysis of restriction fragment length polymorphism (RFLP) patterns in chloroplast DNA (Harris *et al.*, 1994b), construction of genomic library of *L. leucocephala* and isolation of a gene encoding seed storage protein (Pan *et al.*, 1990). Although numerous chromosome numbers have been reported for the genus and its breeding lines derived from interspecific hybrids, the amount of DNA content per nucleus (C-value) has been estimated for only 7 species using microspectrophotometry of Feulgen-stained nuclei in squashed tissues (Pan and Brewbaker, 1988; Cardoso de Freitas *et al.*, 1988; Palomino *et al.*, 1995). Apart from the utility of genome size data for future molecular studies in this importance genus, the amount and distribution of

nuclear DNA may give insights into genomic evolution that underlies or parallels speciation (Price, 1988).

CHAPTER TWO

VARIATION OF DNA CONTENT IN *LEUCAENA* SPECIES

Abstract

Nuclear DNA contents were quantified by flow cytometry in diploid (2n=2x=52, 56) and polyploid (2n=4x=104, 112) species of the genus *Leucaena*. A total of 90 accessions were evaluated including all 17 validated *Leucaena spp.* and several interspecific hybrids. Nuclei isolated from meristem tissues of seedlings were assayed with chicken red blood cells used as an internal standard. Estimates of 2C nuclear DNA content for diploid *Leucaena* species ranged from 1.33 pg for *L. esculenta* to 1.74 pg for *L. retusa*. Tetraploid species ranged from 2.67 pg for *L. diversifolia* (4x) to 3.09 pg for *L. cuspidata*. The range of nuclear DNA content in the genus corresponded to approximately 650 megabase pairs for diploid species to 1,500 megabase pairs for tetraploid species. Three groups of diploid species were distinguished from DNA analysis, that were similar to groupings based on the leaflet size of the species. The first group had large leaflet size and a high average DNA content of 1.57 pg/2C; it included *L. lanceolata*, *L. macrophylla*, *L. multicapitula*, *L. retusa*, and *L. trichodes*. The second group comprised medium leaflet size genotypes and averaged 1.51 pg/2C; it consisted of *L.*

greggii, *L. shannonii*, and *L. salvadorensis*. The third group had small leaflet size and a low average of 1.42 pg/2C; it consisted of *L. collinsii*, *L. diversifolia* (2x), *L. esculenta*, and *L. pulverulenta*. Ploidy level and DNA content were highly correlated ($r=0.97$, $P<0.001$). Interspecific triploid hybrids (2n=3x=78 or 80) possessed an intermediate DNA content between respective parental species.

Introduction

Leucaena Bentham (*Leguminosae: Mimosoideae*) is a very diverse genus. It is native to Central America and is distributed from southern Texas to northern Peru. The genus includes 17 validated species (Sorensson and Brewbaker, 1994; Hughes, 1993).

Leucaena species are among the most productive and versatile tree legumes available in tropical agriculture (Brewbaker, 1987). Their applications include providing sources for forage and fuelwood, reforestation trees, and green manure for soil conservation (NAS, 1984; Brewbaker, 1987; Shelton and Brewbaker, 1994). It has been reported that *L. leucocephala* is harvested commercially from 2 to 5 million hectares worldwide (Brewbaker and Sorensson, 1990).

Limited studies have been conducted on molecular genetics of *Leucaena* species. Published research includes studies of isozyme polymorphism (Sun, 1992; Schifino-

Wittmann and Schlegel, 1990; Harris *et al.*, 1994a), a phylogenetic analysis of restriction fragment length polymorphism (RFLP) patterns in choroloplast DNA (Harris *et al.*, 1994b), and construction of a genomic library of *L. leucocephala* and isolation of a gene encoding seed storage protein (Pan *et al.*, 1990). DNA content estimation using Feulgen densitometry has been conducted on only seven species in the genus (Palomino *et al.*, 1995).

Nuclear DNA content assays are useful for determining ploidy level of plants (Ozias-Akins and Jarret, 1994) and for some types of molecular studies (Grattapaglia and Bradshaw, 1994). The amount and distribution of DNA content variation among related taxa may offer insights into their evolutionary relationships (Price, 1976; Dickson *et al.*, 1992), their ecological and environmental adaptation (Grime and Mowforth, 1982; Cecarelli *et al.*, 1992), and their growth rates and generation times (Bennett, 1987; Rayburn *et al.*, 1994).

The present study was conducted to estimate the DNA contents of 17 *Leucaena* species. Flow cytometric estimates of nuclear DNA content variation are reported for the genus and related to the evolution of tetraploid *Leucaena* species.

Materials and Methods

Seventeen *Leucaena* species consisting of 90 accessions from the University of Hawaii *Leucaena* germplasm collection were used in this study (Table 2.1). Seeds were scarified by hand-nicking and germinated in dibble tubes containing a medium of 75% peat and 25% perlite in the greenhouse. Seedlings were watered three times daily, and fertilized once every week with foliar 19-19-19 (N_2-P_2O_5-K_2O). About 50 mg (fresh weight) of not-fully-expanded leaves were collected from three or four 135-day-old seedlings of each accession, mixed, and chilled until use.

Preparation of nuclei for flow cytometric analysis followed the protocol of Arumuganathan and Earle (1991a), with the following exceptions. The concentration of propidium iodide (Sigma P-4170) was increased to 0.175 mM; the homogenate was transferred with a Pasteur pipet to a modified 1 ml pipet with a 30 μm nylon mesh at cut tip for filtering; and pipet pressure was employed to facilitate filtering.

Nuclear DNA content was analyzed using an EPICS 753 profile flow cytometry (Coulter Electronics, Hialeah, Fla., USA) with an air cooled argon-ion laser. The amount of DNA was estimated by the fluorescence of the nuclei with propidium iodide relative to that of chicken red blood cells (CRBC) and a triploid (2n=3x=80) *Leucaena* clone K1001 [diploid *L. diversifolia* (K11) x *L. leucocephala*].

Table 2.1. *Leucaena* **collections, origin and mean DNA content determined by flow cytometry, K number refer to accession number used by the University of Hawaii and code used by the Oxford Forestry Institute.**

K number and code	Origin	Latitude	Longitude	DNA content (pg/2C)	SD
Diploid Species					
L. collinsii ssp. collinsii (2n=52) [COLL]†					
461	Chiapas, Mexico	16.45N	93.07W	1.35	0.06
456	Chiapas, Mexico	16.45N	93.07W	1.35	0.08
905	Chiapas, Mexico	16.45N	93.07W	1.48	0.13
L. collinsii ssp. zacapana (2n=52) [COLL]					
740	El Progreso, Guatemala	15.02N	89.40W	1.50	0.10
914	Zacapa, Guatemala	14.56N	89.31W	1.38	0.05
917	Chiquimula, Guatemala	14.36N	89.40W	1.34	0.10
995	Guatemala, Guatemala	14.54N	90.07W	1.50	0.09
L. diversifolia ssp. stenocarpa (2n=52) [DIV2]					
399	Cameroons			1.43	0.06
749	Australia			1.38	0.05
907	Quetzaltenango, Guatemala	14.43N	91.32W	1.49	0.08
909	Guatemala, Guatemala	14.40N	90.26W	1.39	0.08
919	Jalapa, Guatemala	14.38N	89.48W	1.48	0.09
926	Jutiapa, Guatemala	14.17N	89.59W	1.46	0.07
927	Santa Rosa, Guatemala	14.24N	90.26W	1.38	0.09
936	Comayagua, Honduras	14.15N	87.28W	1.47	0.07
964	Guatemala, Guatemala	14.44N	90.21W	1.44	0.06
L. esculenta (2n=52) [ESCU]					
812	Puebla, Mexico	18.20N	97.25W	1.33	0.08
948	Guerrero, Mexico	18.18N	99.45W	1.42	0.07
949	Michoacan, Mexico	18.38N	100.48W	1.44	0.09
950	Guerrero, Mexico	17.51N	99.40W	1.33	0.07
L. greggii (2n=56) [GREG]					
853	Nuevo Leon, Mexico	24.40N	99.55W	1.44	0.07
855	Nuevo Leon, Mexico	24.50N	100.05W	1.48	0.10
856	Nuevo Leon, Mexico	24.55N	100.05W	1.45	0.07
859	Coahuila, Mexico	24.50N	100.05W	1.45	0.09
862	Nuevo Leon, Mexico	24.45N	100.50W	1.47	0.09
L. lanceolata (2n=52) [LANC]					
10	Nayarit, Mexico	21.16N	103.10W	1.63	0.12
385	Oaxaca, Mexico	16.36N	94.57W	1.66	0.09
468	Chiapas, Mexico	16.15N	93.54W	1.63	0.11
772	Veracruz, Mexico	19.15N	96.25W	1.66	0.12
773	Veracruz, Mexico	19.15N	96.25W	1.49	0.07
951	Oaxaca, Mexico	16.02N	97.35W	1.54	0.14
952	Oaxaca, Mexico	15.40N	96.30W	1.50	0.06
L. macrophylla (2n=52) [MACR]					
158	Veracruz, Mexico	18.25N	95.17W	1.53	0.08
836	Morelos, Mexico	18.54N	99.58W	1.47	0.13
902	Oaxaca, Mexico	15.59N	97.16W	1.39	0.07
39/89‡	Oaxaca, Mexico	15.58N	97.10W	1.46	0.00
55/88‡	Guerrero, Mexico	18.00N	101.18W	1.42	0.11

Table 2.1.(Continued) Leucaena collections, origin and mean DNA content determined by flow cytometry, K number refer to accession number used by the University of Hawaii and code used by the Oxford Forestry Institute.

K number and code	Origin	Latitude	Longitude	DNA content (pg/2C)	SD
Diploid Species					
L. multicapitula (2n=52) [MULT]					
880	Guanacaste, Costa Rica	11.05N	85.37W	1.60	0.10
955	Los Santos/Hrre, Panama	7.55N	80.25W	1.48	0.12
959	Penas Blancas, Costa Rica	11.10N	85.37W	1.55	0.11
L. pulverulenta (2n=56) [PULV]					
957	Tamaulipas, Mexico	23.36N	99.14W	1.39	0.08
958	Texas, USA	27.35N	97.50W	1.41	0.06
960	Texas, USA	27.45N	97.57W	1.49	0.07
L. retusa (2n=56) [RETU]					
502	Texas, USA	29.33N	103.05W	1.70	0.09
506	Texas, USA	29.51N	102.48W	1.72	0.07
899	Texas, USA	29.44N	102.43W	1.74	0.11
900	Texas, USA	30.37N	104.03W	1.69	0.09
23/86‡	Coahuiia, Mexico	28.44N	102.20W	1.71	0.08
L. salvadorensis (2n=52, 56) [SALV]					
904	Choluteca, Honduras	13.26N	87.11W	1.45	0.09
933	Choluteca, Honduras	14.01N	87.05W	1.43	0.11
7/91‡	Esteli, Nicaragua	13.12N	86.29W	1.50	0.09
34/88‡	Choluteca, Honduras	13.15N	87.06W	1.46	0.07
L. shannonii (2n=52) [SHAN]					
441	Campeche, Mexico	19.47N	90.30W	1.51	0.11
916	Chiquimula, Guatemala	14.36N	89.38W	1.54	0.08
924	Jutiapa, Guatemala	14.22N	89.43W	1.40	0.10
925	Jutiapa, Guatemala	14.16N	89.56W	1.42	0.08
930	Honduras			1.49	0.09
941	Chiquimula, Guatemala	14.36N	89.34W	1.55	0.12
954	Campeche, Mexico	19.20N	90.43W	1.65	0.14
L. trichodes (2n=52) [TRIC]					
90	Venezuela	10.35N	66.56W	1.45	0.12
738	Cesar, Colombia	10.33N	73.12W	1.57	0.13
751				1.57	0.12
903	Trujillo, Venezuela	9.38N	70.18W	1.56	0.13
L. sp (2n=?) [LSP]					
5/91‡	Olancho, Honduras	15.25N	86.50W	1.50	0.07
87/92‡	Sonara, Mexico			2.73	0.13
Tetraploid Species					
L. cuspidata (112) [CUSP]					
745	Puebla, Mexico	13.38N	97.38W	3.09	0.11
89/92‡	Hidalgo, Mexico			2.22	0.11
L. diversifolia ssp. diversifolia (2n=104) [DIV4]					
156	Veracruz, Mexico	18.56N	97.00W	2.67	0.09
783	Veracruz, Mexico	18.52N	97.03W	2.74	0.11
785	Veracruz, Mexico	18.52N	97.03W	2.72	0.11
946	Veracruz, Mexico	19.26N	96.45W	2.81	0.09

Table 2.1.(Continued) Leucaena collections, origin and mean DNA content determined by flow cytometry, K number refer to accession number used by the University of Hawaii and code used by the Oxford Forestry Institute.

K number and code	Origin	Latitude	Longitude	DNA content (pg/2C)	SD
Tetraploid Species					
L. diversifolia ssp. diversifolia (2n=104) [DIV4]					
947	Veracruz, Mexico	19.32N	96.55W	2.72	0.12
L. leucocephala ssp. leucocephala (2n=104) [LEUC]					
26	St. Croix, Virgin Islands			2.85	0.10
209	Valle, Colombia	4.31N	76.01W	2.90	0.10
335	Yucatan, Mexico	20.27N	90.02W	2.94	0.24
400	Cameroons	5.56N	10.10E	2.83	0.14
997	Oahu, USA	22.50N	157.45W	2.95	0.11
L. leucocephala ssp. glabrata (2n=104) [LEUC]					
420	Morazan, Salvador	13.37N	88.01W	2.77	0.10
500	Australia			2.80	0.13
565	Colima, Mexico	19.13N	103.42W	3.00	0.15
584	Veracruz, Mexico	19.46N	96.25W	2.86	0.10
636	Coahuila, Mexico	25.25N	101.00W	2.85	0.11
L. pallida (2n=104) [PALL]					
376	Oaxaca, Mexico	17.08N	96.46W	2.96	0.12
748	Oaxaca, Mexico			2.87	0.11
804	Puebla, Mexico	18.37N	97.24W	2.96	0.13
806	Puebla, Mexico	18.37N	97.24W	2.71	0.14
953	Puebla, Mexico	18.38N	97.24W	2.80	0.13
819	Oaxaca, Mexico	17.21N	96.50W	2.75	0.12
Leucaena interspecific hybrids (Triploid and tetraploid)					
1000	ESCU x LEUC (838 x 636, 3n=78)			2.15	0.08
1001	DIV2 x LEUC (11 x ?, 2n=78)			2.20	0.08
KX2	PALL x LEUC (748 x 584 2n=104)			2.93	0.15
KX2	PALL x LEUC (748 x 636, 2n=104)			2.80	0.16
KX3	DIV4 x LEUC (156 x 636, 2n=104)			2.90	0.13

†: Abbreviation of the species
‡: Code used by Oxford Forestry Institute

Controls of CRBC and the triploid clone were analyzed as standards every 10 samples for calibration purposes. Results were presented in the form of a linear-light scatter histogram. DNA contents were calculated using 256 channels of linear-light scatter. The DNA content was obtained by multiplying the ratio of the fluorescence mean of the sample nuclei to CRBC by the amount of DNA per CRBC (2C=2.33 pg). Each reading was based on propidium iodide fluorescence from 1000 to 5000 plant nuclei. The standard deviation for the mean 2C-value of each sample was calculated following Dickson et al. (1992):

Sample 2C-value standard deviation = σ(samp/crbc) x 2.33

σ^2(samp/crbc) = σ^2(samp)/M(crbc)^2 + [σ^2(crbc) x M(samp)^2]/M(crbc)^4

where, σ^2 = fluorescence variance

M = fluorescence mean

samp = sample nuclei

crbc = chicken red blood cell

The General Linear Model procedure was used to perform analysis of variance among the species (SAS, 1990). Mean separations of DNA content between species and between different leaflet size groups within similar ploidy levels were tested with Waller-Duncan's test (SAS, 1990). Grouping of leaflet size of *Leucaena* species was according to Sorensson (1987).

Results

A typical linear-light scatter histogram of relative fluorescence peaks representing G1/G0 nuclei from *L. macrophylla* (2n=52, K902) and CRBC is presented in Figure 2.1. Only one sample peak for G1 phase cells representing the 2C DNA value of K902 was observed. The coefficients of variation for peaks of sample K902 and CRBC were 3.65% and 3.72%, respectively. These values varied between 2.0% to 6.0% in most of the histograms obtained from the *Leucaena* species analyzed. The DNA content of the triploid clone (K1001) was consistent between 2.18-2.21 pg/2C over many runs and in different experiments. Flow cytometry was found to be an efficient and reliable method to estimate DNA content of *Leucaena* species.

DNA content estimates and standard deviations of *Leucaena* species evaluated are summarized in Table 2.1. The diploids ranged from 1.33 pg for *L. esculenta* (K812 and K950) to 1.74 pg for *L. retusa* (K899). Two triploids, K1000 and K1001, had 2C values of 2.15 and 2.20 pg, respectively. The tetraploids ranged from 2.67 pg for *L. diversifolia ssp. diversifolia* (K156) to 3.09 pg for *L. cuspidata* (K745). The range of nuclear DNA content in the genus corresponded to approximately 650 megabase pairs for diploid species to 1,500 megabase pairs for tetraploid species.

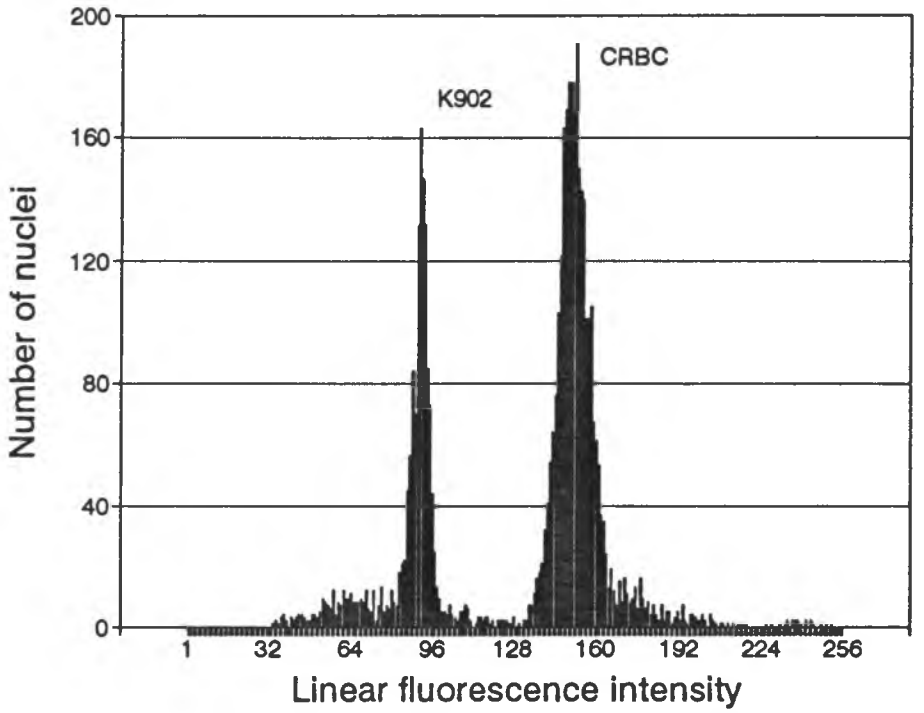


Figure 2.1. Histogram of nuclei per channel resulting from the flew-cytometery analysis

Analysis of variance for DNA content showed significant ($P<0.01$) difference between ploidy levels. Variance component analysis showed that ploidy level explained 99.9% of observed variation. Within the ploidy level, significant ($P<0.05$) differences were observed among diploid and tetraploid species (Table 2.2). Among diploid species, average DNA content of *L. retusa* was significantly higher than other species. Among tetraploid species, average DNA content of *L. diversifolia ssp. diversifolia* was significantly lower than other species. *Leucaena cuspidata*, with 112 chromosomes, showed significantly higher 2C values than the species with 104 chromosomes. However, no significant difference of DNA content was observed between the species with 52 chromosomes and the ones with 56 chromosomes. Intraspecific percentage variation was low, ranging from 3% to 18% (Table 2.3). Ploidy level and DNA content were significantly correlated within the genus ($r^2=0.97$, $n=98$, $P<0.001$).

Differences in 2C values were observed in *Leucaena sp.*, a poorly studied species of Mexico and Central America. *L. sp.* from Olancho, Honduras (5/91) had a 2C value of 1.50 pg, while one from Sonora, Mexico (87/92) had a 2C value of 2.73 pg, indicating that 5/91 is a diploid and 87/92 is a tetraploid species. Unexpectedly, we found that one accession (89/92) of *L. cuspidata* (2n=112) from Hidalgo,

Table 2.2. Analysis of variance for DNA content of ploidy level and species.

Variable	df	Sums of squares	Mean square	F values
Ploidy	1	31.80	31.800	7406.30***
Species	14	0.63	0.045	10.45***
Residual	74	0.32	0.004	

*** Significant at P< 0.001

Table 2.3. Average DNA content, standard deviation (SD), and percentage variation (PV) of Leucaena species within ploidy level.

Species	No. of accessions	DNA content (2C pg)	SD	PV (%)
Diploid species		1.49		31
COLL	7	1.41 ef†	0.06	10
DIV2	9	1.43 ef	0.04	8
ESCU	4	1.38 f	0.05	8
GREG	5	1.46 de	0.02	3
LANC	7	1.59 b	0.07	11
MACR	5	1.46 de	0.05	10
MULT	3	1.54 bc	0.05	8
PULV	3	1.43 ef	0.04	7
RETU	5	1.71 a	0.02	3
SALV	4	1.46 de	0.02	5
SHAN	7	1.51 cd	0.08	18
TRIC	4	1.54 bc	0.05	8
Tetraploid species		2.82		16
CUSP	1	3.09 a		
DIV4	5	2.73 c	0.04	5
LEUC	10	2.87 b	0.09	8
PALL	6	2.82 bc	0.10	10

†: Means with the same letter are not significantly different (P<0.05)

Mexico had a 2c value of 2.22 pg, suggesting it to be a triploid hybrid.

DNA contents differed significantly among both diploid and tetraploid species from different leaflet size groups (Table 2.4). The diploid species with large leaflet size

Table 2.4. Average DNA content of Leucaena species based on leaflet size groups within ploidy level.

Leaflet size	Species	DNA content (2C pg)
Diploid species		
Large	MACR, TRIC, MULT, RETU, LANC	1.57 a†
Medium	SHAN, SALV, GREG	1.48 b
Small	DIV2, PULV, COLL, ESCU	1.42 c
Tetraploid species		
Medium	LEUC	2.87 a
Small	DIV4, PALL	2.77 b

†: Means with the same letter are not significantly different (P<0.05)

included *L. macrophylla*, *L. trichodes*, *L. multicapitula*, *L. retusa*, and *L. lanceolata*. They had an average DNA content of 1.57 pg/2C and varied in mean DNA content from 1.46 (*L. macrophylla*) to 1.71 (*L. retusa*). Except *L. macrophylla*, the other species in this group had a DNA content above 1.54 pg/2C. Species with medium leaflet size included *L. shannonii*, *L. salvadorensis* and *L. greggii*; they had an average DNA content of 1.48 pg/2C and varied from 1.46 (*L. greggii*) to 1.51 (*L. shannonii*). Species with small leaflet size included diploid *L. diversifolia*,

L. pulverulenta, *L. collinsii* and *L. esculenta*; they had an average DNA content of 1.42 pg/2C and varied from 1.38 (*L. esculenta*) to 1.43 (*L. pulverulenta*). Tetraploid *L. leucocephala*, with a medium size leaflet, had a significantly higher DNA content than tetraploid *L. diversifolia* and *L. pallida* of small leaflet size.

Discussion

Flow cytometry was found to be efficient, fast, and reliable method to estimate DNA content of *Leucaena* species. A similar conclusion was drawn by Lodhi and Reisch (1995) in the genus *Vitis* and by Arumuganathan and Earle (1991a) in other plants. One sample peak for G1 phase cells (representing the 2C DNA value) was observed throughout *Leucaena* species analysis. Similar results were reported in rice, wheat, grape, and cacao (Arumuganathan and Earle, 1991b; Figueiro *et al.*, 1992; Lodhi and Reisch, 1995). Presence of a simple peak is attributed to the developmental stage of the leaves. It is assumed the young leaves analyzed had already finished cell division. Two peaks were obtained for G1 and G2 phase cells in another legume species, *Acacia koa*, when cotyledons were used 36 hours after germination. Seed germination in early stages involves cell division and expansion in all tissues, resulting in both G1 and G2 phase cells.

DNA contents estimated in this study were quite similar to those of Palomino *et al.* (1995), with the exception of one accession of *L. diversifolia* and two of *L. esculenta subsp. esculenta*. Dissimilarity of *L. esculenta* species appears to be due to different taxonomic classifications made by Sorensson and Brewbaker (1994) and Zarate (1994). In Sorensson and Brewbaker's classification, *L. esculenta* only included the species with 2n=52, however, in Zarate's classification, *L. esculenta subsp. esculenta* includes both diploid and tetraploid species (Palomino *et al.*, 1995).

DNA content variations are influenced by polyploidy, but also by subsequent evolution that tends to reduce genomic size. *Leucaena* species have a base chromosome of n=26 or n=28, apparently derived from ancestors with n=13 or n=14 chromosomes (Goldblatt, 1980; Pan and Brewbaker, 1988; Sorensson and Brewbaker, 1994). Palomino *et al.* (1995) suggested that tetraploid *Leucaena* species (n=52 or 56) may be of recent origin, as they have not yet gone through the process of reduction of their genome sizes (Grant, 1976).

Intraspecific variations among *Leucaena* species were quite low, compared with those of other plant species (Cavallini and Natali, 1991). This may be due to the reduced experimental error of flow cytometry vs. Feulgen technique. Low variation was reported using flow cytometry for *Pisum sativum* compared with early findings using Feulgen (Baranyi and Greilhuber, 1995). Low intraspecific variation

found in this study may also be related to the geographical distribution of the species. *Leucaena greggii* is restricted to Coahuila, Mexico while *L. retusa* is found in regions between Coahuila and southern Texas. Both of these open-pollinated species showed very low intraspecific varation. However, self-pollinated species of *L. leucocephala* with a wider distributions in lowland Mexico had intermediate intraspecific variation of 8%, which was similar to some other open-pollinated diploid species. Intraspecific variation of DNA content may support the nucleotypic effect theory of Price (1976) and Bennett (1985) that DNA content is related with adaptability to various ecological conditions.

The significant correlation between ploidy level and DNA content indicated that flow cytometry analysis could be used to identify ploidy levels of unknown *Leucaena sp.* and hybrids. Similar results were also found in *Leucaena* by Palomino *et al.* (1995), in *Ranunculus marsicus* by Sgorbati *et al.* (1989), and in *Ipomea* by Ozias-Akins and Jarret (1994). A natural interspecific hybrid between *L. leucocephala* and *L. esculenta* was verified using morphological and DNA evidence (Hughes and Harris, 1994). Flow cytometry could also be used to identify natural *Leucaena* interspecific hybrids provided that the parents have significantly different 2C values.

Groups of *Leucaena* species based on DNA content and leaflet measurement of Sorensson (1987) were quite consistent with the taxonomic groups suggested previously by Zarate (1984), Pan (1985), and Harris *et al.* (1994b). Zarate (1984) designated the large leaflet group as Section *Macrophylla,* and included *L. shannonii*. Harris *et al.* (1994b) called the medium leaflet size group the "*L. shannonii*-complex", and excluded *L. greggii*. Pan (1985) called the small leaflet size group "the *Leucaena diversifolia* complex". The results suggest that the tetraploid species, *L. diversifolia* and *L. pallida*, with small leaflet size may derive from diploid species with small leaflet size and low 2C values. The finding supports evidence that *L. diversifolia* (2x) and *L. esculenta* (2x) are putative parents of both *L. diversifolia* and *L. pallida* (Pan, 1985; Sun, 1992; Harris *et al.*, 1994b).

Estimates of DNA content are important for genome structure analysis and genetic mapping of qualitative and quantitative trait loci. They are useful in devising strategies to isolate and clone genes of interest, particularly through map-based cloning. DNA content information is also useful for understanding the evolution of plant polyploids. This study has shown that the DNA content in *Leucaena* accessions within a species is variable, and that difference in DNA content between species and between groups based on the leaflet size are significant.

The results clearly indicated that there are significant changes in DNA content during the process of speciation in *Leucaena*.

CHAPTER THREE

HETEROSIS AND RECURRENT SELECTION FOR FORAGE, AND TOTAL DRY MATTER YIELDS, AND FOR PSYLLID RESISTANCE IN *LEUCAENA* INTERSPECIFIC HYBRIDS

Abstract

The leguminous genus *Leucaena* includes 17 species, of which two have become pantropical as multipurpose trees. Large-scale *Leucaena* plantings have been limited due to damage caused by psyllids (*Heteropsylla cubana* Crawford), which have spread globally since 1984. This study evaluated performance of interspecific hybrids between *L. pallida* (psyllid-resistant) and the widely planted *L. leucocephala* (susceptible). Replicated experiments were conducted to evaluate forage and woody biomass yield of 17 F_1 hybrids between *L. pallida* and *L. leucocephala* accessions, advanced generations (F_2, F_3, and F_4) from a cross of these species, and 11 hybrid parents and other hybrids.

Seven harvests were taken during an 18-month period for the forage trial. Total dry matter (DM) yield of all replicated entries averaged 18.3 Mg $ha^{-1}yr^{-1}$ and ranged from 2.6 to 39.1 Mg $ha^{-1}yr^{-1}$. Forage DM yield averaged 9.2 Mg ha^{-1} yr^{-1} and ranged from 1.6 to 18.8 Mg $ha^{-1}yr^{-1}$. The best yielding entries were the interspecific hybrids of K748xK584, K748xK865, K748xK8, K748xK481, K748xK636, and

K806xK865. All were relatively psyllid-tolerant. These hybrids produced over 36 Mg $ha^{-1}yr^{-1}$ of total DM and over 16 Mg $ha^{-1}yr^{-1}$ of edible forage DM.

After 26 months' growth of the wood trial, the total biomass yields averaged 22.0 Mg $ha^{-1}yr^{-1}$, and ranged from 2.0 to 41.6 Mg $ha^{-1}yr^{-1}$. Generally, the highest biomass yielding genotypes were 3-way crosses, KX2 F_1 and KX3 F_1. These genotypes produced over 80 Mg ha^{-1} of total dry biomass in two years.

After two cycles of recurrent selection of K376xK8, genetic gains were significant (98%) for the harvested edible forage yield, but not for woody biomass. Heterosis based on the mean of two parents (MP) for forage yield ranged from -75% to 160% with an overall average of 48%, and for woody biomass, heterosis ranged from -99% to 223% with an overall average of 85%.

Leucaena interspecific hybrids and advanced K376xK8 F_4 selections proved promising for forage production and greatly/consistantly outyielded the widely planted *L. leucocephala* K636.

Introduction

Leucaena leucocephala (Lam.) de Wit (2n=104) is a versatile tree species widely grown in the tropics and subtropics. It can be used as livestock forage and fuelwood, or for reforestation and green manure for soil conservation in agroforestry (Brewbaker, 1987). It is the most widely used forage plant among woody legumes due to its high forage yield (20-30 Mg ha^{-1} yr^{-1}), high crude protein content (20-35%), high forage digestibility (55-65%), and drought tolerance (Shelton and Brewbaker, 1994).

About 2-5 million hectares worldwide were planted with *L. leucocephala* (Brewbaker and Sorensson, 1990). However, large-scale plantings of *L. leucocephala* have been limited, due in part to its narrow genetic base and susceptibility to psyllids (*Heteropsylla cubana* Crawford) that have spread globally since 1984.

Prior to the psyllid infestation in the tropics, high biomass yields for *L. leucocephala* were widely recorded. Brewbaker *et al.* (1972) reported the best *L. leucocephala* yielded 30 Mg $ha^{-1}yr^{-1}$ of forage DM. Van Den Beldt *et al.* (1980) reported that *L. leucocephala* reached 8.5 m in height and 7.5 cm in diameter of breast height in two years with 10,000 trees ha^{-1} in Hawaii, and observed biomass yield of 4-year harvests was 73.4 m^3 ha^{-1} yr^{-1}. Hu *et al.* (1982) reported that the "giant" *L. leucocephala* trees could reach 8 m in height and 6 cm in DBH in two years with 1.5 x 1.5 m^2

spacing in Taiwan. Shih *et al.* (1984) reported that *L. leucocephala* could yield about 80 Mg ha^{-1} yr^{-1} of total fresh weight biomass and 40 Mg $ha^{-1}yr^{-1}$ of edible fresh forage. The NAS (1984) stated that *L. leucocephala* could reach heights of 18 m in 4 to 8 years, and vigorous trees could have diameters of 21 to 37 cm in 8 years, and yield 40 to 50 m^3 $ha^{-1}yr^{-1}$ in many tropical sites.

Damage by the *Leucaena* psyllid can greatly reduce biomass yield and forage quality. Othman and Prine (1984) reported a 15% reduction in biomass yield among the top ten *L. leucocephala* accessions in 1983 season due largely to psyllid attack. Beardsley (1987) reported that psyllid infestations caused *Leucaena* defoliation, deformation, stunting and die-back of new growth. Other damage to *Leucaena* by psyllids in the tropics was also widely reported (Bray *et al.*, 1987; Hollenbeck, 1987; DeGuzman, 1987).

Several genetic control options for psyllid resistance exist in *Leucaena*, and breeding for resistance is belived the best way to control damage. One option is direct use of interspecific hybrids between resistant and susceptible species. Some species in the genus, e.g., *L. pallida* (2n=104), are resistant (or tolerant) to the insect psyllid (Sorensson and Brewbaker, 1986). Interspecific compatibility within the genus is high (Sorensson and Brewbaker, 1994). *Leucaena* interspecific hybrids showed resistance to psyllid and heterosis for wood and forage

biomass (Bray, 1984; Sorensson and Brewbaker, 1986; Brewbaker and Sorensson, 1990; Austin *et al.*, 1995).

Objectives of this study were: 1) to improve *Leucaena* forage DM yield, total biomass yield, and psyllid resistance level through direct use of interspecific hybrids and recurrent selection from the cross of *L. pallida* x *L. leucocephala* (K376 x K8), 2) to assess biomass yield, psyllid resistance, and heterosis of interspecific hybrids between different genotypes of *L. leucocephala* and *L. pallida* in both forage and wood management trials.

Materials and Methods

Seven accessions of *L. leucocephala*, four of *L. pallida*, and KX3 hybrids between *L. leucocephala* (K8 or K636) and *L. diversifolia* (K156) were used as parents for making interspecific crosses. *L. leucocephala* accessions included K8, K420, K481, K584, K608, K636, K865, and K997. *L. pallida* included K178, K376, K748, and K953.

Hybridization techniques followed those of Sorensson (1988). Emasculation was not employed, and inflorescences were bagged before use. After pollination, inflorescences were re-bagged to prevent further contamination by insects. Selfed contaminants were identified using leaflet number and size and other seedling traits (Sorensson and Sun, 1990). Crosses were made in Waimanalo, Hawaii, and are listed in Table 3.1. In most cases, the self-sterile *L. pallida* was used as the female parent.

Table 3.1. Interspecific KX2 and 3-way crosses among *L. pallida*, *L. leucocephala*, and KX3 (*L. leucocephala* x *L. diversifolia*), here, *L. pallida* was usually used as the female parent.

	Male parents									
K no.	8	420	481	584	608	636	865	997	KX3	806
Female parents										
178	X†		X			X			X	
376	X									
748	X		X	X	X	X	X	X	X	X
806	X			X		X	X	X	X	

†: X represents cross between two parents

A total of 40 entries was evaluated (Table 3.2). They included 18 interspecific hybrids, 4 3-way crosses, 5 advanced KX2, KX3, and DxC selections, 12 parents, and 1 intraspecific hybrid. Forty entries were divided into ten groups according to their genetic background.

The experiments were conducted at the Waimanalo Research Station, Hawaii. The station is located at 21°20'N and 158°20'W with an elevation of 20 m above sea level. Precipitation averages 1380 mm yr^{-1}, and the annual mean temperature is 24.6°C. Seeds were scarified by hand-nicking and germinated in dibble tubes. The medium for growing seedlings consisted of 75% peat and 25% perlite. A total of 100 seeds per genotype were transplanted into dibble tubes on 10 February 1993. Seedlings were transplanted into an Isohyperthermic Vertic Haplustoll soil on 3 June 1993. Plants were watered during the first three months to ensure good establishment but received no irrigation thereafter. Weeds were controlled by hand-weeding until trees were well established.

The experimental design was an augmented randomized complete block. For the forage trial, a total of 40 entries was evaluated (Table 3.2). These entries included twenty-six entries replicated three times, six entries replicated twice, and the remaining eight entries unreplicated. Sixteen trees were planted in each plot in two rows. Spacing was 1.25 x 0.25 m, or 32,000 trees ha^{-1},

Table 3.2. Entries of interspecific hybrids, parents, and advanced selections, grouped genotypes, and replications in forage and wood trials at Waimanalo, Hawaii.

Entries	Grouped genotypes	Replications Forage Trial	Wood Trial
K8xK376	KX2-F_1	3	2
K8xK748	KX2-F_1	2	
K178xK636	KX2-F_1	3	3
K178xK8	KX2-F_1	1	
K178xK481	KX2-F_1	1	
K748xK636	KX2-F_1	3	3
K748xK8	KX2-F_1	3	3
K748xK481	KX2-F_1	3	
K748xK584	KX2-F_1	3	3
K748xK608	KX2-F_1	2	
K748xK865	KX2-F_1	2	
K748xK997	KX2-F_1	3	1
K806xK8	KX2-F_1	3	3
K806xK584	KX2-F_1	2	
K806xK636	KX2-F_1	3	2
K806xK865	KX2-F_1	2	1
K806xK997	KX2-F_1	1	1
K376xK8(F_2)	KX2-F_2	3	2
K376xK8(F_3)	KX2-F_3	3	3
K376xK8(F_4)	KX2-F_4	3	3
K156xK636(F_1)	KX3-F_1	3	3
K156xK636(F_2)	KX3-F_2	3	3
K178x(K156xK636)	3WAY-F_1	1	
K748x(K156xK8)	3WAY-F_1	3	3
K748x(K156xK636)	3WAY-F_1	3	3
K806x(K156xK636)	3WAY-F_1	3	2
K8	LEUC	3	3
K8a	LEUC	1	
K420	LEUC	2	2
K481	LEUC	3	2
K636	LEUC	3	3
K584	LEUC	3	
K997	LEUC	3	1
K584xK636(F_2)	LEUC	3	3
K178	PALL	1	
K376	PALL	1	
K748sib	PALL	1	
K806xK748	PALL	3	3
K953	PALL	3	
K399xK445(F_2)	DxC-F_2	3	2

1.25 m apart between rows, 0.25 m between trees in the row, and plot size was 5 m^2.

For the wood biomass trial, a total of 26 entries was tested (Table 3.2). These included fifteen entries replicated three times, seven entries replicated twice, and the remaining four unreplicated. Fourteen trees were planted in each plot in two rows. Spacing was 1 x 1 m, or 10,000 trees ha^{-1}.

For the forage trial, psyllid damage level was first recorded on 30 November 1993 according to a resistant-susceptible scale (1 represented highly resistant, 9 represented highly susceptible) developed by Glover (1987). Plant height was measured twice on 30 August 1993 and 1 November 1993 before the first forage harvest.

Forage harvests occurred approximately once every three months. A total of eight harvests was conducted from November 93 to June 1995. Harvest dates were 31 November 1993, 3 Feb. 1994, 9 May 1994, 13 July 1994, 26 Sept. 1994, 24 January 1995, 11 April 1995, and 30 June 1995. All plants in the first harvest were cut to 50 cm above ground level, and thereafter, harvests were cut above the previous cut. Average plant height was about 2 m at every harvest. A total of eight trees from each plot was harvested, four from each side of the double-row plot. Samples from each plot were weighed fresh, and subsamples from one or two harvested shoots were taken to determine the edible forage

DM (leaves plus stem < 5 mm diameter). Due to missing data of the first harvest, only seven harvests were analyzed.

Plot means of total dry matter, dry forage yield and percent forage were analyzed as a split-plot with *Leucaena* as the main plot and harvest date as the subplot using the General Linear Model (GLM) of SAS (1990). A single degree of freedom contrast comparison among the ten grouped genotypes using GLM procedures of SAS (1990) was also conducted. If an entry by harvest interaction was significant, data were reanalyzed by harvest as an RCB. Mean separations were done within each harvest of the replicated entries by using the least significant difference (LSD, $P<0.05$).

For the wood biomass trial, psyllid damage level was recorded after six month planting. Individual tree height was measured at a half-year, one-year, and two-year duration. Diameter at breast height (DBH) was measured at one- and two-year duration. Calculation of a total dry biomass was based on the allometric equation: Yield = $0.0135*DBH^{1.85}*HT^{1.50}$ (Austin, 1995).

Heterosis calculation for both forage and wood trials was based on the equation: Heterosis = $F_1 - [(P_1 + P_2)/2]/(P_1 + P_2)/2$.

Results

Forage trial

1. Seedling growth rate

Tree height among the replicated entries averaged 2.38 m, and ranged from 0.87 to 3.23 m after five months growth (Table 3.3). The six fastest growing entries were K748xK865, K748xK481, K748xK8, K8xK748, K806xK584, and K178x(K156xK636), which exceeded 3.20 m in height. Five of them were interspecific hybrids, and the remaining entry was a 3-way cross. The poorest growing genotype, K748xK997, was also an interspecific hybrid between *L. pallida* and *L. leucocephala* (K997), a common-type (shruby and slow-growing) leucaena. Seedlings of K748xK997 were characterized by slow growth in the greenhouse relative to other hybrids (Table 3.3), despite being fit. Some seedlings of K806xK997 (71%) and K806xK865 (57%) showed deformation of meristem development and later died.

Significant ($P<0.01$) differences between groups were found for plant heights before the first harvest. The average heights of the interspecific hybrids, *L. pallida*, and three-way crosses after five months growth were significantly ($P<0.01$) taller than those of all *L. leucocephala* accessions. No significant difference for plant height was found among three selected advanced KX2 lines (Figure 3.1).

Table 3.3. Tree height (m) and psyllid damage ratings of KX2 F_1 interspecific hybrids and its parents at Waimanalo, Hawaii before first forage harvesting in 1993.

Grouped Genotypes	Entries	Height			Psyllid Damage Rating
		3 month in greenhouse	3 month after planting	5 month after planting	
Replicated					
KX2-F_1	K178xK636	0.25	1.07	2.43	4.50
	K748xK481	0.41	1.87	3.20	1.28
	K748xK584	0.30	1.63	3.10	1.50
	K748xK636	0.33	1.72	3.17	1.33
	K748xK8	0.38	1.72	3.23	1.67
	K748xK997	0.18	0.40	0.87	1.00
	K806xK636	0.28	1.47	3.07	2.17
	K806xK8	0.30	1.15	2.53	1.83
	K8xK376	0.25	1.03	2.40	2.11
KX2-F_2	K376xK8(F_2)	0.20	1.03	2.00	3.94
KX2-F_3	K376xK8(F_3)	0.20	0.77	2.27	3.61
KX2-F_4	K376xK8(F_4)	0.20	0.90	2.13	2.94
KX3-F_1	K156xK636(F_1)	0.20	1.23	2.87	6.67
KX3-F_2	K156xK636(F_2)	0.15	0.83	2.10	7.56
3way	K748x(K156xK636)	0.46	1.60	2.70	1.72
	K748x(K156xK8)	0.46	1.30	2.57	2.00
	K806x(K156xK636)	0.25	1.50	2.97	1.72
LEUC	K8	0.10	0.47	1.43	7.94
	K481	0.13	0.63	1.73	7.17
	K584	0.15	0.53	1.97	4.94
	K584xK636(F_2)	0.13	0.70	2.17	6.78
	K636	0.15	0.67	2.17	7.00
	K997	0.10	0.43	1.23	7.78
PALL	K806xK748	0.46	1.67	2.83	1.00
	K953	0.38	1.73	2.87	2.67
DxC-F_2	K399xK455(F_2)	0.25	0.77	1.97	2.72
AVG.			1.11	2.38	3.68
LSD 0.05			0.60	1.09	0.93
Augmented					
KX2-F_1	K178xK481	0.25	1.30	2.80	6.17
	K178xK8	0.18	0.70	2.00	8.00
	K748xK608	0.18	0.60	1.15	1.00
	K748xK865	0.23	1.90	3.50	1.67
	K806xK584	0.30	1.50	3.20	2.08
	K806xK865	0.18	0.88	2.45	1.08
	K806xK997	0.23	1.50	2.60	2.67
	K8xK748	0.36	1.65	3.20	2.00
3way	K178x(K156xK636)	0.20	1.10	3.40	4.00
LEUC	K8a	0.15	-	-	-
	K420	0.18	0.75	2.25	7.00
PALL	K178	0.20	1.30	2.50	4.83
	K376	0.25	1.70	2.80	1.00
	K748sib	0.30	1.80	3.00	1.00

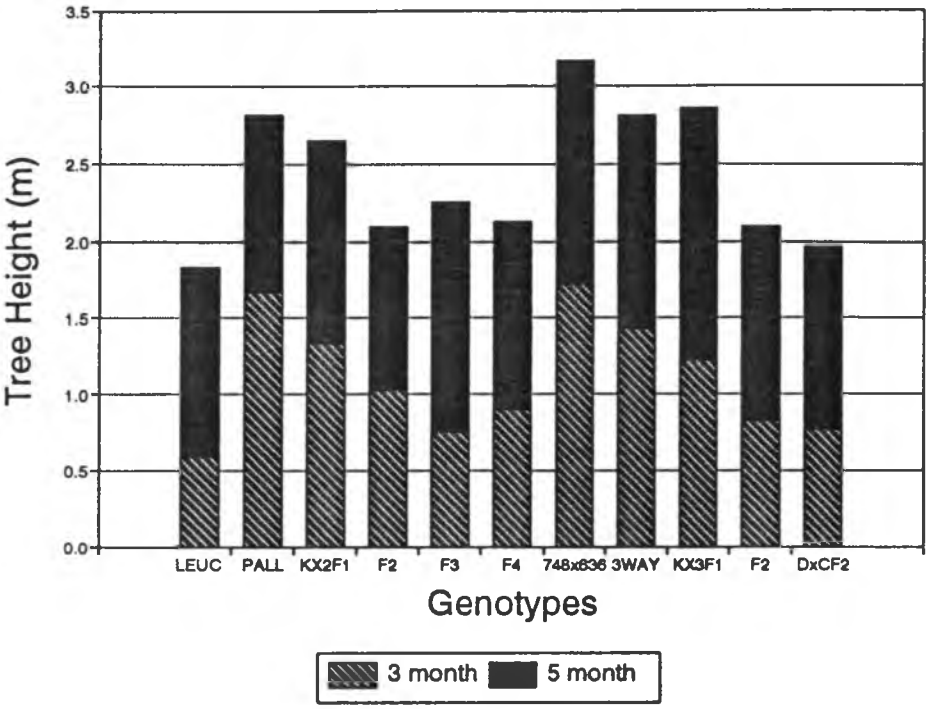


Figure 3.1. Average plant height of ten *Leucaena* groups at three and five months after planting in a forage trial at Waimanalo, Hawaii. LEUC: *L. leucocephala*; PALL: *L. pallida*; KX2F1: *L. pallida* x *L. leucocephala*; F2, F3, and F4: Advanced selections of KX2F1; 748x636: a specific cross between K748 and K636; KX3F1: *L. diversifolia* (4x) x *L. leucocephala*; F2: advanced selection of KX3F1; 3WAY: *L. pallida* x KX3F1; DXCF2: *L. diversifolia* (2x) x *L. collinsii*.

2. Psyllid resistance

Psyllid damage rating averaged 3.68 and ranged from 1.00 to 7.78 among 39 entries during the highest infestation seasons at Waimanalo, Hawaii (Table 3.3). Significant differences for psyllid damage rating were found among the replicated entries with $LSD_{0.05}$ at 0.93. Significant differences for psyllid damage were also observed among ten genotypic groups (Figure 3.2). Interspecific hybrids of KX2 F_1 and 3-way crosses (*L. pallida* x KX3) showed high psyllid resistance transfered from resistant lines of *L. pallida*, and their resistance level was significantly ($P<0.01$) different from their susceptible parent *L. leucocephala*. Level of resistance in the advanced KX2 lines was improved through recurrent selection, but no significant differences among these lines were found.

3. Forage and total dry matter yield

Analysis of the seven harvests over the 18-month period of the forage trial showed that the total DM yield of the replicated entries averaged 27.7 Mg ha^{-1} and ranged from 4.0 Mg ha^{-1} to 58.6 Mg ha^{-1}. Edible forage DM yield averaged 13.8 Mg ha^{-1} and ranged from 2.4 Mg ha^{-1} to 28.2 Mg ha^{-1} (Table 3.4). Significant ($P<0.05$) differences for forage DM yield, total DM, and forage percentage of replicated entries were found for six harvests, with the exception of the 6th harvest.

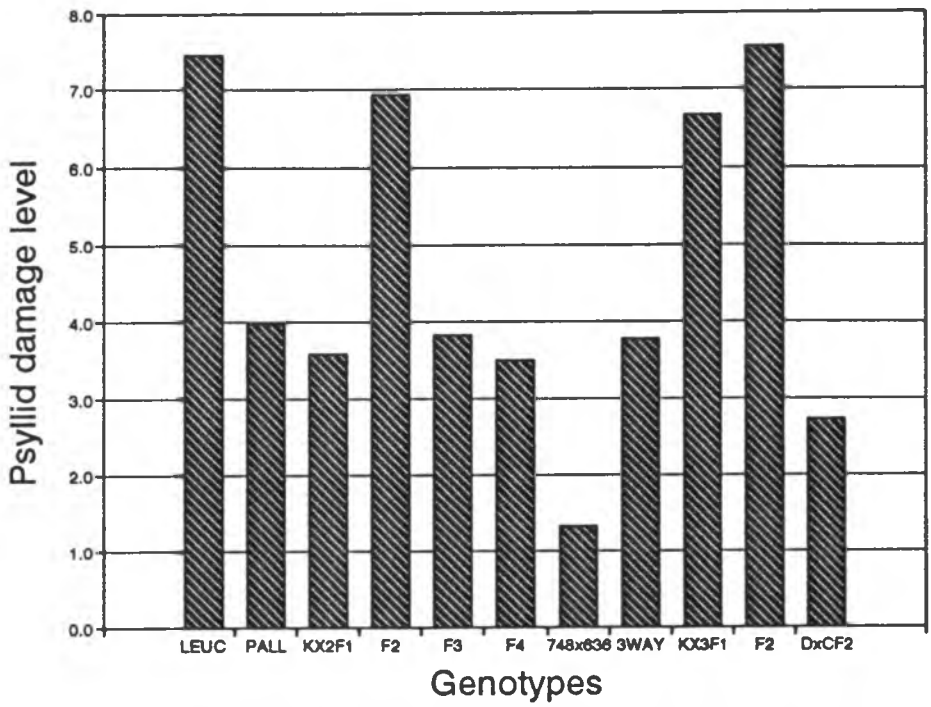


Figure 3.2. Average psyllid damage level (1 represents psyllid resistance with no damage and 9 represents psyllid susceptible with complete defoliation) of ten *Leucaena* groups on 31 November 1993 in a forage trial at Waimanalo, Hawaii.

Table 3.4. Biomass yields of forage DM, total DM (Mg ha^{-1}) and forage percentage(FP) for Leucaena interspecific hybrids and parents in Waimanalo, Hawaii from Feb. 1994 to July 1995.

Entries	Harvest # 2	3	4	5	6†	7	8	Forage DM	Total DM	FP (%)
Replicated										
K178xK636	2.1	2.6	2.9	1.2	1.1	2.8	3.8	16.4	33.1	50
K748xK8	3.8	4.9	3.6	2.4	1.1	3.9	4.0	23.7	54.1	44
K748xK481	3.1	4.5	4.5	2.4	1.6	4.1	5.0	25.1	50.7	50
K748xK584	4.1	4.4	5.2	2.3	1.7	5.6	4.9	28.2	58.6	48
K748xK636	2.9	5.9	3.9	1.9	1.5	4.1	3.9	24.1	51.8	46
K748xK997	0.4	0.3	0.5	0.2	0.1	0.4	0.5	2.4	4.0	61
K806xK636	4.2	2.5	3.8	2.6	0.9	3.1	2.7	19.7	40.7	48
K8xK376	2.2	3.7	3.1	2.1	1.2	3.1	3.7	19.0	39.8	48
K376xK8(F_2)	1.0	1.1	1.4	0.5	0.4	1.5	2.1	7.9	14.8	54
K376xK8(F_3)	1.3	2.4	2.0	1.0	0.7	2.7	2.6	12.7	24.4	52
K376xK8(F_4)	2.3	2.2	2.7	1.2	0.8	2.6	3.9	15.7	31.4	50
K156xK636	1.3	1.5	2.8	1.5	1.3	2.1	2.7	13.1	24.7	53
K156xK636(F_2)	1.0	0.6	1.2	0.8	0.4	1.4	1.6	7.1	11.7	61
K748x(K156xK8)	2.4	4.5	3.1	1.4	1.5	3.9	3.4	20.2	41.3	49
K748x(K156xK636)	1.9	2.6	2.3	1.2	0.7	3.6	3.4	15.7	32.0	49
K806x(K156xK636)	1.5	2.8	2.7	1.9	1.1	3.2	2.8	16.0	32.0	50
K8	0.5	0.2	0.4	0.6	0.3	0.6	0.9	3.4	5.8	59
K481	1.0	0.5	1.0	1.1	1.4	1.1	2.5	8.5	15.2	56
K584	1.6	1.1	1.7	1.1	0.9	2.6	2.6	11.6	20.5	57
K584xK636(F_2)	1.0	0.6	1.2	1.1	1.1	1.3	1.9	8.2	14.8	55
K636	1.1	1.0	1.5	1.1	0.7	1.1	2.6	9.1	16.7	55
K997	0.4	0.3	0.9	0.1	0.2	0.4	0.7	3.2	4.8	66
K806xK748	1.9	4.5	2.1	0.8	0.3	3.2	3.1	16.0	32.1	50
K953	1.0	1.5	1.8	1.1	0.3	0.9	1.7	8.2	17.7	46
K399xK455(F_2)	0.8	2.6	1.3	1.0	0.9	1.6	1.7	9.8	18.1	54
AVG	1.8	2.4	2.3	1.3	0.9	2.4	2.7	13.8	27.7	52
LSD0.05	1.0	1.4	1.3	1.0		1.1	1.3	2.9	5.6	3.6
Augmented										
K8xK748	2.0	4.9	3.8	2.4	2.0	4.8	3.7	23.5	45.5	52
K178xK8	0.6	0.2	0.9	0.7	0.6	1.0	1.2	5.3	8.2	64
K178xK481	1.7	2.6	2.7	2.0	1.7	2.9	4.2	17.7	34.0	52
K748xK608	0.8	0.8	1.1	0.6	0.8	0.9	1.0	6.0	9.4	64
K748xK865	2.2	6.5	5.1	3.4	2.2	5.4	4.5	29.3	53.1	55
K806xK584	2.2	2.0	4.3	2.9	2.2	2.9	2.2	18.5	33.0	56
K806xK865	1.9	5.1	4.8	2.3	1.9	3.6	5.6	25.3	47.0	54
K806xK997	2.0	2.3	5.9	2.5	2.0	3.0	1.9	19.5	35.9	54
K178x(K156xK636)	2.1	3.5	3.9	3.8	1.0	3.2	3.5	21.0	42.4	50
K8a	0.5	0.3	1.1	0.6	0.6	1.2	1.6	5.9	11.5	51
K420	0.9	0.6	1.8	0.9	0.9	1.7	1.7	8.3	13.8	60
K178	2.4	2.6	3.4	3.4	1.0	3.7	4.6	21.0	40.3	52
K376	1.5	3.5	2.7	1.0	1.5	2.3	3.6	16.0	29.5	54
K748sib	1.4	3.3	3.8	2.4	1.4	2.6	2.8	17.7	35.5	50

†: The sixth harvest did not show significant difference among treatments

Significant differences ($P<0.05$) among KX2 F_1 hybrids were also found. The best-yielding KX2 F_1 hybrids were K748xK584, K748xK865, K748xK8, K748xK481, K748xK636, and K806xK865, which produced an average of over 54 Mg ha^{-1} total DM and 24 Mg ha^{-1} of forage DM. The lowest-yielding KX2 F_1 hybrid was K748xK997, which produced only 4.0 Mg ha^{-1} of total DM and 2.4 Mg ha^{-1} of forage DM.

Among the four 3-way crosses, total DM averaged 35.9 Mg ha^{-1} and ranged from 32.0 to 42.4 Mg ha^{-1}, and the forage DM averaged 17.7 Mg ha^{-1} and ranged from 15.7 to 21.0 Mg ha^{-1}. The crosses of K748x(K156xK8) and K178x(K156xK636) had significantly ($P<0.05$) higher yield than both of K748x(K156xK636) and K806x(K156xK636).

An average yield of 13.0 Mg ha^{-1} of total DM and 7.4 Mg ha^{-1} of forage DM was produced among the eight *L. leucocephala* entries. Significant ($P<0.05$) differences among these entries were also observed. The best yielding *L. leucocephala* accessions were K584 and K636, which produced 20.5 and 16.7 Mg ha^{-1} of total DM and 11.6 and 9.1 Mg ha^{-1} of forage DM, respectively.

Among the four *L. pallida* collections, average yields were 27.4 Mg ha^{-1} for the total DM and 13.16 Mg ha^{-1} for the forage DM. K178 had significantly higher yields than the others, which produced a total DM of 40.3 Mg ha^{-1} and forage DM of 21.0 Mg ha^{-1}.

KX2 F_2, F_3, and F_4 selections produced 14.8, 24.4, and 3.4 Mg ha^{-1} of total DM and 7.9, 12.7, and 15.7 Mg ha^{-1} of forage DM over seven harvests, respectively. Significant ($P<0.05$) genetic gain for total DM and forage DM yields after two cycle recurrent selections of the KX2 hybrid (K376xK8) was found from this forage trial. Gains were 65% ($P<0.05$) for total DM and 60% ($P<0.05$) for the forage DM from the first cycle selection, and 29% ($P<0.05$) for total DM and 24% for forage DM from the second selection.

Significant differences of percent forage were also found among 39 entries. Average forage percentage of replicated entries was 52%, and ranged from 43% for *L. pallida* cross (K806xK748) to 65% for *L. leucocephala* (K997). Overall, the forage fraction percentages for *L. pallida*, KX2 F_1, 3-way crosses, and KX2 F_4 selection were significantly lower than *L. leucocephala*.

Overall, KX2 F_1 hybrids and 3-way crosses consistently performed well with significantly ($P<0.01$) higher forage yield than all other genotypic groups over the six harvests (Table 3.5). *L. leucocephala* produced significantly lower ($P<0.01$) forage yield than the other genotypes.

Significant differences for total DM yield among 10 grouped genotypes were shown in Figure 3.3. Interspecific hybrids of KX2 F_1 and 3-way crosses (*L. pallida* x KX3) showed significantly ($P<0.05$) higher total DM yield than the other groups. *L. leucocephala*, KX2 F_2, and KX3 F_2 produced

Table 3.5. Contrast of edible dry forage among the ten grouped Leucaena genotypes from November 1993 to June 1995 at Waimanalo, Hawaii.

Contrast	Harvest #							
	Overall	2	3	4	5	6	7	8
LEUC vs. the others†	**	**	**	**	**	NS	**	**
KX2 F_1 vs. KX2 F_2, KX2 F_3, KX2 F_4, 3way, KX3 F_1, KX3 F_2, DxC, and PALL	**	**	**	**	**	NS	*	*
3WAY vs. KX2 F_2, KX2 F_3, KX2 F_4, KX3 F_1, KX3 F_2, DxC F_2, and PALL	**	NS	**	NS	NS	NS	NS	NS
PALL vs. KX2 F_2, KX2 F_3, KX2 F_4, KX3 F_1, KX3 F_2, and DxC F_2	NS	NS	NS	NS	NS	NS	NS	NS
DxC F_2 vs. KX2 F_2, KX2 F_3, KX2 F_4, KX3 F_1, and KX3 F_2	NS	NS	NS	NS	NS	NS	NS	NS
KX2 F_2, F_3, and F_4 vs. KX3 F_1 and F_2	NS	NS	NS	NS	NS	NS	NS	NS
KX2-F_4 vs. KX2-F_2 and KX2-F_3	*	NS	NS	NS	NS	NS	NS	NS
KX2-F_2 vs. KX2-F_3	NS	NS	NS	NS	NS	NS	NS	NS
KX3-F_1 vs. KX3-F_2	*	NS	NS	NS	NS	NS	NS	NS

*: Significant at the 0.05 level of probability.
**: Significant at the 0.01 level of probability.
NS: not significantly different.
†: The others included KX2 F_1, KX2 F_2, KX2 F_3, KX2 F_4, 3-way, KX3 F_1, KX3 F_2, DxC F_2, and PALL.

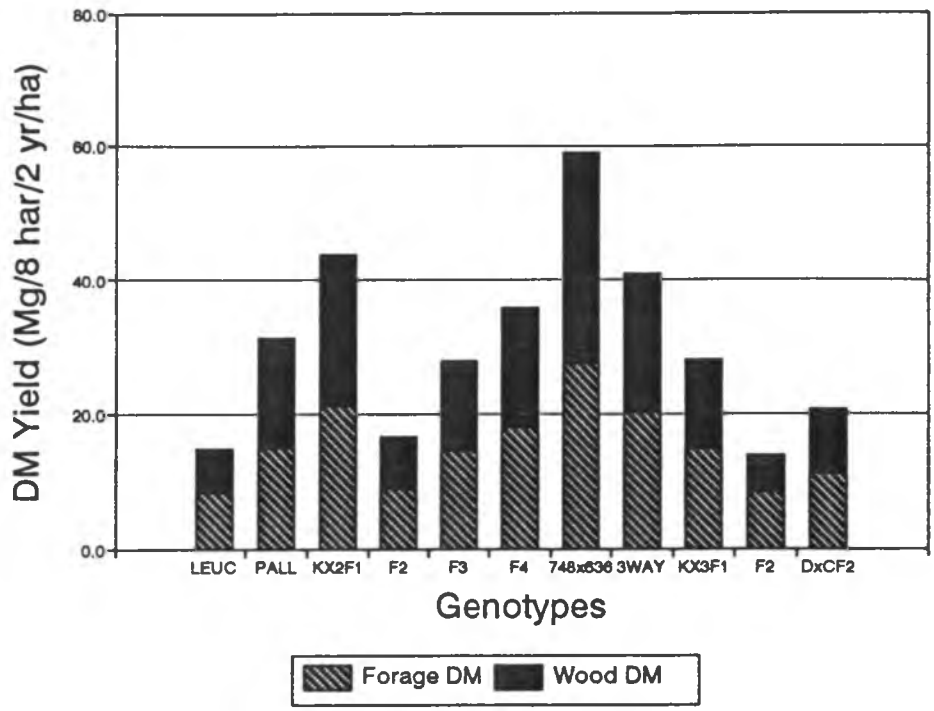


Figure 3.3. Average edible dry forage and total dry biomass including dry woody stem yield (MG ha^{-1}) of ten *Leucaena* groups after seven harvests in one and half year period in a forage trial at Waimanalo, Hawaii.

significantly (P<0.05) lower total DM yield than the other groups.

4. Heterosis of forage yield

Heterosis for forage DM was found among interspecific hybrids (Table 3.6). Heterosis over means of two parents (MP) for forage yield ranged from -75% to 160% with an overall average of 48%. General combining ability (GCA) among seven *L. leucocephala* entries and one KX3 F_1 ranged from -97% for K608 to 97% for K865. Among four *L. pallida* parents, GCA ranged from -50% for K178 to 31% for K376.

Table 3.6. Heterosis and general combining ability (GCA) of forage yield among interspecific hybrids between L. leucocephala and L. pallida accessions.

	L. leucocephala									
L. pallida K no.	8	481	584	608	636	865	997	KX3	Means	GCA
178	-0.56	0.17			0.09			0.23	-0.02	-0.50
376	0.79								0.79	0.31
748	1.17	1.05	1.10	-0.49	0.92	1.60	-0.75	0.23	0.60	0.12
806	0.26		0.38		0.45	1.26	1.14	0.11	0.60	0.12
Means	0.42	0.61	0.74	-0.49	0.49	1.43	0.19	0.19	0.48	
GCA	-0.06	0.13	0.26	-0.97	0.01	0.97	-0.27	-0.27		

5. Correlations among growth traits

Correlation coefficients among seedling height, psyllid damage level, forage DM yield, and total DM yield were significant (P<0.01) for 40 entries (Table 3.7). Plant height after 5 months planting was more positively

Table 3.7. Correlation coefficients and observation numbers among seedling heights, psyllid damage, edible dry forage yield, and total biomass yield of *Leucaena* interspecific hybrids, its parents, and advanced selection lines.

Traits	Height at ages after planting		Psyllid damage	Forage DM yield	Total biomass yield
	3 month	5 month			
Ht. of 3 month-old seedling in greenhouse	0.77 ** 39	0.61 ** 39	-0.66 ** 39	0.53 ** 39	0.58 ** 39
Ht. of 3 month-old plant in field		0.88 ** 39	-0.61 ** 39	0.75 ** 39	0.77 ** 39
Ht. of 5 month-old plant in field			-0.42 ** 39	0.83 ** 39	0.83 ** 39
Psyllid damage				-0.58 ** 39	-0.60 ** 39
Forage DM yield					0.99 ** 40

**: indicates significant level at P<0.01

correlated with edible forage yield and total biomass yield ($r = 0.83$, $P<0.0001$, $n=39$). Overall, psyllid damage was negatively correlated with growth variables of seedling height, total DM yield, and forage DM yield. However, K748xK997, one of KX2 F_1, was highly resistant to the insect psyllid, but it grew very slowly and had very low forage yield.

Wood biomass trial

1. Survival rate

The average survival percentage of the wood trial was 94% after six months, varying between 86% and 100% for the replicated entries. The survival rate decreased to 86% after two years. There were significant ($P<0.05$) difference in survival rate among entries. Overall, *L. leucocephala* collections and KX2 F_1 had a high survival rate (Table 3.8).

2. Psyllid resistance

Significant ($P<0.05$) differences for psyllid damage were found among the replicated entries during the high psyllid infestation season at Waimanalo after six months growth (Table 3.8). Average psyllid damage level was 4.0, varying from 1.0 to 9.0.

Overall, both interspecific hybrids of KX2 F_1 and 3-way crosses showed high resistance with an average psyllid damage score of 1.25 and 1.58, respectively. *L.*

Table 3.8. Average psyllid damage rating and survival percentage at different ages of growth in a wood trial with LSD's for the replicated entries at Waimanalo, Hawaii.

Genotypes	Psyllid damages 6 months	Survival (%) 6 m	12 m	24 m
Replicated				
K178xK636	4.4	93	93	90
K748xK8	1.0	100	95	95
K748xK584	1.0	93	81	81
K748xK636	1.0	98	98	98
K806xK8	1.2	93	81	81
KX2-F_3	4.2	83	79	74
KX2-F_4	4.9	98	86	86
KX3-F_1	6.8	93	93	71
KX3-F_2	8.3	88	83	74
K748x(K156xK8)	1.0	86	83	74
K748x(K636xK156)	1.0	100	100	93
K8	9.0	100	98	95
K584xK636(F_2)	7.6	100	95	95
K636	7.3	100	98	98
K806xK748	1.0	93	93	88
AVG	4.0	94	90	86
LSD 0.05	1.2	15	17	25
Augmented				
K8xK376	1.0	93	89	93
K748xK997	3.0	86	86	86
K806xK636	1.0	86	86	86
K806xK865	1.0	64	64	64
K806xK997	1.0	71	71	71
KX2-F_2	4.6	68	43	43
K806x(K636xK156)	2.0	54	50	46
K420	7.1	100	100	100
K481	8.8	96	96	96
K997	9.0	100	100	100
K399xK455(F_2)	3.0	79	79	79

leucocephala accessions, KX3 F_1, and KX3 F_2 all showed high susceptibility with average damage scores of 8.0, 6.83, and 8.5, respectively (Figure 3.4).

3. Early growth performance

There were significant differences ($P<0.05$) in early tree growth height and DBH among the replicated entries (Table 3.9). Tree height after two years' growth averaged 6.4 m, and varied from 4.0 to 7.9 m. DBH averaged 4.2 cm, and varied from 1.8 to 5.9 cm. The fast-growing entries were K748xK636, K748xK8, and K748x(K156xK8). Significant differences ($P<0.05$) for tree height and DBH among KX2 F_1 was also observed.

Significant ($P<0.05$) differences for tree height and DBH were also found among ten grouped genotypes. The fast-growing genotypes for height were 3-way crosses and KX2 F_1, which attained a height of 3.5 m after six months, 5.7 m after one year, and 7.1 m after two years and a DBH of 4.0 cm after one year and 5.0 cm after two years (Figure 3.5).

4. Total biomass yield

Based on the allometric equation estimation, significant ($P<0.05$) variation for the total DM yield was observed among these entries. After 12 months, the total DM yield for the 15 replicated entries averaged 18.9 Mg ha^{-1}, and ranged from 1.63 Mg ha^{-1} for K8 to 39.1 Mg ha^{-1} for

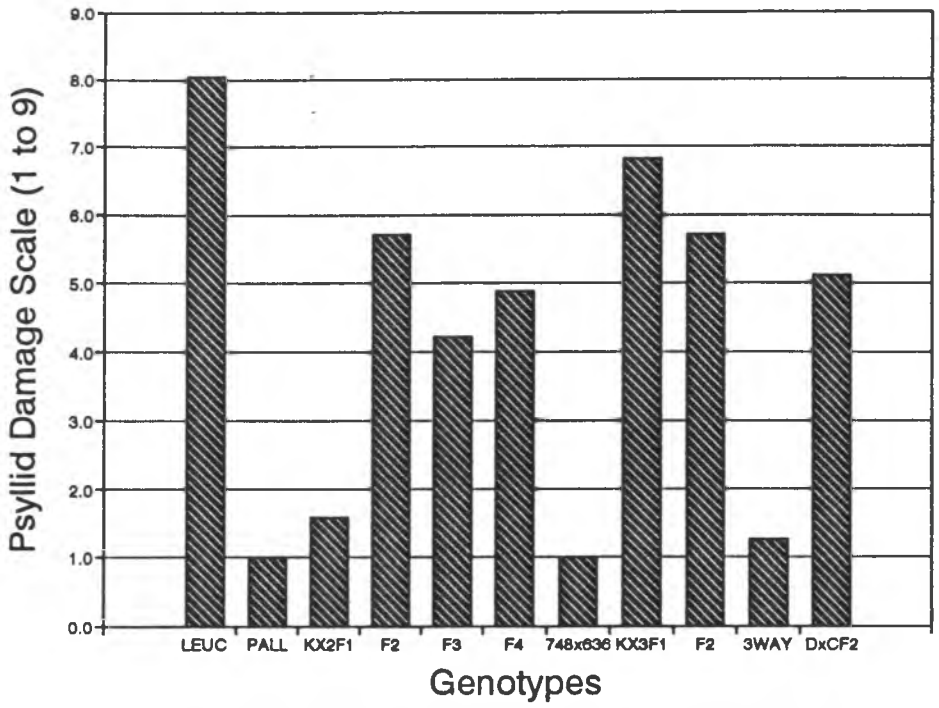


Figure 3.4. Average psyllid damage level (1 represents psyllid resistance with no damage and 9 represents psyllid susceptible with complete defoliation) of ten *Leucaena* groups on 31 November 1993 in a wood trial at Waimanalo, Hawaii.

Table 3.9. Average tree height (m), DBH (cm), and estimated total dry matter biomass yield (MG/ha^{-1}) in a wood trial with LSD's for the replicated entries at Waimanalo, Hawaii.

Entries	Height			DBH		Biomass	
	0.5 yr	1 yr	2 yr	1 yr	2 yr	1 yr	2 yr
Replicated							
K178xK636	2.7	5.2	6.9	3.4	4.4	18.3	49.8
K748xK8	3.8	6.2	7.9	4.5	5.9	37.4	90.2
K748xK584	3.1	4.9	6.2	4.0	5.4	26.5	71.3
K748xK636	4.1	6.5	7.9	4.6	5.7	39.1	78.6
K806xK8	3.2	4.9	6.3	3.8	4.6	21.2	45.1
KX2-F_3	2.1	4.0	5.2	2.6	3.2	9.1	22.4
KX2-F_4	2.3	4.0	5.8	3.1	4.2	10.8	33.8
KX3-F_1	2.6	5.0	7.3	3.2	4.2	19.0	72.9
KX3-F_2	1.5	3.4	4.7	2.0	2.5	4.5	11.0
K748x(K156xK8)	4.1	6.2	7.3	4.2	5.6	35.1	80.5
K748x(K636xK156)	3.9	5.8	7.4	3.8	4.7	27.6	66.2
K8	0.9	3.0	4.0	1.5	1.8	1.6	4.3
K584xK636(F_2)	2.2	4.4	6.2	2.8	3.8	9.6	33.5
K636	2.5	4.6	6.2	2.7	3.6	9.1	28.2
K806xK748	3.4	4.8	6.4	3.1	3.4	14.2	27.0
AVG	2.8	4.9	6.4	3.3	4.2	18.9	47.7
LSD 0.05	0.5	0.9	1.3	0.8	1.4	6.2	34.0
Augmented							
K8xK376	3.4	4.8	6.1	4.1	5.3	25.6	64.1
K748xK997	1.5	1.5	1.5	1.0	1.0	0.1	0.1
K806xK636	3.3	5.0	6.4	4.1	4.8	25.6	56.0
K806xK865	3.6	4.6	6.6	3.5	5.1	16.2	58.4
K806xK997	3.8	5.3	6.9	4.3	5.1	27.2	57.0
KX2-F_2	1.9	2.8	5.2	3.0	4.6	8.3	42.7
K806x(K636xK156)	3.2	5.0	6.3	4.3	5.4	27.1	41.5
K420	2.5	4.5	6.6	3.0	4.2	11.0	37.4
K481	1.3	3.6	4.8	1.7	2.7	2.6	10.0
K997	0.8	2.0	3.2	1.4	1.7	0.7	3.1
K399xK455-F_2	4.3	8.5	5.4	2.9	4.4	23.7	45.0

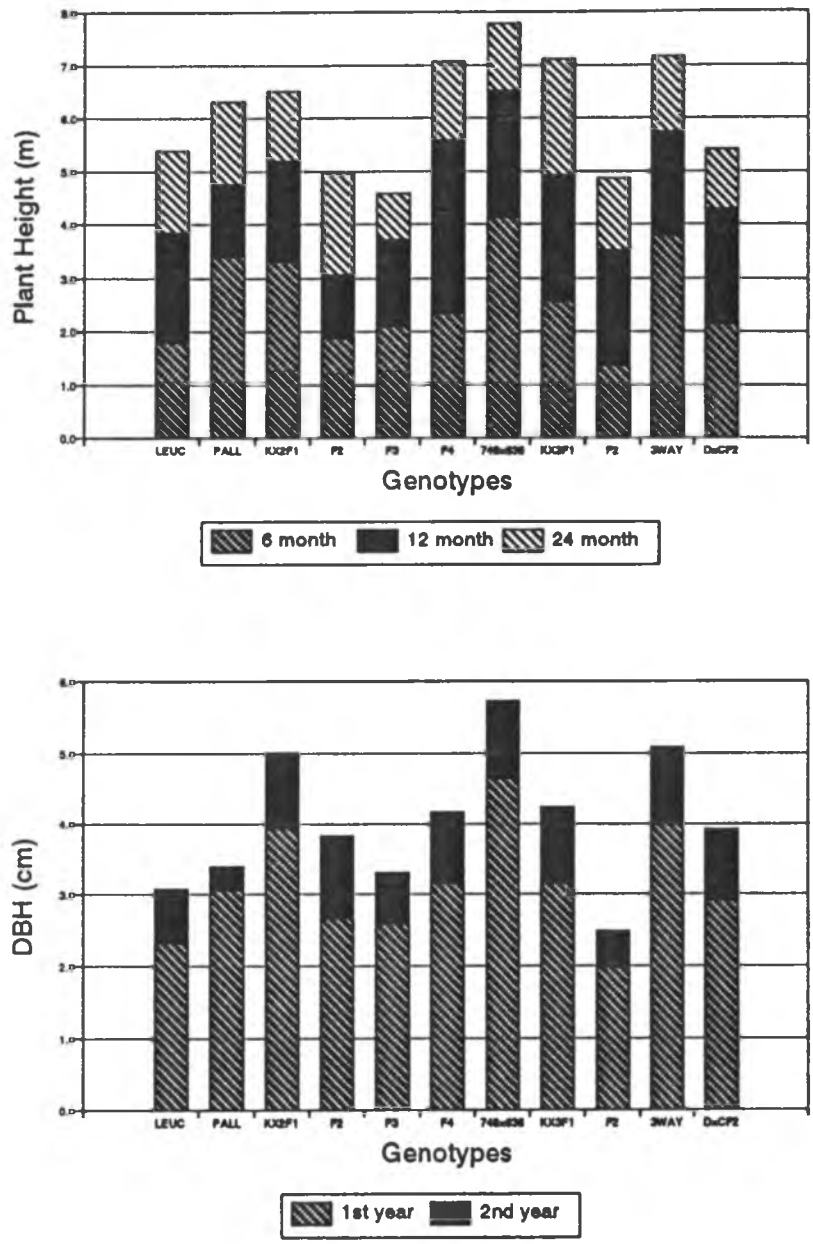


Figure 3.5. Average plant height (above) and diameters at breast height (below) of ten *Leucaena* groups at one half, one and two year growth in a wood trial at Waimanalo, Hawaii.

K748xK636. After 26 months, the total DM yield averaged 47.7 Mg ha^{-1}, and ranged from 4.3 Mg ha^{-1} to 90.2 Mg ha^{-1} (Table 3.9).

Significant ($P<0.05$) differences among the grouped genotypes were also observed. Overall, 3-way crosses, KX2 F_1, and KX3 F_1 produced significantly higher biomass yields than the other groups (Figure 3.6).

No significant genetic gains for total biomass and DBH were made after two cycles of recurrent selection. However, significant genetic gain for height after two cycles of recurrent selection was seen after two years growth. A gain of 40% ($P<0.05$) for height from the second cycle selection was observed, but no gain was made for height from the first cycle selection.

5. Heterosis

Average heterosis of DBH and the estimated total DM yield were found among the twelve interspecific crosses after 26 months growth, but not for plant height (Table 3.10). Average heterosis for total biomass and DBH was 85% and 37%, respectively. GCA for the estimated total biomass among five *L. leucocephala* accessions and one KX3 F_1 ranged from -81% for K997 to 54% for K8, and GCA for DBH ranged from -44% for K997 to 14% for K8. Among the four *L. pallida* accessions, GCA for the biomass ranged from -4% for K806 to

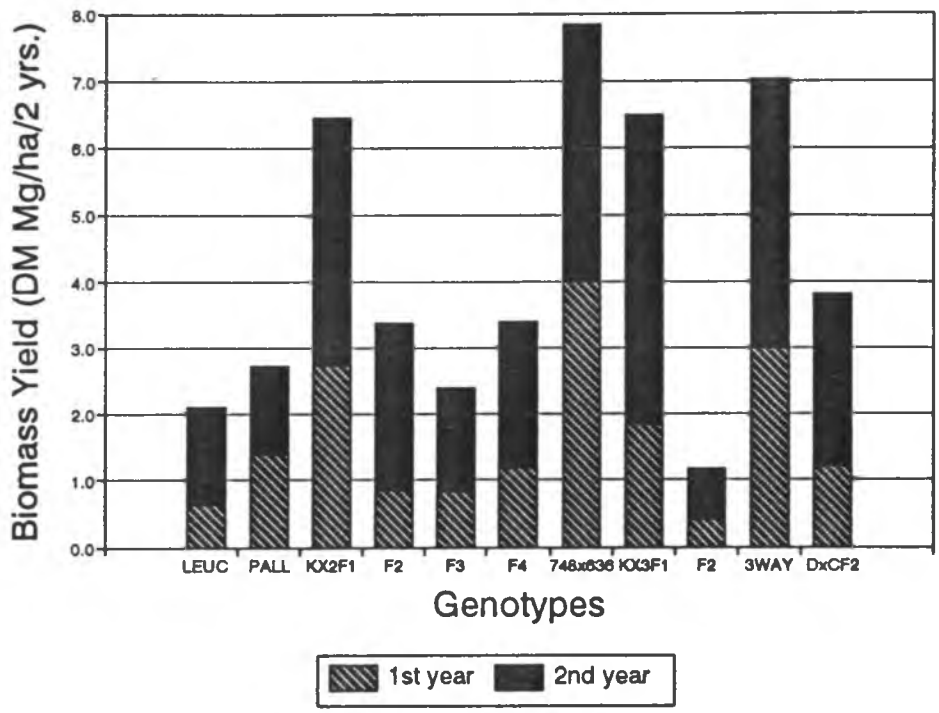


Figure 3.6. Average total dry biomass yield (MG ha^{-1}) of ten *Leucaena* groups at one and two year after planting in a wood trial at Waimanalo, Hawaii.

Table 3.10. Heterosis and general combining ability (GCA) of total estimated MD, plant height, and DBH among interspecific hybrids between *L. leucocephala* and *L. pallida* accessions after two years at Waimanalo, Hawaii.

L. pallida	*L. leucocephala*							
K no.	8	584	636	865	997	KX3	Means	GCA
Total DM								
178			0.81				0.81	-0.04
376	1.32						1.32	0.47
748	2.23	1.36	1.85		-0.99	0.46	0.98	0.13
806	0.63		1.03	0.58	1.07	-0.17	0.63	-0.22
Means	1.39	1.36	1.23	0.58	0.04	0.15	0.85	
GCA	0.54	0.51	0.37	-0.27	-0.81	-0.15		
Plant Height								
178			0.09				0.09	0.09
376	-0.03						-0.03	-0.03
748	0.25	-0.02	0.25		-0.69	0.08	-0.03	-0.03
806	0.00		0.02	0.05	0.09	-0.0	0.01	0.01
Means	0.07	-0.02	0.12	0.05	-0.30	-0.00	0.00	
GCA	0.07	-0.02	0.12	0.05	-0.03	-0.00		
DBH								
178			0.27				0.27	-0.10
376	0.51						0.51	0.14
748	0.69	0.50	0.64		-0.61	0.35	0.31	-0.06
806	0.33		0.37	0.45	0.48	0.42	0.41	0.04
Means	0.51	0.50	0.43	0.45	-0.07	0.39	0.37	
GCA	0.14	0.13	0.06	0.08	-0.44	0.02		

47% for K376, and GCA for DBH ranged from -10% for K178 to 14% for K376.

Discussion

KX2 F_1 hybrids between *L. pallida* and *L. leucocephala* and 3-way crosses between *L. pallida* x KX3 (*L. diversifolia x L. leucocephala*) clearly show considerable potential as forage and wood biomass production plants.

Hybrids of KX2 F_1 and 3-way crosses displayed definite heterosis, exceeding the means of the two parents in forage yield and wood biomass by an average of 48% and 139%, respectively. These crosses not only showed higher biomass yield, but also showed increased resistance to the insect psyllid and seedling vigor, compared with the widely planted *L. leucocephala* (K636).

Field observations of the two trials over two years showed that the KX2 F_1 hybrids and 3-way crosses were consistently more resistant to psyllid damage. The genetic control option proved to be practical after infusing psyllid resistant genes from *L. pallida* through interspecific hybridization. Tetraploid hybrids between *L. pallida* and *L. leucocephala* also performed well for psyllid resistance and showed high seedling vigor and higher biomass yield in studies of Sorensson (1988), Brewbaker and Sorensson (1990), and Austin *et al.* (1995).

Seedling growth rate is a matter of concern for plantations in large-scale plantings of *L. leucocephala* as a forage crop for animals in Australia due to weed competition (Bray, 1984; Shelton and Brewbaker, 1994). Interspecific hybrids between *L. pulverulenta* and *L. diversifolia* did not show fast seedling growth (Bray, 1984). The increased seedling vigor of interspecific KX2 F_1 hybrids and 3-way crosses should overcome competition with weeds for *Leucaena* plantation establishment.

Out of the present study, twelve crosses out-yielded K636 by more than 100% for forage yield. The same result was found for KX2 F_1 hybrids (K748xK636 and K748xK584) and 3-way cross (K748xKX3), which showed a three-fold increase in forage yield over K636 and a local "giant" *L. leucocephala* variety in Hainan, China (Liu and Sun, unpublished). Austin *et al.* (1995) also reported that KX2 F_1 hybrids had forage DM yield of about 30 Mg ha^{-1} in thirteen months in Waimanalo, HI. These crosses exceeded or had the same yield as improved tropical pasture (11 to 19 Mg $ha^{-1}yr^{-1}$) with a heavy input of fertilizers [300 Kg (N), 50 Kg (P), and 100 Kg (K) $ha^{-1}yr^{-1}$] (Hassan *et al.* 1990).

The crosses of KX2 F_1 hybrids not only performed well in the lowland tropics for forage production, but also grew very well at higher elevation. Austin *et al.* (1995) reported that KX2 hybrids of K748xK636 and K584xK636 yielded edible forage DM of 4.4 Mg $ha^{-1}yr^{-1}$, which exceeded that of

the high elevation *Leucaena* species, e.g., *L. diversifolia* (4n). The result indicated that KX2 hybrids could be extended to the highland tropics and the lowland sub-tropics for forage production.

Significant differences found in forage DM yield and total DM yield among these crosses emphasize the necessity for adequate testing and selection of any hybrid created, especially the crosses between *L. pallida* (K748 and K806) and some of the *L. leucocephala* acccessions (K865 and K997). The correlation between early tree height and edible forage or total biomass yield was highly significant ($P<0.01$). This finding indicated that early selection of interspecific hybrids is possible for superior forage and biomass yield based on early plant growth rate, e.g., height.

The results of the wood biomass production trial in this study were quite the same as that for the forage trial. Entries including six KX2 F_1 hybrids, two 3-way crosses, and one KX3 F_1 hybrid out-yielded K636 by more than 100% for the estimated total DM biomass yield. These hybrids produced about 35-40 MG $ha^{-1}yr^{-1}$ DM biomass yield, which is much higher than the average tropical forest biomass yield of 25 MG $ha^{-1}yr^{-1}$ (Austin, personal comm. 1995). The tree growth rates of these hybrids were the same as the "giant" *L. leucocephala* performance prior to the psyllid infestation in 1980 (Van Den Beldt and Brewbaker, 1980).

Significant genetic gains were made for forage yields from recurrent selection of advanced KX2 lines. However, no genetic gains for the woody biomass were made. This was perhaps due to the selection criteria based on early stage tree vigor rather than later tree traits. The advanced KX2 F_4 populations were still showing segregation. This suggests that further selection for forage and woody biomass from these advanced lines should be carried out continuously and separately.

Overall, the interspecific hybrids of KX2 between *L. pallida* and *L. leucocephala*, and the 3-way crosses between *L. pallida* x KX3, proved to be fast growing trees with high biomass yield potential, which could be used as a forage crop and a wood biomass production crop. The advanced KX2 F_4 selection showed promise for forage production in Hawaii. The best parentage for making interspecific hybrids from this study were K748 and K376 of *L. pallida*, and K865, K636 and K584 of *L. leucocephala*. Future success in the use of F_1 hybrids and the advanced KX2 selections will depend on the degree to which seed production and vegetative propagation can be mastered.

There are several ways to produce KX2 hybrid seed, which include cloning the self-incompatible parent (*L. pallida*) and creating a self-incompatible (*L. leucocephala*) line (Brewbaker and Sorensson, 1990). Cloning of *L. pallida* using cuttings proved to be quite difficult (Austin, 1995),

and creation of a self-incompatible *L. leucocephala* line is currently underway at UH (Chapter 4). Cloning of *L. pallida* using grafting was successful only with some *L. pallida* accessions (K953 and K804), but not with K748 (Brennan, 1995). *Leucaena pallida* clones of K953 and K804 produced quite high percentage of selfing when surrounded by *L. leucocephala* for hybrid seed production (Brennan, 1995). Mechanism of selfing from *L. pallida* clones was not known, but may be due to mentor pollen effect.

The other alternative is to make a backcross between KX2 F_1 and *L. leucocephala*. The KX2 F_1 hybrids have been proven self-incompatible, but are easy to propagate vegetatively using cuttings. Yield prediction of these backcrosses could be estimated using Jenkins' theory (1934), if we assume that average heterosis for forage yield is 50% for KX2 F_1 and 10% for intraspecific hybrid between *L. leucocephala* acccessions. The backcross at least has 30% heterosis over means of all parents. Due to a 75% dose of *L. leucocephala* gene constitution in the backcross, better forage quality could exist relative to the KX2 F_1.

Future research should focus on seed production of identified high-yielding KX2 F_1 hybrids and testing of backcrosses between KX2 F_1 and *L. leucocephala*. These steps are prerequisites for exploitation of their economic potential as a new forage and wood biomass crop.

CHAPTER FOUR

HETEROSIS AND CORRELATION OF GROWTH TRAITS AMONG INTRASPECIFIC *LEUCAENA LEUCOCEPHALA* PROGENIES

Abstract

A progeny test was established from a partial-diallel mating design to evaluate intraspecific heterosis in *L. leucocephala* and to estimate correlations for growth traits. The 27 entries included six *L. leucocephala* parents, twenty intraspecific F_1 hybrids, and one F_2 of *L. leucocephala* hybrid (K584xK636). These were grown in a replicated trial for two years at the University of Hawaii Waimanalo Research Station. Significant ($P<0.01$) differences were observed in height, diameter at breast height (DBH), and estimated total dry biomass yield after one and two years of growth. Heterosis over the superior parent averaged 16 % for biomass yield. Heterosis values ranged from -12% to 16.6% for plant height; -11.8% to 25.9% for DBH; and -28.0% to 80.3% for the biomass yield. Among the six parents, K565a and K608 had higher general combining abilities. The best progenies were from crosses involving K397, K565a, and K608. They outyielded a widely planted *L. leucocephala* check, K636. The F_2 population of K584xK636 performed well and was among the five fastest-growing entries. Genotypic and phenotypic correlations were high between first- and second-year growth

characters. It is suggested that a *Leucaena* composite based on F_2s from the fastest-growing hybrids identified in this study may be used for distribution in addition to K636.

Introduction

Leucaena leucocephala (lam.) de Wit is a perennial and multi-purpose leguminous tree. It is a source of high-quality animal feed, fuel wood, and green manure, and has been used extensively for reforestation (NAS, 1984; Brewbaker, 1987; Shelton and Brewbaker, 1994).

L. leucocephala is tetraploid (2n=104) and self-fertilized (Pan, 1985; Sorensson and Brewbaker, 1994). Three major morphological types are commonly found (NAC, 1984). The common type, *L. leucocephala* ssp. *leucocephala*, is a shrub with many branches that can reach 8 m in height (Brewbaker, 1987). The giant type, *L. leucocephala ssp. glabrata*, is a tall, single-branched tree that can reach up to 16 m, and given the name "Hawaiian giant" by Brewbaker (1987). The Peru type is one that is intermediate between the common and the giant. *Leucaena* plantations, mostly of the giant type, reportedly occupy between two and five million hectares worldwide (Brewbaker and Sorensson, 1990).

There are a number of limitations in *L. leucocephala* plantings. These include the lack of tolerance to cold temperatures, slow seedling growth, poor growth on acid soils, heavy seed set, and susceptibility to the psyllid

insect, *Heteropsylla cubana* (Hughes, 1993; Shelton and Brewbaker, 1994). These limitations may be due, in part, to the narrow genetic base of *L. leucocephala* outside of Latin America (Hughes, 1993). It is obvious that *L. leucocephala* plantations can be traced to a few cultivars, e.g., K8, K28, K67, and K636, which were released by Dr. Brewbaker at the University of Hawaii beginning in the early 1970s. Narrow genetic background plantations are vulnerable to the outbreak of pathogens and pests.

The genetic improvement of *L. leucocephala* has long been sought by many tree breeders in several institutes worldwide. Early studies on *Leucaena* improvement in Hawaii, Australia, and elsewhere concentrated on locating variability in self-pollinated species, e.g., *L. leucocephala* (Brewbaker and Hutton, 1979). These studies reported high genetic variation for plant growth rate among the "giant" *L. leucocephala* collections and low genetic variation among the "common" *L. leucocephala* collections (Brewbaker *et al.*, 1972; Wheeler *et al.*, 1987). Genetic variation was also found using isozyme and restriction fragment length polymorphisms (RFLP) in chloroplast DNA (Sun, 1992; Harris *et al.*, 1994). The success of UH *Leucaena* breeding programs were in formal release of the "giant" K8 and K636 from the University of Hawaii (Brewbaker, 1975; Brewbaker and Sorensson, 1994), and

informal release of many other cultivars, e.g., K28, K29, K67, K72, K156, and K743.

Leucaena leucocephala tree improvement also included progeny tests and generation mean analysis for estimating genetic parameters and recurrent selection. Gray (1967a) reported low heterosis for both plant height and date of flowering from a progeny test of intraspecific hybrids among *L. leucocephala* collections. Gupta (1986) revealed no heterosis in forage or fuelwood yield among the intraspecific *L. leucocephala* hybrids. In Hawaii, the *L. leucocephala* hybrid of K584xK636 showed an extraordinarily uniform and fast growth performance over 8-year period relative to its parents at Waimanalo (personal obs.). High general combining abilities were found for stem height and stem number in four *L. leucocephala* varieties of contrasting growth habit in a diallel study (Gray, 1967b). The presence of additive genetic components and the absence of non-additive genetic components were also reported for plant growth traits through variance component analyses of F_2 and F_3 families of intervarietal crosses in *L. leucocephala* (Gray 1967c). High general combining ability and presence of additive genetic components indicated that fast-growing *L. leucocephala* could be made through breeding and selection. Recurrent selection of Gray's lines proved to be successful for forage and wood production with 29% and 49% yield increments, respectively, compared with the superior

L. leucocephala parent, and resulted in the release of the "Cunningham" cultivar for forage production (Hutton and Beattie, 1976). Therefore, it is important to test heterosis between some newly found "giant" *L. leucocephala* collections, and evaluate segregation of the extraordinarily fast-growing F_1 of the K584xK636 cross.

A long generation interval is a major limitation for genetic improvement of most tree species (Haines, 1994). Strong juvenile-mature correlations are essential for an early accurate selection. Where correlations are weak, selection has to be delayed until the trees mature. This is not only economically expensive but also delays the availability of genetically improved seed.

In the present study, intraspecific hybrid progenies between six "giant" *L. leucocephala* collections were made to evaluate parents and heterosis for the plant growth variables of height, DBH, and the estimated total dry biomass yield, and to estimate genetic correlations between first- and second-year traits.

Materials and Methods

A total of twenty-seven entries including 20 F_1 hybrids of *L. leucocephala*, six parents, and one F_2 progeny from K584 x K636 were evaluated. The 20 F_1's were from a partial-diallel mating design involving six *L. leucocephala* selections and ten parent trees (Table 4.1). The six *L. leucocephala* parents included K397, K565, K584, K608, and K636, and K638. The ten trees included four from K584 (designated K584a, b, c, and d), two from K565 (designated 56a and b), and one from each of K397, K608, K636, and K638.

Table 4.1. Progenies produced from the indicated parent trees.

	Female							
K No.	397	565a	584a	584b	584c	584d	608	638
Male								
397			x				x	x†
565a	x				x	x	x	
565b			x					
584a	x				x			x
584d	x							
608	x		x	x	x			
636	x	x			x			
638	x							

†: indicates the cross between two parent trees

Progeny seeds were produced following the pollination technique developed by Sorensson (1988), but with some modifications. The ready-to-open flowers were emasculated one day before pollination, and then bagged. About ten

flowers in an inflorescence were emasculated and the remainder removed from each inflorescence head.

Seeds were scarified by hand-nicking, and germinated in dibble tubes. The media for growing seedlings consisted of 75% peat and 25% perlite. A total of 60 seeds per each entry were sowed into dibble tubes on 1 March 1993. Seedlings were watered three times daily, and fertilized once a week with foliar 19-19-19 (N_2-P_2O_5-K_2O), a formulation by Gravio Inc., in the greenhouse. Plants were inoculated with rhizobia one month after sowing. Three and half-month-old seedlings were transplanted into an isohyperthermic Vertic Haplustoll soil at the Waimanalo Research Station, Hawaii, on 17 June 1993. Plants were watered for the first two months to ensure proper establishment but received no irrigation thereafter. Hand-weeding was done until the tree leaf canopy closed.

The experimental design was an augmented randomized complete block. Fourteen entries were replicated three times, ten entries replicated twice, and the remaining three entries unreplicated. Sixteen trees were planted in each plot in double rows. Spacing was 1 x 0.75 m (13,333 trees ha^{-1}), and plot size was 12 m^2.

Psyllid damage level was recorded after 6 months of planting according to a scale developed by Glover (1987), where 1 represented high resistance and 9 represented high susceptibility to the insect. Plant heights were measured

at a half-year, one-year, and two-year growth periods. DBH measurements were taken at one- and two-year growth periods. Biomass estimation was based on the allometric equation: Yield = $0.0135*DBH^{1.85}*HT^{1.50}$ (Austin, 1995). The heterosis calculation for the traits was based on the equation: Heterosis = $(F_1-P_1)/P_1$, where P_1 represented the superior parent. General combining ability from the incomplete diallel design was based on Gilbert *et al*. (1973). The analysis of variance and means separation were based on the plot means of the replicated entries using a computer spreadsheet (Brewbaker, 1993).

Results

Survival rate

The survival rate of *L. leucocephala* parents and progenies were found to be remarkably high. Average survival rate was 98% for the 16 replicated entries, varying between 92 to 100% (Table 4.2). No significant difference among the replicated entries was found. Most of the mortality in the orchard was due to inadequate watering, which resulted in seedling desiccation.

Psyllid damage and Branching

Psyllid damage score averaged 8.0 and ranged from 7.6 to 9.0 (Table 4.2). All *L. leucocephala* parents and its progenies were basically susceptible to the insect psyllid, although a significant difference ($P<0.05$) for psyllid damage was found among the 16 entries during periods of high psyllid infestation in Hawaii. Only one progeny, K584cxK608, showed significantly less insect damage than K636.

Trees averaged 1.1 branches and ranged between 1.0 to 1.5 (Table 4.2). There was a significant difference ($P<0.05$) for branching among the replicated entries. The "giant" *L. leucocephala* parents and its progenies were basically single-branched trees.

Table 4.2. Average psyllid damage rating after six month growth, tree branching and survival rate after two year growth among *L. leucocephala* intraspecific hybrids and parents with LSD's from the replicated entries at Waimanalo, Hawaii.

Crosses & parents	Psyllid Damage	Branching	Survival Rate (%)
Replicated			
K397 x K565a	7.6	1.1	94
K397 x K584d	8.1	1.1	94
K397 x K608	7.8	1.1	100
K397 x K638	8.1	1.2	92
K584a x K397	8.2	1.1	97
K584a x K565b	8.1	1.1	100
K584c x K608	7.3	1.1	100
K584c x K636	8.2	1.0	100
K584d x K565a	7.7	1.0	100
K608 x K397	8.2	1.2	100
K584 x K636(F_2)	7.9	1.1	97
K397	8.1	1.1	100
K565	7.9	1.1	94
K584	8.0	1.2	94
K608	8.0	1.1	100
K636	8.0	1.3	100
AVG	7.9*	1.1*	98
LSD 0.05	0.5	0.2	
Augmented			
K638	7.9	1.2	96
K397 x K584a	9.0	1.5	75
K397 x K636	8.4	1.3	92
K565a x K636	8.1	1.1	100
K584a x K608	8.2	1.3	100
K584b x K608	7.8	1.0	100
K584c x K565a	8.3	1.0	88
K584c x K584a	8.4	1.1	100
K608 x K565a	7.7	1.1	100
K638 x K397	8.8	1.2	100
K638 x K584a	8.7	1.3	100

* : significant level at $P < 0.05$

Growth performances

The average height, DBH, and the estimated total dry biomass yield of the six *L. leucocephala* parents and its progenies are shown in Table 4.3. Heights of replicated entries averaged 6.2 m at 2 years and ranged from 4.9 m for K584 to 6.7 m for K397xK565a. DBH after two years growth averaged 3.6 cm and ranged from 2.55 cm for K584 to 4.3 for K397xK608. The estimated total dry biomass yield after two years growth averaged 36.1 Mg ha^{-1} and ranged from 17.9 Mg ha^{-1} for K584 to 53.1 Mg ha^{-1} for K397xK608.

There were significant differences ($P<0.01$) for height, DBH, and biomass after one and two year growth (Table 3). No significant differences for height ocuured among the 16 replicated entries,however, after six months of growth. The five fastest-growing entries were K397xK565a, K397xK608, K608xK397, K608x565a, and the F_2 of K584xK636. These entries showed exceptional growth performances and averaged about 6.5 m in height, 4.0 cm in DBH, and 45.0 MG ha^{-1} of estimated dry biomass yield in two years of growth. These entries also performed better than the widely planted K636 in growth variables of height, DBH, and biomass. In comparison, K584, the poorest performer, averaged 4.8 m in height, 2.6 cm in DBH, and 17.9 Mg ha^{-1} in estimated total dry biomass. Several trees of K584xK636 F_2 grew very well, and one of them reached 9 m in height and 8.5 cm in DBH in two years.

Table 4.3. Average tree height, diameter at breast height (DBH), and estimated dry biomass yield after two year growth among *L. leucocephala* intraspecific hybrids and parents with LSD's from the replicated entries at Waimanalo, Hawaii.

	Height (m)			DBH (cm)		Biomass ($Mgha^{-1}$)	
Genotypes	6 m.	1 yr.	2 yr.	1 yr.	2 yr.	1 yr.	2 yr.
Replicated							
K397 x K565a	2.3	5.3	6.7	3.4	4.1	15.4	48.8
K397 x K584d	2.2	5.3	6.5	3.0	3.7	12.2	37.8
K397 x KK608	2.2	5.4	6.6	3.5	4.3	16.6	53.1
K397 x K638	1.7	4.4	5.4	2.6	3.2	7.8	23.8
K584a x K397	1.8	4.6	5.7	2.6	3.1	7.9	23.1
K584a x K565b	2.0	4.9	6.2	2.9	3.5	10.2	32.7
K584c x K608	2.2	5.3	6.4	3.4	4.1	14.8	40.8
K584c x K636	2.1	5.3	6.5	3.4	3.9	14.1	40.8
K584d x K565a	2.4	5.1	6.4	3.2	3.7	12.7	39.9
K608 x K397	2.2	5.2	6.3	3.2	4.0	13.4	44.5
K584 x K636(F_2)	1.9	4.9	6.3	3.1	3.9	12.1	46.1
K397	1.9	4.7	6.1	2.8	3.2	9.4	27.2
K565	1.8	4.5	5.9	3.0	3.5	10.3	28.5
K584	1.7	4.2	4.9	2.3	2.6	6.7	17.9
K608	1.6	4.6	6.1	2.9	3.4	9.6	29.4
K636	1.9	4.8	6.6	3.1	3.9	11.0	42.4
AVG	2.0	4.9**	6.2**	3.0**	3.6**	11.5**	36.0**
LSD 0.05		0.5	0.6	0.5	0.7	4.0	15.2
Augmented							
K638	1.5	4.4	5.7	2.8	3.3	8.9	25.0
K397 x K584a	1.9	4.3	5.4	2.8	3.1	9.1	23.6
K397 x K636	1.8	4.3	5.8	2.8	3.5	9.7	34.9
K565a x K636	1.8	4.9	5.8	3.3	3.9	13.8	40.9
K584a x K608	2.0	4.9	6.0	3.2	3.7	11.9	31.3
K584b x K608	2.1	4.0	6.1	2.6	3.1	6.9	27.8
K584c x K565a	2.0	5.0	6.1	3.3	4.0	13.0	40.6
K584c x K584a	2.0	4.6	5.7	2.7	3.2	8.2	21.9
K608 x K565a	1.9	5.4	6.5	3.7	4.3	17.8	47.6
K638 x K397	1.6	4.3	5.3	2.5	2.9	6.9	19.6
K638 x K584a	1.8	4.2	5.4	2.6	3.1	7.3	21.3

** :significant level at $P < 0.01$

Significant ($P<0.05$) difference for tree growth variables were also found between hybrids of K584 and K608 when different K584 parent trees were used.

Heterosis and general combining ability

Overall, intraspecific heteroses for plant height, DBH, and biomass were quite low after two years growth, comparing F1 with soperior parent (Table 4.4). Average heterosis for plant height at two years was 0.2%, and varyed from -12.5% to 16.6%. Average heterosis for DBH was 6.5%, varying from -12% to 26%. Average heterosis for the biomass yield was 16%, varying from -27% to 81%.

The general combining ability for plant height, DBH, and biomass yield after two years for all *L. leucocephala* parent trees are shown in Table 4.5. General combining ability was quite consistent for most of the *L. leucocephala* parents when used as either a male tree or a female tree. The parents with highest general combining ability were K565a, K608, and K636.

Phenotypic and Genotypic correlations

Phenotypic and genotypic correlations were high and positive among the replicated entries of *L. leucocephala* parents and progenies between first- and second-year growth characters (Table 4.6). In general, genotypic correlations were slightly greater than phenotypic ones, and correlation

Table 4.4. Heterosis for plant height, DBH, and the estimated dry biomass yield over the better parent after two year growth among intraspecific *L. leucocephala* progenies at Waimanalo, Hawaii.

Genotypes	Heterosis (%)		
	Height	DBH	Biomass
Replicated			
K397 x K565a	10.45	19.89	70.94
K397 x K584d	7.57	14.75	38.93
K397 x K608	9.02	25.93	80.31
K397 x K638	-10.33	-2.37	-12.59
K584a x K397	-5.54	-1.63	-14.99
K584a x K565b	2.06	2.04	14.45
K584c x K608	5.01	18.52	38.62
K584c x K636	-1.43	-0.51	-3.92
K584d x K565a	9.45	8.34	39.96
K608 x K397	3.97	15.89	51.32
Augmented			
K397 x K584a	-11.58	-3.52	-13.17
K397 x K636	-12.44	-10.49	-17.88
K565a x K636	-12.48	-0.23	-3.56
K584a x K608	-1.55	7.70	6.48
K584b x K608	0.09	-9.51	-5.70
K584c x K565a	4.75	14.35	42.38
K584c x K584a	16.60	24.00	22.20
K608 x K565a	7.41	25.01	61.70
K638 x K397	-12.13	-11.78	-27.95
K638 x K584a	-4.84	-5.57	-14.74
Means	0.20	6.54	16.04

Table 4.5. General combining ability for plant height, DBH, and the estimated dry biomass yield among *L. leucocephala* parents used as male and female in a partial dialell mating design.

Parents	Height			DBH			Biomass		
	Mean	Male	Fem.	Mean	Male	Fem.	Mean	Male	Fem.
K397	-0.1	0.1	-0.3	-0.1	0.0	-0.3	-1.7	2.2	-5.7
K565a	0.1	-0.3	0.4	0.4	0.3	0.4	7.8	6.2	9.5
K565b	0.1		0.1	-0.1		-0.1	-2.1		-2.1
K584a	-0.3	-0.1	-0.5	-0.3	-0.2	-0.5	-9.1	-5.7	-12.5
K584b	0.0	0.0		-0.5	-0.5		-7.0	-7.0	
K584c	0.1	0.1		0.2	0.2		1.3	1.3	
K584d	0.4	0.3	0.5	0.1	0.1	0.0	4.1	5.2	3.0
K608	0.3	0.4	0.2	0.4	0.5	0.2	7.4	11.3	3.5
K638	-0.7	-0.7	-0.6	-0.5	-0.6	-0.4	-12.6	-14.3	-11.0
K636	-0.0		-0.0	0.2		0.2	4.1		4.1

Table 4.6. Genotypic (above diagonal) and phenotypic (below diagonal) correlations among the replicated entries of *L. leucocephala* accessions and its progenies between and within first- and second-year traits.

Traits	HT1†	DBH1	BIO1	HT2	DBH2	BIO2
HT1	1	0.94	0.98	0.97	0.94	0.97
DBH1	0.84	1	0.97	0.97	0.97	0.91
BIO1	0.88	0.96	1	0.93	0.95	0.95
HT2	0.77	0.82	0.76	1	0.99	0.94
DBH2	0.82	0.96	0.92	0.82	1	0.97
BIO2	0.82	0.96	0.91	0.83	0.95	1

†: HT1 and HT2 represent height at one-year and two-year growth period; DBH1 and DBH2 represent diameter of breast height at one-year and two-year growth period; BIO1 and BIO2 represent the estimated biomass yield at one-year and two-year growth period

between traits were also high and positive both within and between ages.

Discussion

Significant differences ($P<0.05$) for plant growth characters among *L. leucocephala* parents and its progenies after one year planting indicates that genetic difference among the six "giant" *L. leucocephala* parents exists. These results are in agreement with reports of high genetic variation for plant growth traits, isozyme, and chloroplast RFLP (Brewbaker *et al.*, 1972; Wheeler *et al.*, 1987; Sun, 1992; Harris *et al.*, 1994).

The fast growing characters among the four progenies could be attributed to additive and non-additive genetic

components, because both K565a and K608 with the high general combining ability showed the high heterosis when these two lines crossed, and both K565a and K608 crossing with low general combining ability K397 also showed higher heterosis. These results suggest there are additive and non-additive components in *L. leucocephala* crosses.

Low heteroses for two-year growth characters among *L. leucocephala* hybrids were found. However, some progenies showed as high as 15%, 25%, and 80% heteroses for plant height, DBH, and the estimated dry biomass yield respectively. This finding was in contrast with the result reported by Gupta (1986) that none of the hybrids exceeded the yield of the superior parent. The contradiction may be due to differences in the materials selected for the study.

Exploiting direct use of these high intraspecifc heteroses, may not be economically feasible at the present time due to the high cost of producing hybrid seeds. However, other alternatives should be considered unless the cost effective seed production method is found.

One of the alternatives is to use F_2 populations from the superior best F_1 progenies. This experiment showed that the F_2 of K584xK636 grew as well as the K636 parent did. Slow segregation of *L. leucocephala* progenies may be due to its tetraploid nature, in which the homozygosity rate decreases following selfing, unlike diploid plants (Bingham *et al.*, 1994). The results suggest that a mixture of seeds

harvested from fast-growing F_1 progenies may be used as composite *L. leucocephala* for distribution. The composite will consist of a diverse genetic background with growth rates greater than or equal to that of K636. K636 was identified as the best growing *L. leucocephala* within the *Leucaena* genus in terms of wood and forage production in Waimanalo, Hawaii (Wheeler *et al.*, 1989; Austin, 1995). Seeds of the four best-growing F_1 identified in this study were collected and germinated, and trees were planted for forage and wood yield production tests at the Hawaiian Agricultural Research Center (HARC) in Hawaii. Preliminary results showed great promise (Austin, personal comm. 1996).

Another alternative is to fix fast growth traits through recurrent selection. Recurrent selection proved to be very successful for forage production from *L. leucocephala* crosses in Australia (Hutton and Beattie, 1976). Only the additive genetic component can be fixed through recurrent selection. High combining ability of K565a and K608 for fast-growing traits indicates additive genetic component is present, and the traits in their progenies could be selected.

Recurrent selection should also include the F_2 population of K584xK636. Several individual F_2 trees of K584xK636 had superior growth rates. One of them exceeded 9 meters in height and 8.5 cm in DBH after two years growth. These individual trees, growing under psyllid pressure, grew

faster or equal to the "giant" *L. leucocephala* previous to the psyllid infestation. Therefore, recurrent selection from some of the best progeny crosses and the K584xK636 F_2 population from this trial should be pursued to select fast-growing *L. leucocephala* varieties.

A cost-effective seed production method would be to use self-incompatible (SI) trees in *L. leucocephala* to produce intraspecifc or interspecific hybrid seeds. SI trees could be identified from the progenies of different *L. leucocephala* backgrounds, although *L. leucocephala* is highly self-compatible (SC) (Brewbaker, 1986). *L. leucocephala* is an amphiploid with a great many S alleles. Different ratios of SI:SC would be expected due to competition of S alleles when different *L. leucocephala* parents are used to produce progenies (Brewbaker, 1986). SI trees of *L. leucocephala* may be used to produce intra- and interspecific hybrid seeds, especially of hybrids between L. *leucocephala* and *L. pallida,* which showed great promise for forage and wood production (Austin 1995; Chapter 3). Therefore, this trial could be used to test Brewbaker's hypothesis (Brewbaker, 1986) that SI trees in F_1 progenies of *L. leucocephala* intraspecifc hybrids may be found by coppicing the trees and checking self-fertility.

Significant difference for tree growth traits were observed between hybrids of K584 and K608 when different K584 trees were used. This result suggested contamination

in K584 and encourages isolation for pure-line *L. leucocephala* seed production. Though *L. leucocephala* is self-fertile, bee pollination is seen. Outcrossing rate in *L. leucocephala* (K636) was about 2 to 5 % (Brewbaker, unpubl.).

Phenotypic and genotypic correlations between traits at different ages have practical applications, since *L. leucocephala* is usually grown on two- or four-year rotations for biomass production. The results indicated that early selection for *L. leucocephala* growth variable of height, DBH, and the estimated biomass yield is reliable.

The results from this experiment clearly showed there was great diversity of the genetic background among the "giant" *L. leucocephala* collections, even though only six collections were tested. Progenies produced from K397, K565a, and K608 were highly productive. Although the direct use of these progenies was not practical at this time, the F_2 of these highly productive progenies could be exploited as a composite variety because of its diverse genetic background. Superior *L. leucocephala* varieties may could be produced through recurrent selection of the identified fast-growing progenies. Looking for self-incompatible *L. leucocephala* trees should be pursued from this progeny trial for exploiting intra- and inter-specific heterosis in *Leucaena*.

CHAPTER FIVE

PROPAGATION OF TRIPLOID *LEUCAENA* CLONES BY CUTTINGS

Abstract

Seedless trees of *Leucaena* have been prized in Indonesia for their fast growth and use as shade trees in coffee and cacao plantations. However, large-scale use of this fast-growing tree has been limited due to the inability to propagate vegetative cuttings on a consistent basis. A series of greenhouse experiments was therefore established in Waimanalo, Hawaii to develop a simple and reliable method to vegetatively propagate triploid *Leucaena* clones. Successful cloning was the result of a combination of treatments including cutting preparation, misting time, shade, medium, and a plant regulator. Binodal cuttings were dipped in solutions of 1% IBA and 0.5% NAA, inserted into a medium consisting of four parts perlite to one part peatmoss, shaded with a 30% sunlight reduction, and placed under mist. The system had a bottom heat of 35°C. The highest percentage of rooted plants was 80 percent from softwood cuttings of K1000 (*L. esculenta* K838 x *L. leucocephala* K636) and K1001 (diploid *L. diversifolia* K11 x *L. leucocephala*). *Leucaena* genotypes have shown differences as to the relative ease of propagation. Cuttings of *L. pallida* (K804) were difficult to root. The cloning methods

used here could be immediately employed to propagate these fast-growing trees and provide clonal material for the *Leucaena* tree breeding program.

Introduction

Leucaena is a small genus under the tribe Mimosoidae, which includes about 17 species, and is distributed from Southern Texas to northern Peru (Hughes, 1993; Sorensson and Brewbaker, 1994). *Leucaena leucocephala* (Lam.) de Wit., one of the best known species in the genus, was recognized as a 'miracle tree' during the 1970s and 1980s because of its fast growth and multiple uses in the tropics. It has a great number of uses including forage, firewood, timber, green manure, shade and reforestation trees, and food for human consumption (NAS, 1984; Brewbaker, 1987). It was reported that 2-5 million hectares of *L. leucocephala* were planted worldwide (Brewbaker and Sorensson, 1990).

Some limitations exist that restrict the usefulness of the self-compatible tetraploid *L. leucocephala*. They include high seeding yield, susceptibility to psyllids (*Heterophylla cubana* Crawford), slow seedling growth, and intolerance to acid soils and cold environments. High seeding yield may be a detriment if the objective is to provide shading for plantation trees since leaf production would be reduced due to competition for available energy by developing pods, and sometimes, it is an annoyance because

of the high population of seedlings that germinate and compete with the crops. Psyllid susceptibility also reduces foliage production. These factors that limit its further expansion may be overcome by a *leucaena* breeding program.

One of the objectives of a *leucaena* breeding program is to produce a triploid cross of a self-incompatible diploid species with a tetraploid species. Triploids are usually seedless, fast-growing, and have shown tolerance to psyllids when appropriate diploid parents are used (Brewbaker and Sorensson, 1990). Seedless triploid crosses between *L. pulverulenta* and *L. leucocephala* showed great potential for wood and forage production in Hawaii (Gonzalez *et al.*, 1967) and Australia (Bray, 1984), but were psyllid-susceptible. Brewbaker and Sorensson (1990) reported that other triploids could be produced by crossing diploid species such as *L. diversifolia* and *L. esculenta* with tetraploid *L. leucocephala*. Since there are no present methods of effectively producing seeds, vegetative propagation of these triploid hybrids forms the only practical means of multiplying these fast-growing trees.

There are several advantages of using a vegetatively propagated clone. These clones could be used to precisely test genotype-by-environment interactions. Clones of self-incompatible tree species could also be used to produce hybrid seeds when isolated for cross with selected parent trees.

Jones *et al.* (1982) and Litzow *et al.* (1991) reported that vegetative propagation of *Leucaena* was difficult. In contrast, Hu and Liu (1981) successfully propagated *L. leucocephala* leafy cuttings in Taiwan using sand as a medium under misting. Bristow (1983) propagated softwood cuttings successfully in Wales using grit and sand as a medium with bottom heat under polythene coverage. Other reports of success in vegetative propagation methods include those done by grafting (Schweizer, 1940; Zabala, 1977; Versace, 1982; Brewbaker, 1988; Brennan, 1992; 1995), use of large stems (Zabala, 1977; Delton, 1980; Austin, 1995), foam-air-layering (Osman, 1995), and tissue culture (Dhawan and Bhojwani, 1985; Goyal *et al.*, 1985).

A series of greenhouse experiments was conducted to establish a simple and reliable method of vegetative propagation for exploiting the potential of *Leucaena* triploid and facilitating *Leucaena* breeding programs at the University of Hawaii.

Materials and Methods

A series of unreplicated cloning experiments was conducted at Waimanalo, Hawaii from November 1994 to July 1995. K1000 (*L. esculenta* K838 x *L. leucocephala* K636), K1001 (diploid *L. diversifolia* K11 x *L. leucocephala*), K748, and K804 (*L. pallida*) were selected for the study. Softwood cuttings were taken from six-week old regrowth shoots of several coppiced eight-year old K1000 trees, one eleven-year old K1001 tree, one ten-year old K748 tree, and several four-year old K804 clones. Cuttings from regrown shoots were trimmed into lengths ranging from 5 to 15 cm, dipped in two commercial plant-rooting growth regulators, DIP'N GROW with 1.0% Indole-3-butyric acid, 0.5% 1-Naphthalenetic acid, and 98.5% inert ingredients (D&G) and Hormodin 3 with 0.8% Indole-3-butyric acid and 99.2% inert ingredients (Horm3), and then inserted into media under shade. The misting frequency was 5 seconds at 5 minute intervals. Black plastic seedling trays contained both 'Oasis' propagation wedge sets and various media combinations. Factors and treatments used in the *Leucaena* rooting experiments are listed in Table 5.1.

The mean monthly temperature of the greenhouse ranged from 22 to 24°C, daylight ranged from 11 to 13 hours, and mean monthly solar radiation ranged from 300 to 450 cal m^{-2} day^{-1}.

Table 5.1. Factors and treatments tested in a series of *Leucaena* rooting experiments from cuttings between November 1994 and July 1995 at UH Waimanalo Greenhouse, Hawaii.

Factors	Treatments
Pair of pinnae in the cuttings	One pair, two pairs, and no leaves
Number of nodes in the cuttings	One and two nodes
Plant rooting regulator	DIP'N GROW (D&G)†, Hormodin 3 (Horm3)†, and Control (only water)
Medium	Oasis wedge set, perlite, vermiculite & perlite, and perlite & peatmoss
Bottom heat	With and without bottom heat

†: See text

On 11 November 1994, the first experiment (Ex1) was established with about one-hundred-and-eighty cuttings from two triploid clones of K1000 and K1001. The cuttings were trimmed to about 6 cm in length with only one node and inserted into an Oasis wedge set with four treatments under 50% shade. Four treatments involved leaf retention on the cutting and concentration of rooting hormones: retaining two pairs of pinnates vs. leafless cuttings; and D&G vs. only water.

In December 1994, three more experiments were set up to test the effects of the number of pinnae (one pair vs. two pairs) left on the cutting, different genotypes, and plant regulator treatment (D&G vs. water). All other conditions

were the same as the first experiment. The second experiment (Ex2) was set up on 8 December 1994, and it included four combination treatments of one pair of pinnates vs. two pairs of pinnates and D&G vs. the control. K1000 and K1001 were evaluated. Each treatment had 18 cuttings for each genotype. The third experiment (Ex3) was set up on 18 December 1994. Treatments of D&G vs. the control and two genotypes of K1000 and K1001 were tested. Each treatment had 18 cuttings for each genotype, which were trimmed to one node with one pair of pinnates. The fourth experiment (Ex4) was established on 30 December 1994. Treatments and number of cuttings were the same as that of the Ex3, with the exception of the genotypes evaluated, which in this case were K748 and K804 of *L. pallida*.

On 1 March 1995, the fifth experiment (Ex5) was set up using a mixture of two parts vermiculite and one part perlite as a medium (2V1P). Three hundred K1000 and two hundred K1001 cuttings were placed under 30% shade. All cuttings were trimmed to about 6 cm in length with one node and dipped in D&G.

On 8 April 1995, the sixth experiment (Ex6) was carried out using a mixture of four parts perlite and one part peatmoss as a medium (4P1M) with bottom heat treatment of 35°C. Seventy-two K1000 and fifty K1001 cuttings were inserted into the medium and placed under 30% shade. All cuttings were binodal and dipped in D&G.

On 30 May 1995, the seventh experiment (Ex7) was established using the same medium and shade condition as in Ex6. Treatments were bottom vs. no bottom heat (control). Each treatment included fifty K1001 binodal cuttings with one pair of pinnates and dipped in D&G.

On 6 June 1995, the eighth experiment (Ex8) was installed using the same medium and shade conditions as Ex6. Seventy-five K1001 cuttings were inserted into the medium with bottom heat applied. The cutting preparation was the same as the Ex6.

On 9 June 1995, the ninth experiment (Ex9) was established using the same medium and shade condition as Ex6. Treatments were a combination of bottom heat vs. no heat (control) and D&G vs. Horm3. Each treatment included twenty-five K1000 binodal cuttings, having one pair of pinnate leaves.

On 15 June 1995, the tenth experiment (Ex10) was carried out under 30% shade with two different media. One media formulation consisted of a mixture of four parts perlite and one part peatmoss and the other consisted of using pure perlite. Two genotypes were tested: K1000 and K804. Each treatment contained twenty-five binodal cuttings, having one pair of pinnate leaves and dipped in D&G.

The number of rooted cuttings in all experiments was recorded, and they were transplanted into 12-cm diameter pots after five weeks of rooting. Forty rooted seedlings of

two triploid clones were transplanted at the Hamakua Station, Hawaii in May 1995 and the Waimanalo Research Station, Oahu in November 1995. Individual tree heights were measured and the Student-t test was applied to test the tree growth differences between the two genotypes.

Results

Rooting percentages from the different treatments in Ex1 are presented in Table 5.2. The best rooting result was 43% for K1001 and 24% for K1000. These results were from the cuttings with two pairs of pinnae and no plant regulator treatment. No mortality of cuttings was noticed three weeks after transplanting. The results indicated that leaves left on the cuttings were important for successful propagation.

Table 5.2. Percentage of cuttings rooted (Ex1) under four treatments using Oasis wedge set as a medium under five second misting at five minute intervals and 50% shade at Waimanalo greenhouse, Hawaii.

Treatments		K1000			K1001		
Leaves		Inserted			Inserted		
	D&G		Rooted	%		Rooted	%
Ex1							
Yes	Yes	21	4	19	21	4	19
No	Yes	21	0	0	21	0	0
Yes	No	21	5	24	21	9	43
No	No	21	0	0	21	0	0

All cuttings from Ex2 to Ex4 did not root. The wedge sets used were evidently too wet resulting in a high incidence of rotting at the basal cut. It was observed, however, that cuttings with one pair of pinnae situated at the edge of rooting bed and exposed to more light showed an increase in the length of time that leaves remained turgid relative to the other cuttings in the rooting bed.

The percentage of cuttings rooted from Ex5 to Ex8 are presented in Table 5.3. Rooted cuttings of K1000 from the Ex6 are shown in Figure 5.1. Average rooting percentage for the K1001 clone with bottom heat treatment was 68% and it ranged from 48% to 91%, which was higher than the treatment without bottom heat (46%). Average rooting percentage for both triploid clones was 6% for Ex5, but increased to 40% in Ex6. These results clearly indicated bottom heat treatment, two node cuttings, and the medium of 4P1M improved rooting percentage for both triploid clones under 30% shade.

Rooting percentages for K1000 and K804 of the Ex9 and Ex10 are presented in Table 5.4. The best rooting percentage for K1000 was about 80%, which was from the combination treatment of D&G, bottom heat, and the medium of four parts perlite: one part peatmoss. Average rooting percentage for K1000 cuttings with D&G treatment was 50%, and it was higher than the Horm3 treatment of Ex9. Average rooting percentage for K1000 with bottom heat was 58%, and it was higher than the control. However, no rooting was

Table 5.3. Percentage of cuttings rooted (Ex5 to Ex8) under different media and notes with or without bottom heat, all cuttings having one pair of pinnae treated with D&G plant regulator under five second misting at five minute intervals and 30% shade at Waimanalo greenhouse, Hawaii.

Treatments			K1000			K1001		
Medium	Bottom heat	Nodes	Inserted	Rooted	%	Inserted	Rooted	%
Ex5								
2V1P†	No	One	300	9	3	200	24	12
Ex6								
4P1M	Yes	Two	72	24	33	50	24	48
Ex7								
4P1M‡	Yes	Two				50	29	59
4P1M	No	Two				50	23	46
Ex8								
4P1M	Yes	Two				75	68	91

†: 2V1P indicates that medium consists of 2 parts vermiculite to 1 part perlite; ‡: 4P1M indicates that medium consists of 4 parts perlite to 1 part peatmoss.

Figure 5.1. Rooted cuttings of K1000 from the EX6 treated with D&G plant regulator and inserted in the medium of 4 parts peatmoss: 1 part perlite under five second misting at five minute intervals and 30% shade.

Table 5.4. Percentage of cuttings rooted (Ex9 and Ex10) under different media and hormones with or without bottom heat, all cuttings having one pair of pinnae treated under five second misting at five minute intervals and 30% shade at Waimanalo greenhouse, Hawaii.

Treatments			K1000			*L. pallida* (K804)		
Medium	Bottom heat	Hormone	Inserted	Rooted	%	Inserted	Rooted	%
Ex9								
4P1M[†]	Yes	D&G	25	20	80			
4P1M	No	D&G	25	5	20			
4P1M	Yes	Horm3	25	9	36			
4P1M	No	Horm3	25	2	8			
Ex10								
4P1M	Yes	D&G	25	19	76	25	0	0
1P00[‡]	Yes	D&G	25	0	0	25	0	0

[†]: 4P1M indicates that medium consists of 4 parts perlite to 1 part peatmoss; [‡]: 1P00 indicates 100% perlite medium

observed for cuttings of *L. pallida* (K804) in two different media and for K1000 in pure perlite medium. Results clearly showed that D&G treatment was better than Horm3 for triploid clone K1000, and that bottom heat treatment improved rooting percentage.

No mortality was noticed at two sites after five months planting, average plant height was 2.22 m for K1000 and 2.48 m for K1001 at Hamakua, and 1.80 m for 1000 and 1.90 m for K1001 at Waimanalo. However, no significant difference was found for plant height between the two clones at two sites.

Discussion

Leucaena vegetative propagation has considered easy by some and difficult for others. The problems facing *Leucaena* researchers to repeat the reported successful vegetative propagation methods could be attributed to materials they selected and experimental environments they are under. The cutting materials include different cutting genotypes and different cutting parts from the plant. Environments include shade condition, misting (size of droplets), and site.

The results of the present study regarding cutting genotypes and positions were in accordance with the early studies. Austin (1995) reported that there was a great difference of rooting ability from *Leucaena* species and interspecific hybrids with different genetic background, and found that *L. pallida* was difficult to root. Austin (1995) also reported that the second and the third cuttings from the top of a *Leucaena* branch (relative to the fourth to eighth nodes or soft-green tissues) more easily rooted. I also noticed that new growth softwood cuttings with thinner stems were easier to root than cuttings below the softwood with thicker stem. Litzow and Shelton (1991) suggested that stem cuttings may possess a stronger inhibitory barrier than the leafy twigs or softwood cuttings.

The right environmental conditions for *Leucaena* cuttings are very important. Bottom heat treatment and 30% shade

were essential for improving rooting ability of triploid *Leucaena* clone cuttings. In Ex1 without bottom heat treatment, signs of small white spots at the basal end of some cuttings were observed four days after installation of the experiment at which time shoots also began to develop. However, no obvious callus was noticed. In Ex6 with bottom heat treatment, callus formation was noticed one week after cuttings were inserted into the medium. Hartmann and Kester (1975) suggested that warm-temperature partially overcomes the internal barrier of cuttings. It may also be possible that warm temperature may be involved with calcium concentration changes in cells where callus and root initiation take place. The role of calcium adjusted by higher temperature treatments was clear for the germination of the purple nutsedge (*Cyperus rotundus*) from dormancy (Sun, 1996).

In general, very high light intensity is detrimental to newly planted cuttings and fifty percent direct sunlight is desirable for most tropical timber species (Darus and Aminah, 1994). Results of the present study suggested that triploid *Leucaena* clones are light-loving plants and need higher light intensities for cuttings to root.

The critical environmental conditions for successful *Leucaena* vegetative propagation from cuttings included sanitation of facilities and condition of misting systems. Rotting of *Leucaena* cuttings began earlier than callus

initiation, possibly because *Leucaena* has a high protein and phenolic content in its softwood tissues. Sources of infection could include cutting materials, media, and water. Standard procedures should include fungicide treatment of cuttings and media.

It was clear that a fine misting system improved the rooting ability of triploid *Leucaena* clones. Fine misting may reduce the exposure of cuttings to microbes, because the large water drops usually carry surface microbes to the wounded cutting area and cause cuttings to rot earlier.

Interspecific F_1 hybrids between *L. pallida* and *L. leucocephala* show great promise for forage and wood production (Chapter 3; Brewbaker and Sorensson, 1990; Austin, 1995). Utilization of these hybrids relies on the hybrid seed production by employing the use of self-incompatible clones, e.g., *L. pallida*. Cuttings of *L. pallida* (K804 and K748) have been very difficult or impossible to root. Another problem facing direct use of this interspecific hybrid was low percentage of pure hybrid seed production from *L. pallida*, when used as the female parent surrounded by *L. leucocephala* (Brennan, 1995; Shelton, personal. comm. 1995). Some interspecific KX2 F_1 hybrids between *L. pallida* and *L. leucocephala* are self-incompatible (Sun and Kamemoto, unpubl.) and could be rooted (Austin, 1995). In order to use *Leucaena* hybrid heterosis, KX2 F_1 hybrid may be used as a female parent to produce

backcrosses with *L. leucocephala* for hybrid seed production, instead of direct use of F_1 hybrid seeds from *L. pallida* and *L. leucocephala*. Productivity of this backcross can be estimated according to Jenkins (1934). However, the backcrosses should be tested in the field before any large quantity of backcross seed production is made.

The success of vegetative propagation of triploid *Leucaena* clones with high percentage rooting from cuttings on a consistent basis is a breakthrough for our *Leucaena* improvement program and the new use of *Leucaena* as a gum tree. A large quantity of gum exudates was reported from a triploid hybrid between *L. esculenta* K838 x *L. leucocephala* K636 (Brewbaker and Sorensson, 1990). *Leucaena* gum was reported having the same quality as a gum arabica of *Acacia senegal* (Anderson and Douglas, 1989). The technique developed in this study could be employed to vegetatively propagate this clone to test *Leucaena* gum productivity under different management regimes following *Acacia senegal* gum production techniques.

CHAPTER SIX

FORAGE YIELD MANAGEMENT OF *LEUCAENA LEUCOCEPHALA* AND AN INTERSPECIFIC HYBRID KX2

Abstract

The present study was established to estimate two growth parameters: weekly increments (WI) and mean weekly increments (MWI) of edible forage and of wood in forage-managed *Leucaena*. These parameters were used to predict optimal harvest time for forage production. *Leucaena leucocephala* cv. 636 and an interspecific F_1 hybrid, KX2 (K748 x K636), were studied in an experiment at Waimanalo, Hawaii from March to June, 1994. Sample trees were taken every week in three replicated plots from the fifth week after the previous harvesting.

KX2 significantly outyielded K636 under the modest psyllid infestation in this trial. The maximum MWI for KX2 was about 560 kg $ha^{-1}week^{-1}$ between seven to eight week regrowth period with an attained height of about 2 m. The maximum MWI for K636 was about 200 kg $ha^{-1}week^{-1}$ between eight to nine week regrowth period with an attained height of about 1.5 m. More than 30% increase in forage yield was obtained by optimizing the harvest intervals of these two varieties. Allometric equations were calculated using both heights and diameters, and heights alone appeared to suffice

for predictions. The results imply that heights of *Leucaena* tree regrowth can be used to estimate growth parameters and predict optimal harvest time and yield for K636 and KX2.

Introduction

Leucaena leucocephala once was well known as a "miracle tree" of the tropics during the 1970s and 1980s shortly after the release of a series of accessions of the 'Hawaiian giant type' from the University of Hawaii. Because of its fast growth and nitrogen fixing nature, it was widely used as forage, firewood, timber, green manure, shade and reforestation trees, and even food for human consumptions in some countries (NAS, 1984; Brewbaker, 1987). It was reported that *Leucaena*, mostly *L. leucocephala*, occupied about 2-5 million hectares plantations worldwide (Brewbaker and Sorensson, 1990).

Leucaena's greatest attribute appears to be in forage production due to its high forage yield (20 to 30 MG ha^{-1} yr^{-1}), high crude protein content (20 to 35%), high forage digestibility (55 to 65%), drought tolerance, and persistence (Shelton and Brewbaker 1994; Austin, 1995). A *Leucaena* pasture could produce twice the animal live-weight gains per hectare to that of Siratro (*Macroptilium atropurpureum*), when grown in similar soils in Australia (Jones and Jones, 1982). However, large-scale *Leucaena* plantings have been restricted to subtropical areas due to

its narrow genetic base and susceptibility to the psyllid insect (*Heterophylla cubana* Crawford), which has spread globally since 1984 (Hughes, 1993; Austin *et al.*, 1995).

Extensive research on *Leucaena* forage yield trials have focused on tree genetic improvement and field management studies. Tree improvement include the use of interspecific hybrids for desirable characteristics, for example, fast growth and psyllid resistance or tolerance conducted at the university of Hawaii (UH) and provenance assessment among the genus elsewhere. The UH *Leucaena* breeding program has released a series of 'giant' *L. leucocephala*, e.g., K8, K29, K67, K584, and K636, as well as identified promising interspecific hybrids, such as KX2 F_1 hybrid between *L. pallida* and *L. leucocephala*, triploid clones of K1000 (*L. esculenta* K838 x *L. leucocephala* K636) and K1001 (diploid *L. diversifolia* K11 x *L. leucocephala* unknown). The KX2 F_1 hybrid has proven to be a promising crop for forage and wood production (Brewbaker and Sorensson, 1990; Austin, 1995; Chapter 3).

Some of the field management aspects for forage production include planting density (Guevarra *et al.*, 1978; Desai *et al.*, 1988; Ella *et al.*, 1989; Jiang and Liu, 1991; Sampet, 1991), planting geometry (Jeyaraman *et al.*, 1988), cutting height (Guevarra *et al.*, 1978; Jama and Nair, 1989; Sampet, 1991), cutting frequency (Guevarra *et al.*, 1978; Ella *et al.*, 1989; Jeyaraman *et al.*, 1989; de Lucena Costa

et al., 1991; Nyathi *et al.*, 1995), and age of first cutting (Ella *et al.*, 1991).

Cutting frequency or cutting intervals could greatly affect *Leucaena* edible and non-edible yield. Examples of the effect of cutting interval on edible fraction and stem yield are listed in Table 6.1. However, results of the various cuttings trials are difficult to interpret because of the different conditions (i.e., climatic differences) under which the trials were carried out (Stur *et al.*, 1994).

Table 6.1. Examples of the effect of cutting frequency on edible forage yield (MG $ha^{-1}yr^{-1}$), wood stem yield (MG ha^{-1} yr^{-1}), and edible fraction of *L. leucocephala*.

Cutting intervals (week)	Edible forage	Wood stem	Edible fraction	Reference
3	13	8.6	60%	de Lucena Costa *et al.* 1991
6	14.7	11.8	55%	de Lucena Costa *et al.* 1991
6	8.6	2	81%	Ella *et al.* 1989
6	11.5			Jeyaraman *et al.* 1989
7	14.8			Jeyaraman *et al.* 1989
8	9.2	7.8	54%	Feraris *et al.* 1979
9	14.5	14.6	50%	de Lucena Costa *et al.* 1991
9	18.4			Jeyaraman *et al.* 1989
11	9.4	2.6	78%	Guevarra *et al.* 1978
12	10.5	9.2	53%	Ella *et al.* 1989
12	17.1	16.5	51%	de Lucena Costa *et al.* 1991
14	11.5	5.4	68%	Guevarra *et al.* 1978
16	10.3	18.6	36%	Feraris *et al.* 1979
18	12	8.8	58%	Guevarra *et al.* 1978

The growth rate is an increase in size or weight per unit of time, and the cumulative growth rate is an average of plant size or weight over times. Growth parameters have long been studied by plant physiologists and foresters to recommend plant growth needs and harvest dates for maximum biomass yield. For example, mean annual increment (MAI), a cumulative growth rate, is often used by foresters as the criterion for an optimal rotation length for biomass production of fast-growing trees. When annual increments falls below the overall MAI, harvest is recommended.

Few studies regard growth rates of multi-purpose tree species for forage production were reported. Stur *et al.* (1994) reported that two growth rates, weekly growth increment (WI) and mean weekly growth increment (MWI), of edible forage of *Caliandra calothyrsus* during a 24 week regrowth period and suggested that 10 week interval harvest would produce the maximum edible forage yield. Similar information on MWI for *Leucaena* forage production might be equally useful for growers to set a rotation length that maximizes forage yields.

An experiment was conducted to study MWI and WI of *L. leucocephala* (K636) and an interspecific KX2 F_1 hybrid between *L. pallida* and *L. leucocephala* after coppicing for forage management and to predict the optimal harvest intervals for the maximal forage production using these growth parameters.

Materials and Methods

Cultivar K636 of *L. leucocephala* and KX2, an F_1 hybrid between *L. pallida* (K748) and K636, were selected for estimation of MWI (mean weekly increment) and WI (weekly increment) in an experiment conducted at Waimanalo, Hawaii. During the study period (March to June, 1994), mean temperature was 24°C, insolation average was 380 cal cm^{-2} day^{-1}, and plants did not come under drought stress.

The trial SET 94-3 was established on 3 June 1993 (Chapter 3). In this trial, a total of 40 entries were evaluated in an augmented randomized complete block design with three replications. Sixteen trees were planted in each plot in two rows. Spacings were 0.25 x 1.25 m^2/tree or 32,000 trees ha^{-1}.

Sample trees were harvested and cut back at about 0.5 m in height. Three trees were taken weekly beginning with the fifth week after the second forage harvest in trial SET 94-3. Edible forage and woody stems of each sample tree were separated, oven-dried, and weighed. Plant height and base diameter (diameter at the base of cut sample stem or branch) and edible forage dry matter (leaves plus stem < 5 mm in diameter) of each sample were measured. Data were analyzed with the use of a spreadsheet (Brewbaker 1993) for allometric equations.

Results

Mean weekly increment (MWI) and weekly increment (WI)

MWI and WI of edible forage dry matter for K636 and KX2 are presented in Figure 6.1. MWI, an average over the entire seasons, progressed slowly to reach the maximum by about eight to nine weeks. The maximum MWI for KX2 was 560 kg ha^{-1} between seven and eight week regrowth period, and the maximum MWI for K636 was about 200 kg ha^{-1} between eight to nine week regrowth period. WI, value for each week, climbed and then dropped sharply within a three week period from the seventh to ninth week for KX2 and from the eighth to tenth week for K636. The maximum WI for KX2 was about 1,500 kg ha^{-1}, and for K636 it was only 600 kg ha^{-1}. KX2 significantly outyielded K636, in part due to the modest psyllid infestation.

Growth curves of height, DBH, and edible forage DM

The rate of main shoot regrowth of KX2 F_1 exceeded that of K636 (Figure 6.2). In a nine-week regrowth period, the main shoot of KX2 F_1 attained an average of 240 cm in height and 15 mm in base diameter, and that of K636 attained an average of 160 cm in height and 12 mm in base diameter.

Regrowth of forage (F) and wood (W) dry matter after the second harvest are presented for both K636 and KX2 in Figure 6.3. Both varieties distributed more photosynthates to

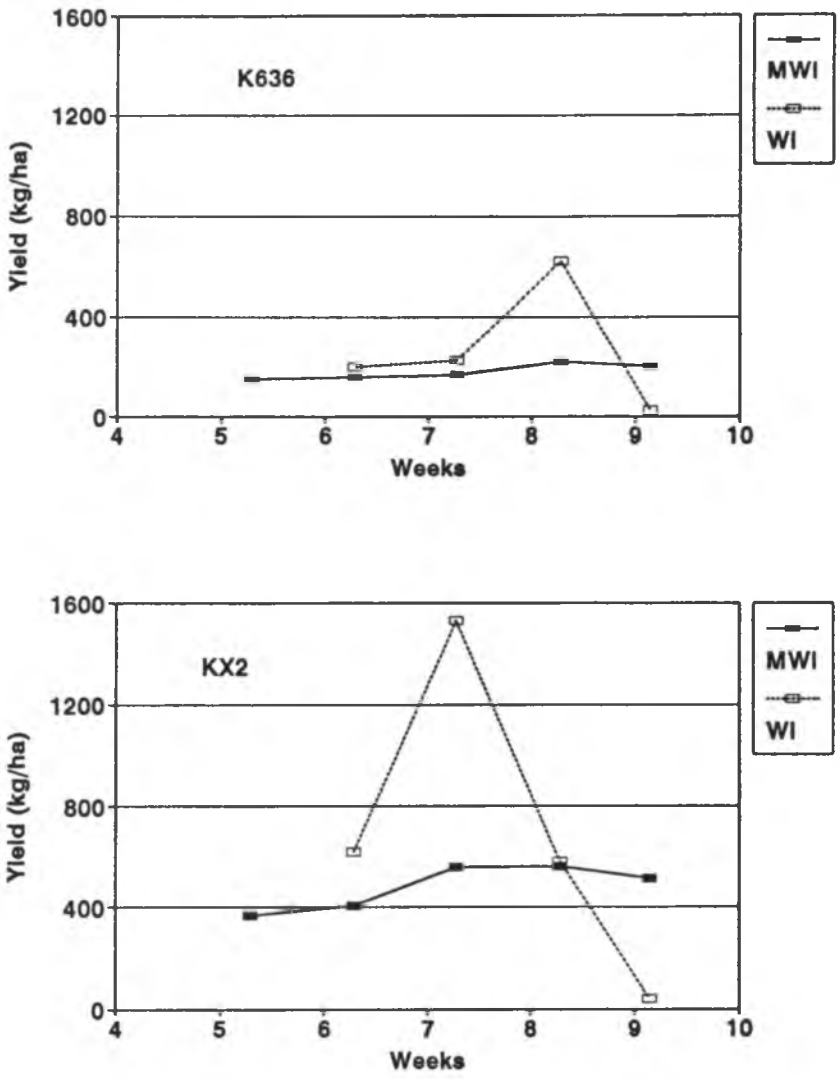


Figure 6.1. Mean weekly increment (MWI) and weekly increment (WI) of forage yield for K636 of *L. leucocephala* and KX2 F_1 between K748 of *L. pallida* x K636 at Waimanalo, Hawaii.

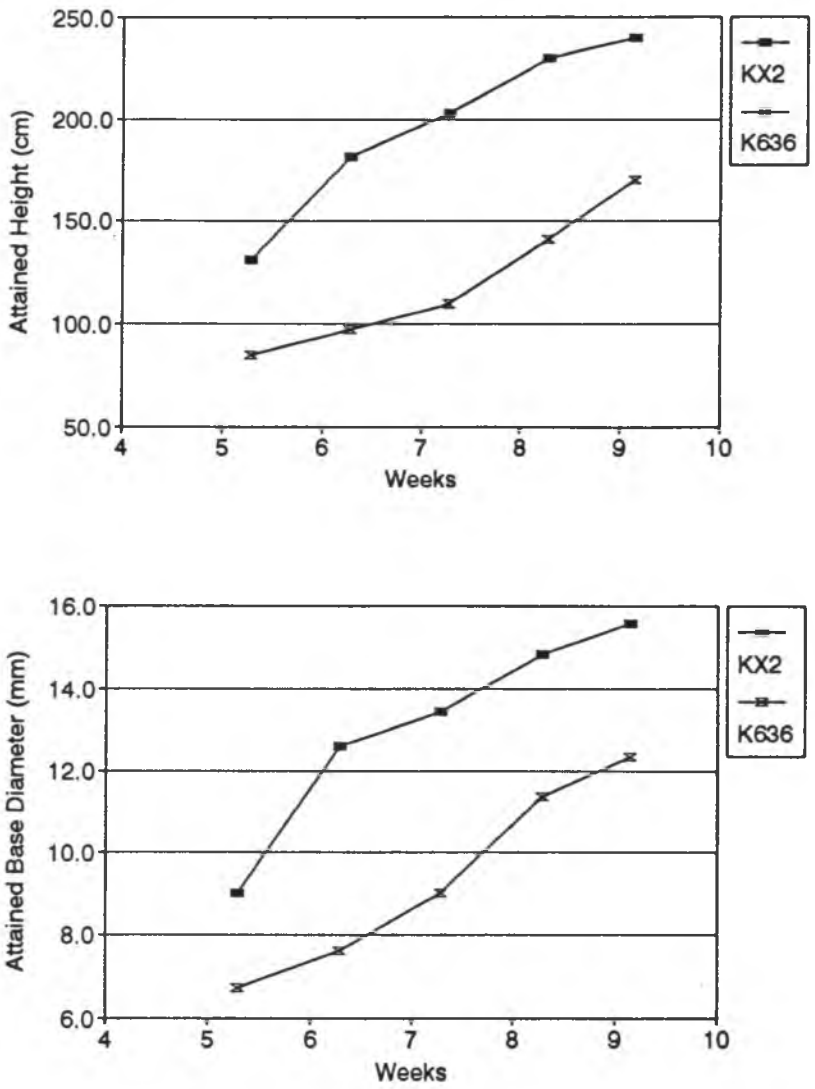


Figure 6.2. Attained shoot height (a) and base diameter (b) of K636 of *L. leucocephala* and KX2 F_1 between K748 of *L. pallida* and K636 during a 10-week regrowth period after the second previous harvest at Waimanalo, Hawaii.

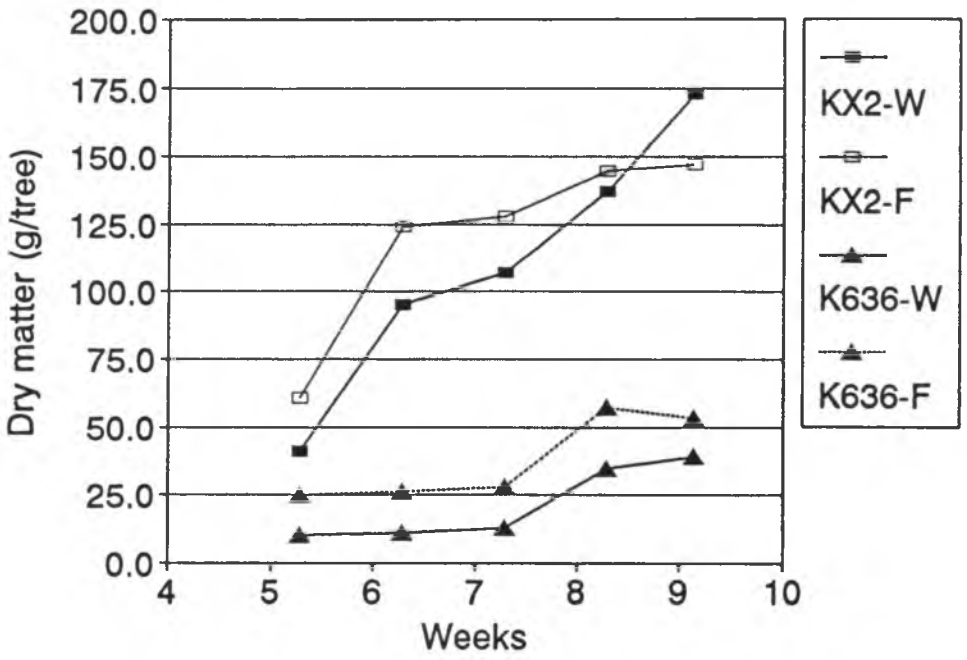


Figure 6.3. Edible forage DM (edible stem to 5 mm diameter)(F) and wood or stem DM production (W)(g tree $^{-1}$) of K636 of *L. leucocephala* and KX2 F1 between K748 of L. pallida and K636 during a 10-week regrowth period after the second previous harvest at Waimanalo, Hawaii.

leaves during first eight to nine weeks, then shifted its distribution to woody stems.

Edible forage percentages are graphed over time for K636 and KX2 F_1 in Figure 6.4. The trend of edible forage percentage for both varieties was the same. High percentages were observed during the early regrowth stage but slowly declined from the fifth to sixth week. KX2 F_1 had a significantly ($P<0.05$) lower edible forage percentage compared with K636 for the same growth period.

Allometric equations

Several allometric equations for K636 and KX2 F_1 were developed (Table 6.2). Total dry matter (DM) and edible forage DM from regrowth tree stems and branches after coppicing were predicted from data for height and the base diameter. For K636, predictions of total DM and forage DM using base diameters were better than using stem heights. However, for the KX2 F_1 hybrid, no difference was observed between the two parameters. Increased precision for prediction did not occur when using both height and base diameter parameters for both genotypes (Table 6.2).

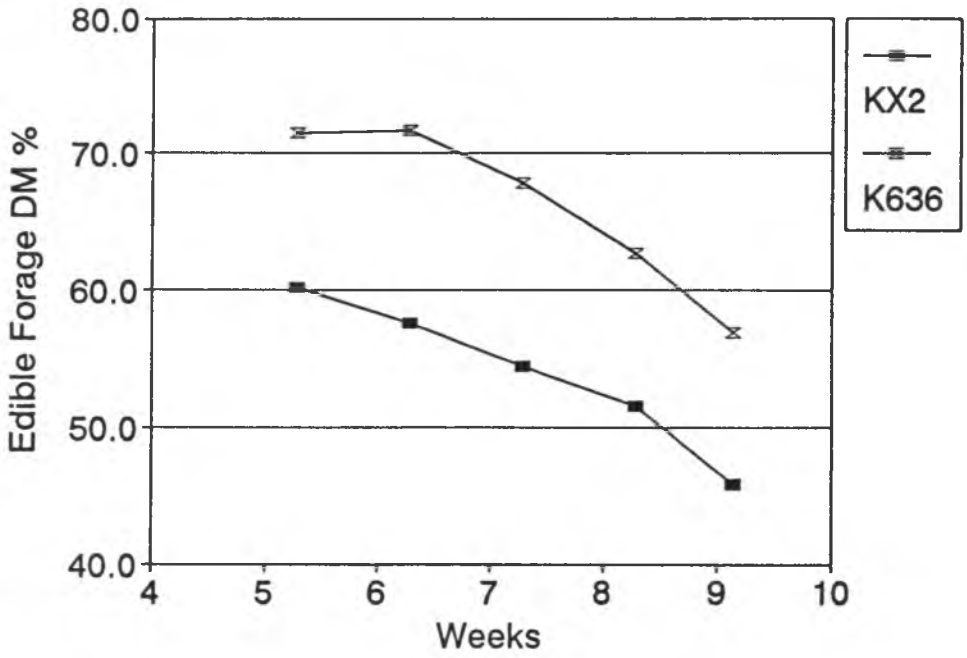


Figure 6.4. Edible forage dry matter percentage changes of K636 of *L. leucocephala* and KX2 F_1 between K748 of *L. pallida* and K636 during a 10-week regrowth period after the second previous harvest at Waimanalo, Hawaii.

Table 6.2. Allometric equations with R^2 for predicting total and forage DM from stem height (H) and base diameter (D) for *L. leucocephala* and KX2 after coppicing.

Allometric equations†	R^2
K636	
Total DM = $9.12*10^{-3}*H^{2.16}$	0.886
Total DM = $0.138*D^{2.41}$	0.957
Total DM = $0.123*D^{2.36}*H^{0.05}$	0.957
Forage DM = $3.02*10^{-3}*H^{1.81}$	0.845
Forage DM = $0.191*D^{2.04}$	0.937
Forage DM = $0.347*D^{2.278}*H^{-0.23}$	0.937
KX2	
Total DM = $5.37*10^{-5}*H^{2.714}$	0.969
Total DM = $9.3*10^{-2}*D^{2.718}$	0.956
Total DM = $7.24*10^{-4}*D^{1.003}*H^{1.743}$	0.975
Forage DM = $1.66*10^{-4}*H^{2.365}$	0.962
Forage DM = $0.107*D^{2.37}$	0.951
Forage DM = $1.73*10^{-3}*D^{0.908}*H^{1.485}$	0.969

†: Allometeric equations for K636 were based on heights ranging between 50 and 180 cm and diameters between 5 and 13.7 mm, and for KX2 heights between 50 and 250 cm and diamters bewteen 5 and 17.5 mm

Discussion

Leucaena leucocephala has long been proven to be a very productive tropical legume for forage production, but optimal harvesting time for maximum forage yield production have not been well studied. The field management for forage production was based on intuition or inflexible schedules rather than on plant physiological principles, because the optimal harvesting time for most leguminous species is not known (Stur *et al.*, 1994).

If the main criterion for managing wood legume forage production is to maximize forage yield on a per-week basis, the time at which WI and MWI lines intersect would optimize harvest. Yields from this study were about 30% higher than the yields obtained from regular three month harvest intervals in one and half year period for these two varities at Waimanalo, Oahu, Hawaii. Optimal rotation lengths for *L. leucocephala* and KX2 F_1 forage production from this study are still difficult to interpret and extrapolate because different growing conditions may account for these results. However, the parameters, such as stem height and diameter, may attain the information with the optimal harvesting time. When combining optimal harvest time information with shoot growth curves of both varieties (Figure 6.2), it clearly indicates that the time which the shoots attained about 200 cm in height for KX2 and about 150 cm in height for K636 were optimal for harvesting. This result was in agreement

with Guevarra *et al*. (1978), who reported that the highest dry matter and forage yield for *L. leucocephala* were obtained when cut at an attained height of 150 cm. The result of the present study clearly suggests that the height of main stem regrowth after coppicing could be used as an indicator for predicting optimal harvest times for *Leucaena*.

The percentage of edible forage was higher under more frequent harvesting of *L. leucocephala* and KX2 F_1. The results were in agreement with the findings of other authors, e.g. Guevarra *et al*. (1978). It was also obvious that KX2 F_1 had a lower edible forage percentage compared with K636 at the same growth period, in concurrence with Austin (1995).

Allometric equations have beeb reported by Austin (1995) for *Leucaena* tree growth prediction, but not for regrowth from 50 to 250 cm in height. Such allometric equations might be used to predict the optimal harvesting time for *Leucaena* forage management. While the parameters MWI and WI could be calculated, this is not practical for field production. An alternative may be to use allometric equations to estimate total and edible forage dry matters from heights or base diameters. Equations developed here can be used to estimate the two growth parameters and predict the optimal harvest time based on height and based diameter.

Successful use of *Leucaena* as a forage crop in pasture is partly dependent on the field management which maximizes the forage yield according to its physiological principles. Determination of optimal harvesting time is essential for exploiting the full potential of *Leucaena* species and hybrids for forage production. Traditionally, the optimal harvesting time could be decided by using growth parameters of edible forage dry matter. Field practices may use allometric equations based on tree regrowth height or base diameter to predict the optimal harvest time. The results of this study, with particular regard to the shoot height for K636 and KX2 F_1 hybrid, may be recommended for *Leucaena* forage management in other areas under different growing conditions.

CHAPTER SEVEN

SUMMARY OF SECTION ONE

The major objects of this study on *Leucaena* genetic improvement are to identify the high biomass yield *Leucaena* lines through inter- and intra-specific hybridization and recurrent selection and to capture heterosis via hybrid seed production, vegetative propagation, and selection.

High heterosis and variation for *Leucaena* forage and biomass yield were found among interspecific and 3-way crosses between *L. pallida* and *L. leucocephala* (or KX3 F_1). Interspecific heterosis for forage yield among interspecific hybrids ranged from -75% to 160% with an overall average of 48% and for wood biomass yield ranged from -99% to 223% with an overall average of 85%. The best-yielding KX2 F_1 hybrids for forage were K748xK584, K748xK481, and K748xK636, which produced an average yield of 16 Mg ha^{-1} yr^{-1}. The best-yielding hybrids for wood biomass were K748xK8, K748x(K156xK8), and K748xK636, which produced a average yield of 45 Mg ha^{-1} yr^{-1} in a two year period. These hybrids yielded about three times the amount of forage and wood biomass produced by the widely planted *L. leucocephala* K636.

There are three ways to capture the interspecific heterosis (Brewbaker and Sorensson, 1990). These include direct cloning hybrids, using clones of self-incompatible

(SI) species (e.g., *L. pallida*) for hybrid seed production, and developing SI (*L. leucocephala*) lines. Direct use of hybrids by cloning seems to be expensive and presently is not practical. Cloning of SI *L. pallida* using grafting was only successful with some accessions (Brennan, 1995), but was very difficult using cuttings (Austin, 1995; Chapter 4). *Leucaena pallida* clones also had considerably high percentage of selfing when surrounded by *L. leucocephala* for hybrid seed production (Brennan, 1995; Shelton, personal comm. 1995). Due to the cost and problems related to direct cloning and use of *L. pallida*, the ideal way of using these heteroses is to create SI lines through S allele recombination among *L. leucocephala* accessions, then use these SI lines to produce hybrid seeds with the desired species, e.g., *L. pallida*. In order to find SI lines in *L. leucocephala*, twenty intraspecific crosses were made among six *L. leucocephala* accessions. More than one thousand of these hybrid trees were planted at Waimanalo, Oahu, for evaluation of SI trait.

The other alternative to capture heterosis is to make a backcross between KX2 F_1 and *L. leucocephala*. KX2 F_1 hybrids have been proven self-incompatible (Kamemoto and Sun, unpublished), and should be easy to propagate vegetatively by cuttings. Yield prediction of these backcrosses could be estimated using the method of Jenkins (1934). The backcross will have more than 30% heterosis

over means of all parents. Several backcrosses between different KX2 F_1 and *L. leucocephala* lines were also made and trees have been planted at Waimanalo, Oahu and Mealani, Hawaii to test this hypothesis.

If some of the interspecific heterosis is controlled by the additive gene effects, this portion of heterosis can be captured through selection. Recurrent selection attempts were made from progenies of K8 x K376 cross in our breeding program. Significant genetic gains (98%) for forage yield were made after two cycles of recurrent selection, but not for wood biomass. These unexpected results could be due to criteria used to select advanced progeny based on psyllid resistance and early growth vigor.

Intraspecific heterosis and variation of tree height, DBH, and wood biomass yield were also found among twenty intraspecific hybrids involving six *L. leucocephala* collections. Intraspecific heterosis for the biomass yield in two years growth ranged from -27% to 81% with an overall average of 16%. The best-yielding hybrids were K397xK608, K397xK565a, K608xK565a, and K584xK636 (F_2) which yielded higher than K636. Exploiting for direct use of intraspecific heterosis may not be economically feasible at the present time, due to the high cost of producing hybrid seeds by hand pollination. However, the results clearly showed the promise of exploiting commercial use of F_2 seeds from these hybrids and advancing selection of K584x636 F_2

and other hybrids. F_2 seeds of the four best-yielding intraspecific hybrids were collected, and were planted for forage and wood yield production trials at Hawaiian Agricultural Research Center (HARC) in Hawaii. Preliminary results showed great promise for forage production and a large quantity of these F_2 seeds were harvested for distribution (Austin, personal comm. 1996).

Standardizing *Leucaena* vegetative propagation technique is a key component to the successful *Leucaena* breeding program. A series of greenhouse experiments were conducted to develop a simple and reliable method for *Leucaena* cloning. A cloning method with 80% successful rooting of triploid *Leucaena* (K1000 and K1001) cuttings was established. The method included using binodal cuttings with one pair of pinnae treated with a solution of 1% IBA and 0.5% NAA, inserted into a medium consisting of four parts perlite and one part peatmoss with bottom heat at 35°C, shaded with 30% sunlight reduction, and placed under five second misting at five minute intervals.

Successful use of these identified *Leucaena* hybrids as a forage crop in pasture is partly dependent on the field management which maximizes the forage yield according to its physiological principles. An experiment was carried out to study tree growth traits of weekly increments and mean weekly increments for K636 and K748xK636(KX2 F_1). The results showed that the maximum forage yield of 30 Mg ha^{-1}

yr^{-1} for K748xK636 could be obtained between seven to eight week regrowth period with an attained height of about 2 m. The maximum forage yield for K636 was 10 Mg ha^{-1} yr^{-1} between eight to nine week regrowth period with an attained height of about 1.5 m. These yields were about 30% higher than the yields obtained from regular three month harvest intervals in one and half year period for these two varieties at Waimanalo, Oahu, Hawaii.

In this study, DNA contents of *Leucaena* species were also analyzed using flow-cytometry. Estimates of DNA content are important for genome structure analysis and genetic mapping of qualitative and quantitative trait loci. DNA content information is also useful for understanding the evolution of plant ploypoids. The study of DNA contents among 90 *Leucaena* accessions showed that the difference in DNA content between *Leucaena* accessions within a species exists, and those between species and between groups based on the leaflet size are significant. Estimates of 2C nuclear DNA content for diploid species ranged from 1.33 to 1.74 pg and tetraploid species ranged from 2.67 to 3.09 pg. The range of nuclear DNA content in the genus corresponded to approximately 650 megabase pairs for diploid species and to 1,500 megabase pairs for tetraploid species. The result also indicated that there are significant changes in DNA content during the process of speciation in the genus.

The results of these studies have several practical applications. The studies identified several fast-growing *Leucaena* hybrids and varieties and recommended cultural practices for maximizing forage yields. The studies also clearly suggest the changes of *Leucaena* breeding directions from the traditional selection and evaluation of collected materials to the use of inter- and intraspecific heterosis. This change requires breeders to find ways to produce the hybrid seeds and to look for other alternatives for capturing inter- and intraspecific heterosis. The alternatives include use of backcrosses between KX2 F_1 and *L. leucocephala*, use of F_2 of intraspecific hybrids among selected *L. leucocephala* accessions, and fixation of some heterosis through selection.

The immediate contribution of these studies is the release of composites of F_2 populations from selected *L. leucocephala* intraspecific hybrids with the cooperation of HARC. These F_2 population not only provide a broad genetic background and wider adaptation, but also produce higher forage yield compared with the widely planted *L. leucocephala* K636.

SECTION TWO

GENETIC IMPROVEMENT OF *ACACIA KOA*

The goal of *Acacia koa* tree improvement is to increase the supply of koa wood by accelerating the growth rates and reducing the harvesting age via selection. However, research regarding the genetic improvement of koa has been almost non-existent, perhaps due in part to a perceived, though questionable notion, that koa growth is affected strictly by environmental conditions. To date, of the few studies aimed at improving koa growth performance, no reports have made on genetic gains of selected provenances and improved progeny. These past studies have been limited to the identification of superior trees based on size and bole straightness (Skolmen, 1977), but have failed in obtaining advanced progenies from a cloned nursery and a half-sib progeny trial of selected superior trees (Skolmen *et al.*, 1991).

Previous morphological studies have implied that koa might have a large genetic base. Morphological differences of natural stands of koa, including phyllode size, tree form, seed size, and flower characteristics are found throughout the Hawaiian islands. These differences in morphology have led to the classification of species and varieties within Hawaiian *Acacia*, namely, that of *A. koa* Gray, *A. koaia,* and *A. kauaiensis* (Hillebrand, 1888; Rock, 1920; Lamoureux, 1971; St. John 1979). The morphological variation among *Acacia koa* varieties observed by early botanists also suggest genetic variation. Brewbaker (1977)

reported isozyme polymorphism and variation of phyllode size and shape among selected koa populations.

Previous attempts of koa reforestation have shown slow growth and poor stand performance (Whitesell and Ishewood, 1971; Ching, 1981; Scowcroft and Adee, 1991). After 10 years, koa attained an average height of only 8.4 m and an average DBH of 12 cm at the Hilo Forest Reserve, Hawaii (Scowcroft and Adee, 1991). These growth rates are inferior to observations made by Judd (1919), who reported that koa attained heights of 9 m in five years. Skolmen (1990) also observed differences in growth between koa progenies grown in the same location. One progeny produced straight and uniformly tall trees, while the other was heavily defoliated and died shortly thereafter. Conrad *et al.* (1995) reported there were significant differences for plant growth among eight koa provenances from four Hawaiian islands.

Genetic variation of koa wood quality and other traits have also been observed. Simmons *et al.* (1991) reported variations in koa wood quality regarding color, grain, and specific gravity. Skolmen (1990) observed differences in koa response to volcanic fumes. Plants from a region distant to an area of volcanic activity was more prone to fume damage than plants proximal to the volcanic region. Further evidence of the genetic variation in koa is suggested by its highly diverse ecological range.

Strategies for *Acacia koa* improvement and selection were outlined by Glover *et al.* (1991). These include an operational component that entails seed collection and genetic conservation to meet the current and future seed demand, and a research component to maintain ongoing genetic studies for long term improvement. The research component was concluded to clarifying koa's breeding system, determining the extent of genetic variations, measuring heritability of specific traits, and evaluating genotype x environment interactions.

Other strategies for koa improvement also include provenance and progeny testing, in order to evaluate sources for commercial seed production, to select materials for breeding objectives, to delineate seed transfer zones, and to elucidate genetic architecture (Loo-Dinkins, 1992). The testing is also used to estimate the breeding value of the selected parents and genetic parameters (genetic variance and correlation) and is often used as a source for the next selection and seed orchard for plantation seed production (Eldridge *et al.*, 1993).

Objectives of this current study were to collect *Acacia koa* germplasm in the Hawaiian islands, to quantify the genetic variability among *Acacia koa* populations, to identify quality seed sources for reforestation, and to select superior koa as the basis for long-term genetic improvement.

CHAPTER EIGHT

LITERATURE REVIEW

Koa (*Acacia koa* Gray) is an indigenous legume tree of the Hawaiian Islands that is valued for many uses and roles. The ancient Hawaiians used koa for utensils and canoe building. It grows in nearly pure stands or in admixtures with ohia (*Metrosiderus polymorpha*) and provides the major ecosystem for many of Hawaii's indigenous bird species. Koa forests provide critical watershed recharge areas and important recreational opportunities (Walker, 1990; Whitesell, 1990). They provide a renewable economic resource for Hawaii's forestry industries. Koa's exceptionally beautiful and durable wood makes it the premier Hawaiian timber for furniture, cabinet work, face veneers, and woodcraft (Whitesell, 1990). Shehata (1993) valued the koa industry in Hawaii at $16 million annually, and Loudat (pers. comm., 1996) estimates it to exceed $50 million by the year 2000. Despite the high value of koa, production has not kept up with the demand.

Coverage of koa forest decreased from 455 to 41 thousand hectares between 1963 to 1991 (Nelson *et al.*, 1963; Brewbaker *et al.*, 1991). Stock volume of commercial koa decreased from 34.1 to 7.1 x 10^5 m^3 between 1960 to 1980 (Nelson *et al.*, 1963; Metcalf *et al.*, 1978). Dwindling of

the koa forest in Hawaii has been attributed to various reasons, namely, due to land clearing for ranch development, poor harvesting practices, animal destruction, insect and disease damage, and forest fires. Skolmen (1990) stressed that cattle are the main cause for the loss and failed recovery of koa forests. Efforts to increase the diminishing supply of koa have been minimal, possibly due in part to the conception that koa is slow-growing (NAS, 1979) and has a long production cycle, estimated between forty and sixty years. Concequently, the slow-growing koa make investment of planting trees not economical.

General Biology

Distribution

Koa (*Acacia koa* Gray) is found on all of the major islands of the Hawaiian chain--Kauai, Oahu, Molokai, Maui, Lanai, and Hawaii. The longitudes range between 154°W and 160°W, and the latitudes range between 19°N and 22°N. Koa grows on volcanic soils of all geologic ages from an elevation of 100 m on Oahu to 2300 m on Hawaii. It grows in environments with a mean annual rainfall ranging from 600 to 5000 mm and with mean annual temperatures ranging from 9 to 23°C (Whitesell, 1990). Koa obviously thrives at the elevation between 800-1600 m to create a "koa belt" around Hawaii's islands.

Taxonomy and Evolution Background

Acacia koa Gray belongs to the thornless, phyllodinous group of the *acacia* subgenus *Heterophyllum* (Guinet and Vassal, 1978; Whitesell, 1990; Wagner *et al.*, 1990). Koa populations display a high degree of morphological variation of traits, e.g, tree forms, phyllode size and shape, seed weight and shape, and flowering habits. Several varieties of *Acacia koa* Gray were recognized and proposed by Rock (1920), Lamoureux (1971), and St. John (1979). These koa varieties include: *Acacia koa* var. *koa*, which grows on all six major islands; *Acacia koa* var. *waianaeensis*, found only on Oahu, and most commonly on the Waianae Range; *Acacia koa* var. *lanaiensis*, which grows only on Lanai; and *Acacia koa* var. *latifolia* (syn. *Acacia koa* var. *hawaiiensis* Rock), which grows on the island of Hawaii at higher elevation rain forest.

Two related native Hawaiian *Acacia* species, *Acacia kauaiensis* and *Acacia koaia*, were recognized by Hillebrand (1888), but later disputed (Wagner *et al.*, 1990). *Acacia kauaiensis* grows in western Kauai and has distinguishing sepals, petals, inflorescence, and a round seed shape (Hillebrand, 1888; Lamoureux, 1971; St. John, 1979). *Acacia koaia* grows on the leeward side below 1000 m of Molokai, Maui, and Hawaii, and has a small tree bushy stature, hard wood, and longitudinal seed orientation in the pod (Rock,

1920; Lamoureux, 1971). St. John (1979) reported that *Acacia koaia* also could be found on Kauai and Lanai.

Cytological studies reveal that *Acacia koa* is a tetraploid with chromosome number 2n=52 (Atchison 1948; Carr, 1978), one of a very few in this large genus (c. 1200 spp.) with such chromosome numbers (Guinet and Vassal, 1978). Three other *Acacia* species outside Hawaii may be related to koa (Whitesell, 1990). They are *Acacia heterophylla* in Reunion and Mauritius island, *Acacia melanoxylon* in Australia, and *Acacia simplicifolia* in Samoa, New Hebrides, New Caledonia, and Fiji. Isozyme analysis revealed that *Acacia simplicifolia* from Fiji had the closest band pattern with *A. koa* among 13 *Acacia* species (Aradhya, pers. comm., 1996).

The recent publication of a manual of Hawaiian plants recognizes only one Hawaiian *Acacia* species, *A. koa* (Wagner *et al.*, 1990). Geesink and Kornet (1990) even argued that *Acacia koa* and *Acacia heterophylla* could be treated as one species as was done for another legume species *Rhynchosia rothii* occurring in Burma and East Java, even though the Mascarene and Hawaiian Islands are 18,000 km apart from each other. The Hawaiian koa was once identified as *Acacia heterophylla* or that species was considered its closest relative by Bentham (1842). Gray (1854) distinguished the two as different species. Vassal (1969) and St. John (1979)

reconfirmed Gray's distinction on the basis of seedling growth characters and better taxonomic specimens.

The origin of *Acacia* Subgen. *Heterophyllum* is not known. Pedley (1975) considered that *Heteropyllum* was fundamentally an Australian group including Tasmania and New Guinea. Guinet and Vassal (1978) reported that eighteen species with plurinerved phyllodes were also located mostly in the Pacific islands, reaching Madagascar and the Mascarene Islands to the west and extending as far east as Hawaii. Ten of them were endemic and absent from Australia. Guinet and Vassal (1978) and Pedley (1975) suggested that Australia or the northern part of Australia was a secondary center of differentiation of these species.

Degener (1945) and other authors suggest koa bears sickle-shaped phyllodes, as an evolutionary result of natural selection. The Australia habitat for koa changed over millions of years under alternating extreme environmental conditions (first wet and fertile, then dry and infertile). Koa ancestor had compound leaves as most legumes do. In order to thrive under these changed conditions, acacias with phyllodes (expended petioles) were favored by natural selection. Walters and Bartholomew (1984; 1990) reported that koa phyllodes transpire only one-fifth as much as do the true leaves after dark. Hansen (1986) and Hansen and Steig (1993) reported that koa phyllodes possessed higher water-use efficiency and lower

internal carbon dioxide concentration than juvenile leaves. Their findings also support Degener's hypothesis that the acacia phyllode, as those of eucalypts, are the result of a natural evolution process.

Phenology

Lanner (1965) studied koa flowering and fruiting on the slopes of Mauna Loa (Island of Hawaii) and reported that the major flowering flush was correlated with the elevation gradient. The flush moved upslope at about 100 meters of elevation per week and lasted about two months. Usually, flowering started at 1300 meters in December, and ended at 2100 meters elevation by May and June. Rosa (1994) reported that koa could flower almost any time of year, depending upon local weather conditions.

Variation in first-time flowering among provenances or individuals has been observed in the present study. Two provenances from Hawaii flowered two years after planting at Hamakua, Hawaii. Atchison (1948) and Skolmen and Fujii (1980) also reported that seedlings were observed in flower and fruit at two and three years, respectively.

Koa populations vary in their seed maturation times. Lanner (1965) observed that one of the recorded koa pods took about two and a half months to reach its full length and about seven months to mature. However, most of koa

seeds took about four to six months to mature from anthesis depending on location and weather conditions (Rosa, 1994).

Breeding Systems and Seed Production

Tree breeding program cannot make much progress without information on the reproductive biology of the species involved, and little is known about the pollination of tropical tree species (Bawa, 1976). Relatively few legumes species are self-compatible, and most of these are polyploids. Arroyo (1981) reported that 75% percent out of twelve tested acacia species and 67% out of 27 tested Mimosoideae species were self-incompatible. The same results were also reported by Brewbaker (1984) that among the tested nitrogen-fixing tree species 17 of the 21 studied genera and 47 of the 63 species were self-incompatible.

The breeding system of koa is a matter of controversy. Early preliminary study indicated that *Acacia koa* was cross-pollinated by insects (Lanner, 1965). The fruit-set level (percentage of seed pods from mature flowering heads) was very low (2.7%) from enclosed branches, compared with about 15% from open-pollinated branches. Lack of fruit set with exclusion of insects cannot be considered conclusive evidence for cross-pollination, since insects may also be important for self-pollination of dichogamous plants.

Brewbaker (1977) reported that koa is basically self-fertile based on the limited hand pollination. Koa flowers

are highly dichogamous, with anthers dehiscing about five days prior to full uncoiling of the style and stigma. This dichogamy effectively prevents selfing of any individual floret or flowering head. However, it would not prevent pollination among heads on the same tree.

Koa flowers are visited frequently by many insects such as bees and flies. Koa pollen is shed as a 16-cell polyad with a diameter of 80 micrometers (Brewbaker, 1977). Such cells are comparatively heavy, and do not remain airborne. It is possible that koa seeds from a single pod may be a full-sib progeny, as occurs in several *Acacia spp.* where only one polyad fits the style and germinates (Moran *et al.*, 1991).

Whitesell (1990) reported that seed production in koa is quite abundant, but seed yield varies year to year. An individual tree can produce numerous seed pods which contain between 6 to 12 seeds. Koa seed pods dehisce while on the tree or fall to the ground unopened, where they either dehisce or disintegrate. Usually, weevils and koa worms of many insect species and birds destroy a large proportion of the seeds in pods. Seeds could survive in the ground for decades. Seed weight or size varies from population to population with a range from 2,650 to 8,150 seeds per kilogram (Whitesell, 1990).

Uses

Wood Products

Koa is the most valuable lumber tree in Hawaii and is used to make various wood products. Koa wood is highly valued for furniture and crafts by consumers in the world, especially for its unique grain, varied color and workability (Whitesell, 1990). Koa wood is widely used for cabinet work, picture frames, bowls, and face veneers. Once koa was carved by Hawaiians into such things as war canoes, paddles, surfboards, and calabashes.

Land Reclamation and Watershed Protection

Koa is an important species for land reclamation, watershed protection, and wildlife habitat (Whitesell, 1990). Koa plantings in Hawaii were made initially to provide vegetative cover on sites degraded by decades of intensive grazing and koa harvesting (Judd, 1916; 1919; Whitesell, 1990). However, koa did not compete with other introduced species on these deteriorated sites.

Forage

Cattle, sheep and pigs browse koa foliage aggressively, especially the juvenile leaves. Niang *et al*. (1995) reported that *Acacia koa* and *Acacia koaia* grew better than *L. diversifolia* in acid soil on the highlands of Rwanda and were the most palatable among eight other legume tree

species. Koa is of little use as a forage tree due to its poor regrowth after coppicing or browsing.

Management and Production

Establishment

Natural regeneration has been the preferred method for koa plantation establishment, as it is least costly. The most common way to recover a koa forest has been to exclude animals and let koa regenerate from seedlings and rootsprouts. Baldwin and Fagerlund (1943) showed that successful and vigorous koa reproduction could be established when cattle were excluded. Several successful examples of this approach were described by Skolmen (1990). An excellent example of koa forest recovery in the state was at the Humuula tract area of Keanakolu, Hawaii. A forest reserve fence was built in 1922, resulting in the exclusion of cattle from the area. A stand of large koa trees of the same age can now be seen. Another example is at Volcanoes National Park, which through the exclusion of cattle and feral animals now accommodates a healthy native forest. About seventy years ago, the National Park Land area was characteristic of today's Keauhou Ranch, which contains only a few remnant old koa and ohia.

Another approach to reforest koa was conducted by the Bishop Estate at Kilauea-Keauhou and Honaunau during the past three decades (Skolmen, 1990). The process involved

removing the vegetation and scarifying the surface soil to cause koa seeds reserved in the ground to germinate and to grow with limited competition and abundant light.

Direct seeding of koa with moderate success on prepared seed spots was reported by Bryan (1929) and Carlson and Bryan (1959). In two trials, the direct seeding spots method had four times higher stocking than a broadcast sowing method on the island of Maui, whereas no difference in the percentage of stocked spots or of height growth was observed on the Island of Hawaii.

Transplanting is another approach for koa plantations. Koa seedlings are raised in a greenhouse and transplanted in the field. An intensive care for the first half year is necessary for a good establishment, mainly concentrating on providing nutrients and suppressing weeds. Early successful transplanting of koa seedlings was described by Judd (1916, 1919). Skolmen (1990) emphasized that seed source was very important for the success of reforestation.

Other methods of providing material for koa reforestation include tissue culture and vegetative propagation. Very limited success of these methods was reported by Skolmen (1986), and large-scale application of these methods have not yet been attempted.

Nutrient and microorganism requirements

Very limited research has been published on koa nutritional requirements and koa-microorganism interaction. Scowcroft and Stein (1986) reported that fertilizer yielded limited response in a stagnated twelve-year koa stands on Haleakala, Maui and suggested fertilization be applied before canopy closure. Silva (1995, unpubl.) showed that application of organic and inorganic fertilizers enhanced tree establishment and early growth. Miyasaka *et al.*, (1993) reported that application of mycorrhizas was beneficial in enhancing seedling growth in greenhouse. Inoculation of *Rhizobium* (Group C) at seedling stages was advised by NifTAL (Nitrogen Fixation by Tropical Agricultural Legumes) and widely practiced in the State.

Insect and Disease Problems

More than a hundred insect and pathogen species associated with koa have been reported, however, most appear to have little affect on koa (Jones *et al.*, 1991). Insect species such as the koa seed weevil, the koa moth, and the koa psyllid cause damage to koa. The weevil and its larva damage koa seeds, while the moth and psyllid cause defoliation of koa trees. Stein (1983a) stated that below 1500 m elevation, most seed damage was caused by the koa weevil (*Araecerus levipennis*) and the bruchid seed weevil (*Stator sp.*). Above 1500 m elevation, seed damage was

basically caused by koa seed worms (*Crytophlebia illepida*). The koa moth caused serious defoliation and some tree mortality on Maui in 1977 (Stein, 1983b).

Various diseases have been reported on koa seedlings and mature koa trees (Skolmen, 1990; Jones *et al.*, 1991). Recently, the most concerned disease is 'koa blight', which is lethal to koa (Jones *et al.*, 1991). The cause of this disease has not yet been identified, but root rot disease to fungi such as *Fusarium spp.* is suspected (Gardner, pers. commun., 1996). The koa rusts are caused by four rust fungi (*Uromyces koae, U. digitatus, Endorraecium acaciae, and E. hawaiiense*), which are common on the Island of Hawaii. Sometimes the rust cause severe damage to koa, especially koa seedlings of new plantations (Hodges and Gardner, 1984; Skolmen, 1990).

CHAPTER NINE

VARIATION OF *ACACIA KOA* SEED AND SEEDLING CHARACTERISTICS

Abstract

A total of 334 *Acacia koa* accessions from across the Hawaiian Islands: Kauai, Oahu, Lanai, Maui, and Hawaii, were collected and documented. Most of the accessions were derived from a single tree. Nursery evaluations were conducted to study variations in seed weight, width, and length, and in germination and greenhouse seedling growth, and to evaluate correlations among seed and seedling growth parameters. Seeds from Kauai and Hawaii were larger and significantly heavier and wider ($P<0.05$) than those from Oahu and Maui. Seeds from Hawaii were significantly ($P<0.05$) longer than those from the other islands. Average germination rate varied significantly from 15 to 84% for seeds with different storage periods of one to seventeen years. Two distinct seed types, a round-shaped and an oblong-shaped, were found among the koa collections. Accessions of the round-shaped seeds from Kauai grew significantly slower in the greenhouse than those of the oblong-shaped seeds from Kauai, Oahu, Maui and Hawaii. Variations of seed weight, seed shape, seed color, and seedling growth rate were found to be essentially genetic in origin, because a large portion of variation for these

traits was attributed to the difference of provenances. Significant ($P<0.05$) negative correlation between seed parameters (seed weight and seed width) and seedling height at eleven weeks were found. The information obtained from the present study could be used for taxonomic classification of the native Hawaiian *Acacia* species.

Introduction

Germplasm collection is a prerequisite to advanced tree improvement programs. The main purposes of germplasm collection are evaluation, conservation, and utilization of available genetic resources (Palmberg, 1985). Evaluation basically involves provenance testing but may also include testing of seed and seedling growth traits.

Variations of seed and seedling characters are important for selection and classification of the species. Past studies have shown that differences in seed weight affect seedling growth. Shiv Kumar and Banerjee (1986) reported that *Acacia nilotica* provenances with heavier seed weight yielded better seedling plants, and that provenances of small seed size took longer to germinate. Bagchi *et al.* (1990) reported that traits of seed width, length, and thickness among *Acacia nilotica* provenances were highly heritable and correlated, and suggested that selection of larger seeds could improve seedling vigor and reduce nursery operation costs. Patterns of variations among seedling

traits were also used to verify the classification and to group the provenances of other *Acacia* species (Vassal, 1969; Bleakley and Matheson, 1992).

Koa seeds have been collected widely for reforestation by the USDA Forestry Service and the Department of Land and Natural Resources, State of Hawaii. Variability of seedling growth was observed (Goo, pers. comm., 1994). Because of the variability of growth associated with provenances, it is critical to determine whether patterns of seed characteristics are correlated with early seedling growth of *Acacia koa*.

The objectives of the present study were to collect *Acacia koa* germplasm in the Hawaiian islands, to study variation of seed and seedling growth traits among *Acacia koa* population, and evaluate correlations among these parameters.

Materials and Methods

Germplasm collection

Acacia koa germplasm was collected from 1991 to 1995, in areas which represent the natural range of *Acacia koa*. Seeds were also collected from trees in koa plantations and from private properties.

Accessions were collected as families from individual trees. In order to assess the natural genetic variation of koa, several individual trees, usually separated by distances of at least 100 meters, were collected in a confined area or provenance. A provenance was defined geologically, *i.e.*, koa trees found on different mountain ridges were given a separate provenance name. A tree pruner was used to collect koa pods. In cases where pods were inaccessible due to extreme tree height, a composite collection was taken, in which pods were often sampled from the ground. Seed sources were documented and stored at the University of Hawaii Seed Facility at 15°C.

Nursery management

Procedures of raising koa seedlings were followed using the techniques adapted from the *Leucaena* breeding program at the University of Hawaii. Seeds were scarified by hand-nicking, and sown into dibble tubes containing a soil-less medium, consisting of a 3:1 peat-perlite mix. Seedlings were grown in a greenhouse at the Waimanalo Research Station

on Oahu, Hawaii, in the middle of February. Seedlings were watered three times daily and inoculated with group C rhizobium at the fourth week after sowing provided by Department of Agronomy and Soil Science, UH. Fertilization with foliar 19-19-19 (N_2-P_2O_5-K_2O) was applied twice every week from the fifth week. Seedlings were transplanted twelve weeks after sowing.

Damping off diseases were controlled by minimizing watering levels and by application of Benlate, a fungicide which was sprayed weekly from the third week to the eighth week after sowing.

In order to enhance survival rate after transplanting, hardening of seedlings was required. Two-and-a-half month old koa seedlings were moved out of the greenhouse for hardening. Irrigation and fertilization applications were adjusted to compensate for the higher transpiration rates of the plants during hardening.

Evaluation of koa seed and seedling traits

Evaluations included: average seed weight (based on a random sample size of 100 seeds), seed width, seed length, germination rate, and seedling growth rate.

A total of 294 koa collections from five Hawaiian islands were weighed (Appendix B). Ninety-two koa collections from Oahu, Kauai, Maui, and Hawaii were selected for the seed parameter measurement (Appendix C). Five

random seeds were measured for width and length to the nearest millimeter.

Of the collected koa populations, 238 koa collections with various storage ages from 1 to 17 years were tested for germination rate. Percentage of germination was recorded two weeks after sowing. Data from several years' tests (1991-1995) were combined to observe the decline of the germination rate after years of seed storage.

Eighty-one koa collections from Oahu, Kauai, Maui, and Hawaii were studied for seedling growth (Appendix D). Seedling heights from five random seedlings of each collection were monitored after two weeks of sowing until transplanting. Leaflet colors (purple and green) were also recorded.

All data were analyzed using a spreadsheet approach (Brewbaker, 1993) and GLM of SAS (SAS, 1990). Significant and highly significant differences were at the $P < 0.05$ and the $P < 0.01$ levels of probability, respectively.

Results

Germplasm collection

By May 1995, a total of 334 koa accessions have been collected and documented (Table 9.1 and Appendix B). This collection includes 116 accessions collected by Dr. Brewbaker during the 1960s and 44 accessions contributed by the USDA Forest Service in Hilo. These collections were mainly from Kauai, Oahu, Maui, and Hawaii islands. Among these collections, 272 were derived from single trees and 63 were composites. Seeds of these collections were stored at the UH HFSF, Waimanalo Research Station, Oahu. A wide range of ecological conditions are represented in these provenance collections, with elevation ranging from 100 to 2300 meters above sea level and mean annual precipitation from 600 to 5000 mm. Collection areas among the Hawaiian islands are shown in Figure 9.1.

Table 9.1. *Acacia koa* collections from the Hawaiian islands through the years.

	ISLANDS						
YEAR	Oahu	Kauai	Lanai	Maui	Hawaii	NO.†	TOTAL
1991 ‡	66	20	5	17	8	4	116
1993	5				7	6	12
1994		42			18		60
1995	2	93		17	34	34	146
Total	**73**	**155**	**5**	**34**	**67**	**44**	**334**

† : Number of collections contributed by USDA Forest Service
‡ : Collections made in the 1960's by Dr. Brewbaker

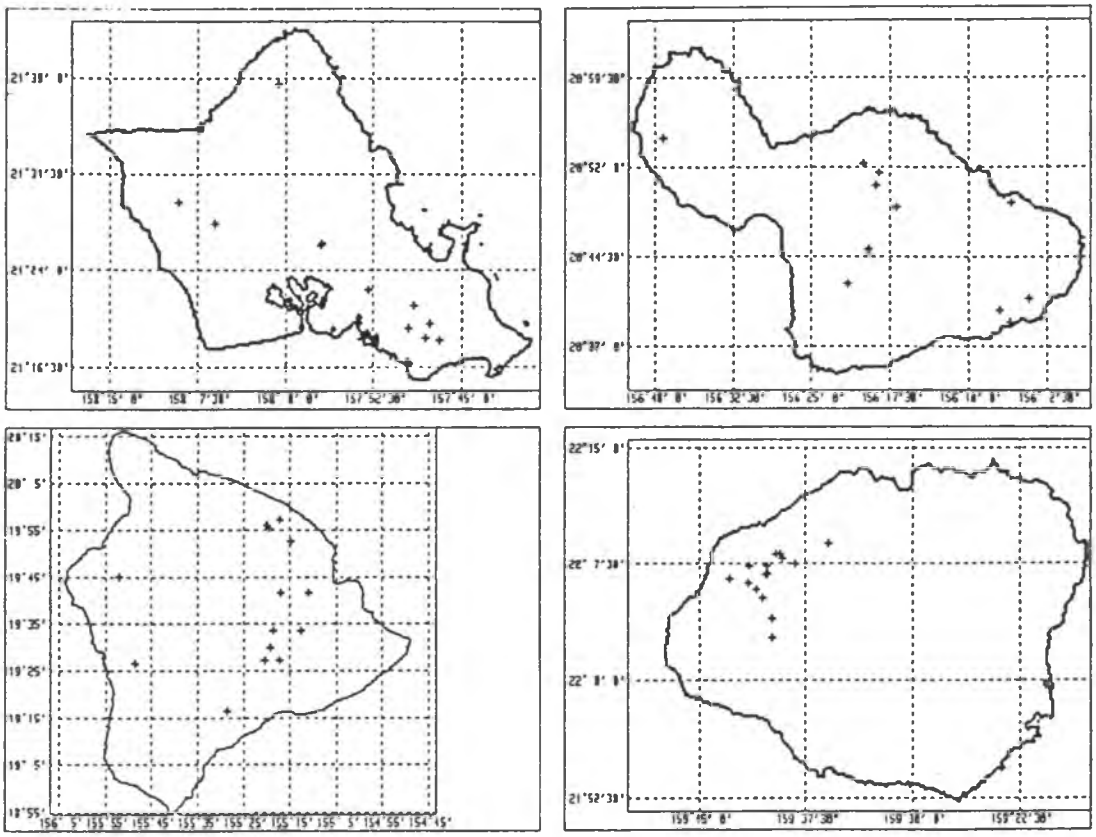


Figure 9.1. *Acacia koa* collection sites from the Hawaiian Islands.

Variations of seed weight, length, width, and L/W ratio

Great variations in seed weight, size and morphology from different koa collections were observed (Figure 9.2 and Appendix B).

Seed weight of *Acacia koa* averaged 8.5 g per 100 seeds and ranged from 1.7 g (about 58800 seeds/Kg) of 2W2-2 (Waimea canyon drive) from Kauai to 17.7 g (about 5650 seeds/Kg) of 6KA5C (Kukaiau) from the Island of Hawaii (Table 9.2). Seeds from Kauai and Hawaii were significantly heavier than those from Oahu, Lanai, and Maui.

Table 9.2. Average seed weight of *Acacia koa* collections from five Hawaiian islands.

Source	No. of coll.	Weight(avg.) 100 seeds	SD	Range
		-g-	-g-	-g-
Oahu	57	4.8	1.2	2.2- 7.5
Kauai	146	9.5	2.8	1.7-15.6
Lanai	5	5.9	1.3	3.9- 7.7
Maui	28	5.6	1.8	2.2- 9.8
Hawaii	58	11.2	3.5	4.6-17.7
Total	294	8.5	3.5	1.7-17.7

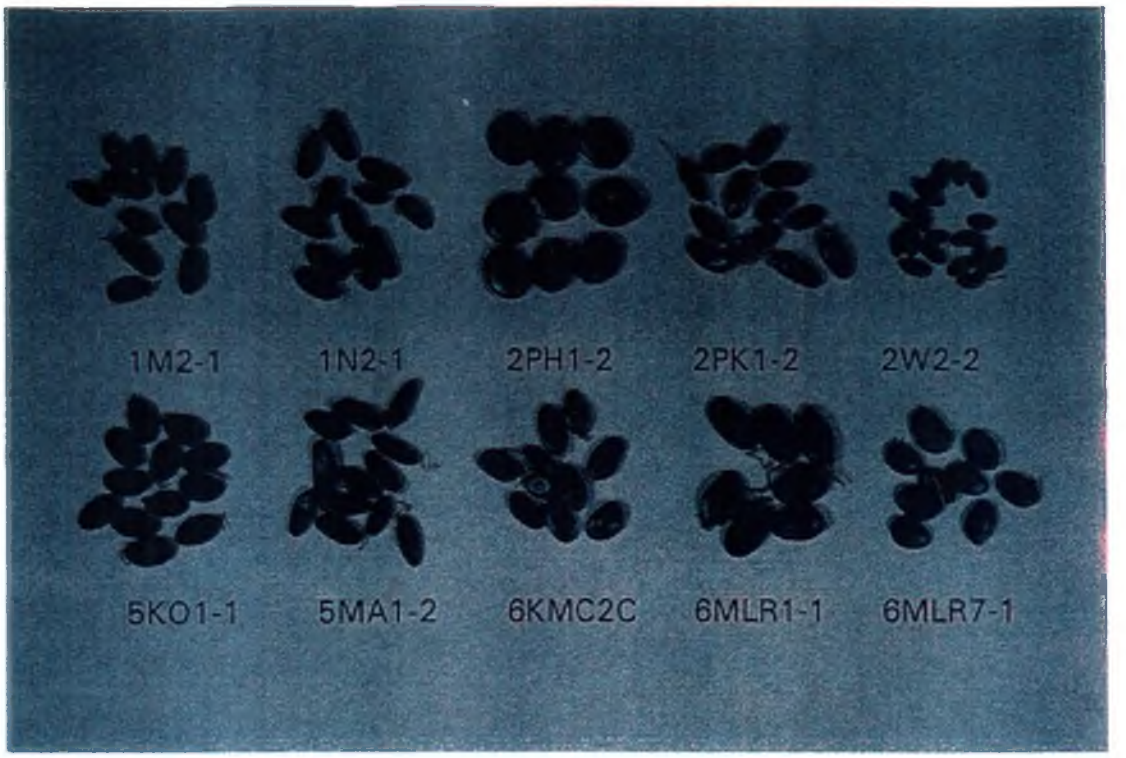


Figure 9.2. Various *Acacia koa* seeds collected from the Hawaiian Islands. 1M2-1: Makiki, Oahu; 1N2-1: Nuuanu, Oahu; 2PH1-2: Puu Hinahina, Kauai; 2PK1-2: Puu Ka Pele, Kauai; 2W2-2: Waimea Canyon Drive, Kauai; 5KO1-1: Kokoma Rd., Maui; 5MA1-2: Hamana Rd., Maui; 6KMC2C: Kilauea Military Camp., Hawaii; 6MLR1-1 and 6MLR7-1: Mauna Loa Rd., Hawaii.

Average seed width (W), length (L), and L/W ratio of 95 koa collections from four Hawaiian islands are presented in Table 9.3 and Appendix C. Koa seed width averaged 6.4 mm and ranged from 3.4 mm of 2W2-2 to 9.3 mm of 2PH1-2. Koa seed length averaged 10.2 mm and ranged from 6.7 mm of 2W2-2 to 13.4 mm of 6KA5C. Seed L/W ratio averaged 1.7 and ranged from 1.1 of 2PH1-1 to 2.5 of 6KL1C. Overall, seeds from Kauai and Hawaii were significantly ($P<0.05$) wider than those from Oahu and Maui. Seeds from Hawaii were significantly ($P<0.05$) longer than those from the other islands.

Table 9.3. Average seed width, length, and ratio of length/width of 95 *Acacia koa* collections from Oahu, Kauai, Maui, and Hawaii.

Source	No. of coll.	Seed width	Seed length	Ratio (L/W)
		-mm-	-mm-	
Oahu	4	4.8 (0.3)†	8.9 (0.6)†	1.9 (0.1)†
Kauai	49	6.7 (1.5)	9.9 (1.1)	1.5 (0.4)
R‡	34	7.5 (0.8)	9.8 (1.0)	1.3 (0.1)
O§	15	4.8 (0.5)	10.0 (1.3)	2.1 (0.2)
Maui	8	5.2 (0.5)	10.1 (1.1)	2.0 (0.3)
Hawaii	34	6.4 (1.1)	11.3 (1.4)	1.7 (0.3)
Total	95			
Means		6.4	10.2	1.7
LSD 0.05		0.6	0.9	

†: Numbers in parenthesis are standard deviations
‡: R represents the round-shaped seed type
§: O represents the oblong-shaped seed type

Two distinct seed types, round and oblong-shaped seeds, were found among the koa collections, based on visual assessment. The round seeds averaged 7.5 mm in width, and were significantly wider than the oblong-shaped seeds among 49 Kauai collections (Table 9.3). However, no difference was found for seed length between the two seed types. When using the ratio of L/W as a criterion, the round seed had a L/W ratio below 1.5. These seeds were predominantly from Kauai, with a few exceptions from Hawaii. The oblong-shaped seeds were found on five Hawaiian islands. Some collections from Hawaii and Kauai showed an intermediate seed shape with a L/W ratio close to 1.5.

Distribution of the oblong-shaped seeds on western Kauai are limited to certain areas compared with the trees with the round seeds. Trees with round seeds are widely distributed on the west of Kauai. Trees with the oblong-shaped seeds could only be found along Waimea Canyon Drive, Kokee Road, Kaaweiki Ridge, and Kumuwela trial, and are presumed to trace to foreatry plantations in 1930s.

Analysis of variance and variance components of these seed variables are presented in Table 9.4. Seed width and seed length were significantly different among seed sources. In these two cases, more than 67% of the variation observed was due to the provenances.

Table 9.4. Analysis of variance and variance components for seed width, length and ratio of Length/Width (L/W) of 95 accessions of *Acacia koa* from Oahu, Kauai, Maui, and Hawaii.

Sources	df	Seed width MS	F	Seed length MS	F	L/W ratio MS	F
Total	474	1.86		1.60		0.14	
Entry	94	8.33	**	5.93	**	0.02	NS†
Error	380	0.25		0.54		0.17	

Variance components and its percentages

	Comp.	%	Comp.	%
Entry	1.62	87	1.08	67
Error	0.25	13	0.54	33

**: Significantly different at $P < 0.01$
†: Not significantly different

Seed germination

Although the germination rate varied among accessions of the same storage age, average germination rate varied significantly between seeds of different storage ages (Table 9.5). For example, 17-year old seeds had only a 15% germination rate, whereas one year old seeds had a rate of 84% germination. These results show that germination rate is affected by the storage age, but the seeds that are stored for many years can still remain viable.

Table 9.5. Average germination rate of *Acacia koa* seeds after years of storage.

Years of Storage	No. of coll.	Germ. Rate (%)	SD	Range (%)
One	117	84	12.7	40-100
Two	7	74	15.8	42-88
Five	12	68	26.1	0-93
Thirteen	23	15	14.0	0-50
Seventeen	79	15	20.0	0-80

Seedling height

Average seedling heights from two to eleven weeks after sowing are summarized in Appendix D for 81 collections from Oahu, Kauai, Maui, and Hawaii. Height of eleven-week old seedlings averaged 24.0 cm ranging from 11.4 to 39.9 cm.

The analysis of variance of seedling heights are presented in Table 9.6. Seedling heights were highly significant between collections after two weeks growth in

Table 9.6. Analysis of variance and variance components for seedling height in 11 weeks of 80 *Acacia koa* collections from Oahu, Kauai, Maui, and Hawaii.

Sources	df	Two weeks MS	F	Five weeks MS	F	Eleven weeks MS	F
Total	399	1.0		8.0		86.5	
Entry	79	2.9	**	34.5	**	643.9	**
R vs. O†	1	92.2	**	1535.3	**	57295.2	**
Error	320	0.5		1.5		25.5	

Variance components and its percentages

	Comp.	%	Comp.	%	Comp.	%
Entry	0.5	49	6.6	81	61.8	71
Error	0.5	51	1.5	19	25.5	29

**: Significantly different at $P < 0.01$
† : Orthognal comparison between the round-shaped seed (R) and the oblong-shaped seed (O) collections

the greenhouse. Variance components showed that more than 70% of the variation in seedling height after four weeks growth was due to the seed source. Highly significant difference for seedling growth between the round-shaped seed and the oblong-shaped seed collection was observed two weeks after sowing.

Average seedling growth rate of accessions from the different islands are presented in Figure 9.3. Accessions of the round-shaped seeds from Kauai grew highly significantly slower than those of the oblong-shaped seeds from Kauai, Oahu, Maui and Hawaii.

Three groups of seedings were observed among these collections based on the color of the seedling stem, the leaf rib, and the leaflets. The first group is from western Kauai and consisted of round-shaped seed, green seedling stems, leaf rib, and leaflets, and slow growth. The second group is distributed on Oahu, Maui, and some areas of Kauai with oblong-shaped seeds, purple seedling stem and leaf ribs, dark green leaflets, and normal seedling growth. The koa within this second group from Kauai was found only at a few sites: Waimea Canyon Drive; Kokee Road; Kaaweiki Ridge; and Kumuwela trial. The third group is from the island of Hawaii with both round- and oblong-shaped seeds, green seedling color with reddish stems, and seedlings of normal growth.

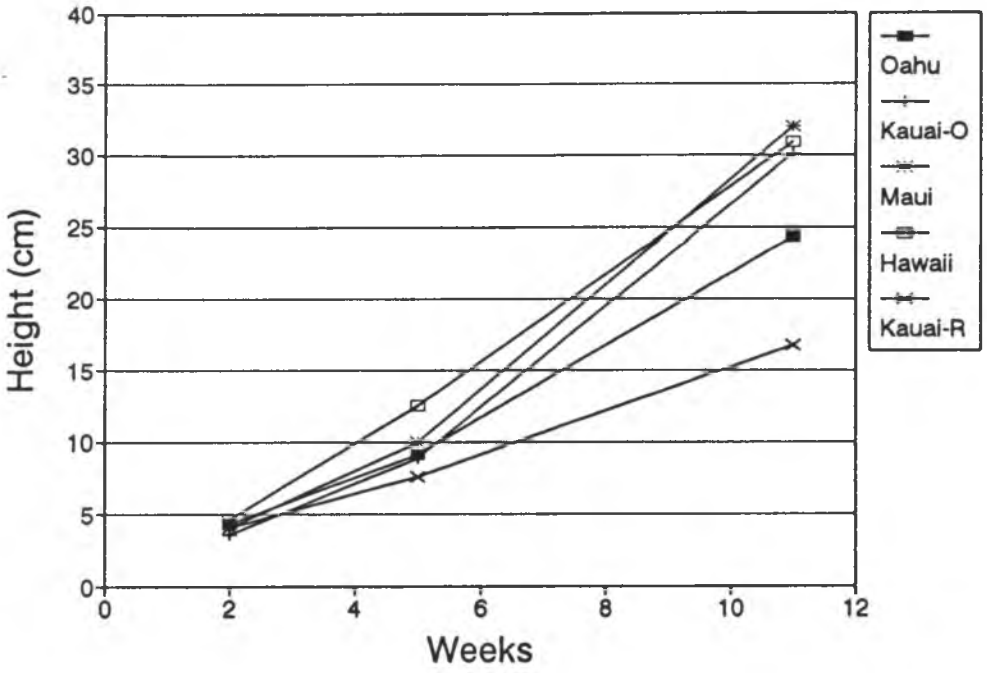


Figure 9.3. Average *Acacia koa* seedling growth rate of koa collections from the Hawaiian Islands. Kauai-O: Seedlings from Kauai with the oblong-shaped seed; Kauai-R: Seedlings from Kauai with the round-shaped seed.

Correlations among seed size and seedling growth traits

Correlation coefficients between seed size and seedling growth parameters are presented in Table 9.7. No significant correlation between seed parameters and seedling height at two weeks and five weeks were observed. However, significant negative correlation between both average seed weight and seed width was found with seedling height at eleven weeks. Within the seed parameters of seed weight, seed width, seed length, and L/W ratio, significant correlations were found among them with the exception between seed length and L/W ratio. Within the seedling growth parameters of two-, five-, and eleven-week growth, significant correlations were found among the two- and five-week growth and the five- and eleven-week growth but not between the two- and eleven-week heights.

Table 9.7. Correlation coefficients (P<0.05) between seed and seedling growth parameters measured for 90 *Acacia koa* accessions.

	Seed			Height at weeks		
Parameters	Width	Length	L/W	2	5	11
100 seed weight	0.82	0.59	-0.55	NS†	NS	-0.37
Seed Width		0.28	-0.87	NS	NS	-0.64
Seed Length			NS	NS	0.33	0.23
Seed L/W				NS	0.26	0.71
Height at 2 weeks					0.60	NS
Height at 5 weeks						0.68

†: Not significantly different

Discussion

Significant variation in seed weight, seed shape, seed width and length, seedling growth, and seedling color is not unique to *Acacia koa*. Significant variation in such seed and seedling traits was also reported in *A. mangium* (Salazar, 1989; Bleakley and Matheson, 1992) and *A. nilotica* (Bagchi et al., 1990; Krishan and Toky, 1996). The variation reported here in Hawaiian *Acacia* species was essentially genetic in origin and may be useful in tree improvement and classification of these species.

One assumption of this experiment was that seed size is correlated with early seedling growth. The results of this experiment, however, showed that in some cases seedlings from small seeds had faster seedling growth than seedlings from the findings of large seeds. This finding is not in accordance with the previous studies (Hendrix *et al.*, 1991; Sorensson *et al.*, 1994), in which seed weight was positively correlated with early seed growth. The contrast was partly associated with the round-shaped seed collections from Kauai incorporated in the experiment. The round seed accessions from Kauai always grew slowly regardless of seed weight. When the round-shaped seed collections were removed from the analysis, a significant relationship of seed size was found with five week-old seedling height, but not with two and eleven week-old seedling height. Variations of seed and seedling growth traits are useful in selection of

provenances with fast seedling growth for nursery management (Shiv Kumar and Banerjee, 1986) provided that significant correlations exist. However, the findings of correlations from the present experiment are not conclusive. The results may be due to the different genotype or species involved in the study.

Variations observed in the present study are useful for taxonomic classification of the species. Due to the different number of collections used to determine variation of seed and seedling parameters, cluster analysis of similarity (or groups) was not performed. However, three koa groups could be distinguished according to their seed shape, seedling growth rate, and seedling color among the evaluated koa populations. Taxonomic classification of koa on the Hawaiian islands is somewhat controversial. The native Hawaiian *Acacia* species was first recognized as *A. heterophylla,* which is also found in the Mascarene Islands in the India ocean (Bentham, 1842), then distinguished as *A. koa* from *A. heterophylla* (Gray, 1854). Three major *Acacia* species: *A. kauaiensis*, *A. koa*, and *A. koaia*, were recognized later by the early botanists (Hillebrand, 1888; Rock, 1920; Lamoureux, 1971; St. John, 1979). However, the most recent publication of Hawaiian islands plants recognized only one Hawaiian *acacia* species, *Acacia koa* (Wagner *et al.,* 1990). The results of the present study support the taxonomy of Hillebrand (1888) with additional

evidence of the seed shape and seedling growth characters for the treatment of *A. kauaiensis* (Table 9.8). The round-shaped seed for *A. kauaiensis* was described by Lamoureux (1971).

Table 9.8. Traits distinguishing koa populations on Kauai

Traits	*A. koa* type	*A. kauaiensis* type
Seed Shape	Oblong	Round
Seed width	4.8 mm	7.5 mm
Seed length	10.0 mm	9.8 mm
L/W ratio	2.1	1.3
Seedling Color	Purple	Green-yellow
Seedling Height		
in 3 months	16.0 cm	35.0 cm
in 12 months	110.0 cm	250.0 cm
Phyllode size	Narrow to medium	Variable
Flowering seasons	Summer	Winter
Location	Along highways	Widely distributed

Variation observed and groups suggested may also clear some confusion for taxonomists due to the movement of koa seeds for reforestation. Degener and Degener (1971) reported that taxonomy problems for Hawaiian *acacia* arose due to the hybridized taxa caused by the practice of reforestation. Foresters in Hawaii used koa seeds collected from all islands for reforestation in 1930s. For example of Kauai acacia populations, natural hybridization between the round-shaped seed population and the oblong-shaped seed

population may not be possible because of difference in flowering times. The round seed population flowers during the summer from May to August, while the oblong-shaped seed population flowers from November to February. The distribution of the oblong-shaped seed population along the main road also suggested that this koa population might have been introduced from Oahu or Maui for reforestation in the 1930s.

Koa grouping suggested in the present study seems to have some geographic partitioning in addition to morphological similarities. This suggests there may be some evolutionary significance to the groups. For this reason, it may be expected that other traits such as phyllode size, wood characters, and even molecular markers may also tend to be classified into the same groups.

There were some difficulties in collecting koa germplasm. Pod and seed development of *Acacia koa* appeared to be heavily influenced by climate, seed weevils (*Araecerus levipennis* and *Stator sp.*), and seed worms (*Crytophlebia illepida*). Healthy pods collected on Hawaii, Maui, and Oahu during a certain season of one year were severely damaged when collected during the same season in another year. Because of this, it was difficult to collect koa germplasm on a consistent basis with respect to seasons. Only on the island of Kauai was there apparently a regular time to

collect seeds, which occurred during the months of December and January.

There were also some difficulties during the period of seedling growth in the greenhouse. The first was the outstanding seedlings which were pushed out of the medium or seed coat by its own embryo growth after three or four days of sowing. The problem was related to the physical scarification at the opposite side of the seed embryo. The solution was to put the outstanding seedlings back into the growing medium. Otherwise, these seedlings would have dessicated and died, since the roots were not in contact with moisture. Because there are variations of koa seed size and ages and consequently germination, this procedure lasted for one week.

The second problem was seedling damping off disease. This occurred from the third week after sowing. The disease is related to wetness or moisture and temperature of the environment, and could be controlled by reducing moisture in the seedling bed and spraying fungicide. The third problem was seed coat attachment to the cotyledon during germination, which was related to physical seed scarification. This was solved by manually taking seed coats off the seedlings when necessary.

CHAPTER TEN

GROWTH PERFORMANCE OF *ACACIA KOA* PROGENY TRIALS

Abstract

A series of six *Acacia koa* progeny trials and one koa/leucaena mixture trial were set up at Hamakua, Hawaii and Maunawili, Oahu to evaluate *Acacia koa* germplasm. A total of 178 accessions were grown and tested for the variables of survival rate, tree height, DBH, phyllode development rate, and tree form. Significant ($P<0.05$) differences for these traits were observed.

Acacia koa was shown to be a fast-growing tree during early growth stages. Koa trees reached 3 m after one year's growth and 6 m after two and half years, with superior progenies to 7.0 m. Accessions from Kauai and Maui generally grew faster than those from Oahu and Hawaii, and also showed a slower rate of phyllode development. Accessions with round-shaped seeds from Kauai grew significantly slower than the other accessions, and died after three year's growth in SET 91-1.

The fastest growing families were from Nuuanu and Makiki Heights on Oahu, along Waimea canyon drive on Kauai, Kipahulu on Maui, and Puu Waawaa, Hamakua, and Kilauea Military camp. on Hawaii. Significant differences were also observed between families from the same area. The results

indicate that there is great potential for genetic improvement in koa.

Introduction

Acacia koa is considered to be a slow-growing tropical leguminous tree species (NAS, 1979) and its production cycle from planting to harvesting is about 50 years (Skolmen, 1990). However, koa was also reported as a fast-growing tree in juvenile stages (Jodd, 1919; Brewbaker and Sun, 1995; Sun *et al.*, 1995).

Genetic variations in koa growth have been suggested by several authors. Skolmen (1990) observed differences in growth between koa progenies grown in the same location. One progeny produced straight and uniformly tall trees, while the other was heavily defoliated and died shortly thereafter. Conrad *et al.* (1995) reported significant differences for plant growth among eight koa provenances from four Hawaiian islands. Previous attempts of koa reforestation have shown slow growth and poor stand performance (Whitesell and Isherwood, 1971; Ching, 1981; Scowcroft and Adee, 1991). After 10 years, koa attained an average height of only 8.4 m and an average DBH of 12 cm at the Hilo Forest Reserve, Hawaii (Scowcroft and Adee, 1991). These growth rates are inferior to observations made by Judd (1919), who reported that koa attained heights of 9 m in five years.

Genetic improvement of koa appears to have been neglected due to the belief that koa is slow-growing and has little genetic variation. Few studies have been undertaken of genetic improvement of koa, and none have been successful in quantifying genetic variation. For example, in 1972, the USDA Forest Service identified 52 koa trees with straight boles as "superior trees" (Skolmen, 1977). Of two nurseries derived from these superior trees, slow establishment and growth were characteristics of many of progenies and clones and no advanced seeds were collected for further test (Skolmen *et al.*, 1991). Genetic differences for plant growth among koa populations between or within the Hawaiian islands remain unknown (Glover *et al.*, 1991).

Strategies for *Acacia koa* improvement and selection were outlined by Glover *et al.* (1991). These include an operational component that entails seed collection and genetic conservation to meet current and future demand, and a research component to maintain ongoing genetic improvement over a long term. The research component consists of clarifying koa's breeding system, determining the extent of genetic variations, measuring heritability of specific traits, and evaluating genotype by environment interactions.

Progeny testing, one of the research components, is a necessity to accomplish long-term genetic gain through selection (Eldridge *et al.*, 1993). The purposes of evaluation include estimating the breeding value of the

selected parents and genetic parameters (genetic variance and correlation), selecting sources for further improvement, and converting trials to seed orchards. Progeny could be selected from natural forest or provenance testing trials (Eldridge *et al.*, 1993).

As a starting point of conducting genetic studies of koa, the present study has achieved the following iimportant objectives: evaluate koa progenies throughout the Hawaiian islands, quantify the genetic variability among *Acacia koa* germplasm, identify quality seed sources for reforestation, and select superior koa as the basis for long-range genetic improvement programs.

Materials and Methods

Progeny testing trials

Six progeny trials and one koa/leucaena mixture trial were established at two locations in Hawaii from 1991-1995 (Table 10.1). Four progeny trials and a koa and leucaena mixture trial were set up at Hamakua Research Station of College of Tropical Agriculture and Human Resources (CTAHR) on the Island of Hawaii, and two progeny trials were carried out at Maunawili Station of Hawaii Agricultural Research Center (HARC) on Oahu. Details of field layout and entries tested for these trials are presented in Appendices E.1-E.7.

Table 10.1. SET progeny trials for koa genetic improvement.

SET No.	No. of Entries	No. of Reps.	Location	Date Planted
91-1	48	2	Hamakua, HI	May 22, 1991
92-2	5(1)	3	Hamakua, HI	May 26, 1996
93-1	14	3	Hamakua, HI	May 27, 1993
94-1	43	2	Hamakua, HI	May 26, 1994
94-2	59	2	Maunawili, Oahu	June 22,1994
95-1	58	2	Hamakua, HI	May 27, 1995
95-2	62	2	Maunawili, Oahu	June 1, 1995

The CTAHR Hamakua Research Station is located at 19°58'56N and 159°23'09W with a mean elevation of 650 m above sea level. Precipitation ranges from 1500 to 2300 mm yr^{-1}, and mean annual temperature is 16°C. The soil series is Maile (silty clay loam) with a pH of about 5.0. The HARC Maunawili Research Station is located at 21°22N and 157°47W with a mean elevation of 170 m above sea level. Precipitation ranges from 1000 to 3500 mm yr^{-1}, and mean annual temperature is 23°C. The soil series is Leilehua with a pH of about 5.0.

Site preparation

Before transplanting, the sites were aprayed with Roundup™ herbicide twice at the Hamakua Station. The sites on the Maunawili Station were plowed to a depth of approximately 30 cm. Lime at 4 Mg ha^{-1} was applied to SET 95-2 due to low pH following Hue and Ikawa (1994).

Experimental designs

An augmented design of the randomized complete block (RCB) with two replications was employed for most progeny trials. Each plot consisted of 10 trees in two rows. Spacing was 1 x 1.5 m^2 or 6,667 trees ha^{-1}, 1 m between trees in the row, and 1.5 m between rows. Each trial contained approximately 50 accessions. SET 93-1 differed in having three replications, and each plot consisted of 16 trees in two rows.

For SET 92-1, a split-plot design with three replications was employed. The main plot was five koa progenies and composites, and the sub-plot was *Leucaena* (Tetraploid *L. diversifolia* K784) (planted vs. not planted). Main plot size was 12 x 8 m. Twenty four koa trees were planted in each main plot. Spacing for koa was 2 x 2 m. Sub-plot size was 6 x 8 m accommodating 12 koa trees. When subplot treatment was to plant K784, thirty six K784 trees were interplanted with twelve koa trees. Spacing for mixed trees was 1 x 1 m.

The overall field layout of each trial was carefully planned prior to tree planting. The process of randomizing progenies or accessions to each plot was made in the field during transplanting following the technique suggested by Wright (1975). One extra tree was planted in each plot for replacement, in case of unpredicted mortality.

Experimental management

Individual trees were watered manually after transplanting if there was no immediate rain on the site. Weeding was done manually several times in the first half year. Whenever necessary, Fusilade™, Paraquat™, and Roundup™ herbicides were used to keep weeds suppressed.

Fertilization was carried out one and half months after planting to aid in establishment and stimulate early growth. Rate of fertilization was 50 g of 16-16-16 (N_2-P_2O_5-K_2O) for each tree (or 333 kg ha^{-1}). Granulated fertilizer was manually distributed around each tree.

Pruning and thinning of the trials were done according to the tree growth and the trial. Pruning of side branches below breast height was undertaken on two-year old trees. Thinning of small trees was only carried out in SET 91-1 trial at two and half-year old trees. Thinning percentage was 50%.

Field data collection and analysis

Individual tree height and DBH (diameter at breast height) were measured at five- and seven-month intervals for most of the trials. Where plants forked below breast height, the diameters DBH_1 and DBH_2 of both or more were assessed as an equivalent single stem diameter by the calculation formula below:

$$DBH = \sqrt{DBH_1^2 + DBH_2^2 + \ldots + DBH_n^2}$$

The rate of phyllode development based on the percentage of phyllode coverage in the tree was scored after one year planting. Survival rate of each plot was also recorded from year to year. Tree forms were recorded based on a scale of 1 to 9 (1 represented trees with a bole height more than 2 m with no branches; 5 represented trees with a bole height about 1.5 m with 2 branches; 9 represented trees with a bole height about 1 m with more than 4 branches) at two year old trees for some of trials. Plot means for the four traits were calculated and analysis of variance was done on these data using a Quattro Pro spreadsheet (Brewbaker, 1993) and GLM of SAS (SAS, 1990), provided that a fixed model for the experiments is selected.

Individual tree heritability estimates for these measured traits were not made from the progeny trials due to the lack of information regarding the breeding system of the species. However, variance components estimates were made, provided that a random model for the experiments is selected.

Results

Among 334 collections of *Acacia koa* at UH, 178 were grown and tested in a series of seven trials from 1991 to 1995. A total of 48 accessions from four islands were planted in SET 91-1 at Hamakua, Hawaii. In SET 92-1, five koa accessions and one *Leucaena* species were planted at Hamakua, Hawaii including 1PU1-1 from Oahu, 2ML1-1 and 2PH2-1 from Kauai, and 6-1188C and 6-0191C from Hawaii, and a high land tetraploid *L. diversifolia* (K784) from Mexico. In SET 93-1, fourteen accessions were tested, and including 2MI1-1 (Kauai) and 1N2-1 and 1PU1-2 (Oahu) that were selected from the previous progeny trial because of their fast growths. In 1994, 62 koa accessions were planted at two sites, including 39 from Kauai, 3 from Oahu, and 20 from Hawaii. Forty were completely replicated at both sites. In 1995, a total of 72 koa accessions were tested at two sites, including 4 from Oahu, 35 from Kauai, 10 from Maui, and 23 from Hawaii. Fifty were completely replicated at both sites.

SET 91-1, Hamakua, Hawaii

Growth performance data including survival rate, tree height, DBH, and phyllode development rate were recorded. The overall survival rate at the age of two months was 95.5%. However, composite 6-1288C from Waikea, Hawaii lost one-third of its seedlings. Other seedling death was due to

careless planting or strong trade wind on the Hamakua coast. The survival rate decreased to 85.7 % after two year's growth. The lowest survival rate was from 6-1288C (Hawaii), 2DT2-1, 2MA2-1, 2MA3-1, and 2OV1-1 (Kauai), with 30, 0, 20, 60, and 60 percent survival, respectively. After three years growth, all four Kauai accessions were dead. All these accessions were the round-shaped seed phenotypes.

Average tree heights and DBH data of 48 koa accessions at different ages are summarized with the LSD at $P < 0.05$ level in Table 10.2. Significant difference in growth among these accessions were found. Overall, the average tree height attained 3 m in the first year, and continued to add a meter in every half year period. After two and half years, heights of these accessions averaged 5.9 m and ranged from 2.0 m of 2MA2-1 from Kauai to 7.6 of 5K1-2 from Maui. After four years, DBH averaged 99 mm and ranged from 55 mm of 5OL1-2 from Maui to 149 mm of 1N2-5 from Oahu. One year after planting, phyllode coverage over the trees averaged 64 % and ranged from 0 of 2DT2-1 to 100 % of the of 1M7-1, 1SL3-1, 5K1-1, and NFTA891C.

Significant differences in growth performance among accessions from the same area were also observed for DBH after four year's growth. DBHs of 1M6-1 and 1M8-4 from Makiki area, Oahu were significantly different at 2.5 years, but not for height. Similar results were observed for

Table 10.2. Average tree height (m) and DBH (mm) of SET 91-1 including 48 Acacia koa accessions at various early growth stages and phyllode development (PD) rate in percentage at 12 months at Hamakua, Hawaii.

	Height at ages (month)						DBH at ages (month)					
Progeny	2	6	14	18	26	31	26	31	37	42	47	PD Rate
1M2-1	0.5	2.0	3.2	4.4		6.7	74	79	92	99	115	90
1M2-2	0.4	1.7	3.5	4.8	5.3	6.6	63	110	115	115	130	90
1M6-1	0.4	1.2	2.3	4.0	5.2	5.8	74	92	109	124	132	64
1M7-1	0.5	2.0	3.4	4.6		6.4	74	92	106	109	113	100
1M8-3	0.5	1.5	3.2	4.3	4.7	5.8	61	71	80	88	92	23
1M8-4	0.5	1.6	3.4	4.4	5.9	6.3	54	65	75	86	87	16
1MC*	0.6	2.2	4.0	4.7	5.8	6.6	72	87	92	99	105	79
1N1-2	0.5	2.3	2.9	4.0	4.3	6.2	38	77	81	84	106	10
1N1-3*	0.4	1.6	3.1	4.4	5.0	6.1	59	82	94	103	111	83
1N1-4*	0.6	2.1	3.2	4.3	5.4	6.3	57	65	76	80	91	54
1N1-5*	0.5	2.1	3.4	4.8	5.5	6.4	60	85	95	103	109	43
1N2-1	0.6	2.1	3.6	4.7	5.3	6.6	71	90	108	118	125	65
1N2-2	0.7	2.0	3.6	4.5		6.4	68	98	109	113	121	80
1N2-4	0.6	2.5	3.7	4.4	5.7	6.4	57	92	95	91	110	90
1N2-5	0.5	1.9	3.5	4.4		6.1	90	105	125	136	149	72
1N4-2	0.5	1.9	3.5	4.1	5.4	6.2	72	69	81	90	91	52
1PU1-2*	0.4	1.6	2.9	4.0	5.0	5.5	55	69	72	78	93	33
1PU2-1	0.5	2.0	3.9	4.7	5.5	6.9	67	95	100		117	57
1SL1-1	0.4	2.0	3.0	4.3	4.1	5.6	45	76	60	61	60	46
1SL3-1*	0.4	1.5	2.6	3.8	4.8	5.5	53	63	70	74	74	100
1SL3-4	0.4	0.8	3.0	3.0		5.7	50	74	93	102	108	28
1SL3-5*	0.4	1.8	2.9	3.9	4.8	5.3	47	65	70	76	81	28
2DT2-1	0.2	1.2	1.4	1.6								0
2HA1C*	0.6	1.9	3.4	4.2	5.2	6.5	66	81	92	101	110	75
2KU1-1*	0.5	1.8	3.5	4.4	5.7	7.0	71	86	94	105	106	66
2KU2-1*	0.6	1.8	3.1	4.1	5.5	6.2	64	79	90	102	102	93
2KU3-1	0.2	0.4	2.1	2.5	2.9	3.2	24	21	27			68
2MA2-1	0.2	1.6	2.4	2.7		2.0	22					80
2MA3-1	0.2	0.5	0.8	1.0			23					17
2ML1-1*	0.5	2.2	3.7	4.4	5.6	6.9	66	87	96	105	112	43
2NU2C*	0.6	2.0	3.9	4.8	5.6	7.0	69	97	111	117	137	69
2OV1-1	0.3	0.6	1.3	2.0	3.2		20					30
2PH1-1*	0.4	1.6	2.9	4.2	5.5	6.3	75	94	107	116	120	57
2PH2-1*	0.5	2.0	3.7	4.5	5.5	6.4	57	76	82	93	74	39
2PK3-1*	0.4	1.6	2.7	4.7	6.1	6.3	77	97	127		137	66
5HM1-1	0.5	0.6	2.2	4.0	4.6	6.4	102	101	106	115	122	90
5K1-1*	0.5	2.0	3.4	4.2	5.7	6.8	70	83	91	100	107	100
5K1-2	0.6	2.4	3.8	5.0	6.5	7.6	74	98	109	119	127	90
5K1-6	0.3	1.4	2.2	3.4	3.7	4.2	40	41	45	61	64	37
5OL1-2*	0.5	1.6	2.2	3.6	3.7	4.7	45	51	57	53	68	32
6-0191*	0.3	1.5	2.9	3.9	4.3	5.2	38	49	54	64	64	92
6-1288b*	0.3	1.9	3.3	4.3	4.5	5.5	49	60	65	70	82	84
6-1288c*	0.2	0.8	2.7	4.0	4.5	5.3	32	54	62	76	76	48
6-1288d*	0.3	1.7	3.0	3.9	4.4	5.1	45	58	64	66	66	81
6KA1-1*	0.3	1.5	3.0	4.5	5.4	6.5	56	73	82		101	92
6WA1-1	0.5	1.8	3.5	4.2		6.1	43	52	58	69	72	88
NFTA890C	0.3	1.9	3.5	4.2	4.7	5.1	44	60	51	54	58	88
NFTA891C	0.4	1.8	3.6	4.4		5.5	48	56	55	55	55	100
AVG.	0.4	1.7	3.0	4.0	5.0	5.9	57	76	85	92	99	63
MAX.	0.7	2.5	4.0	5.0	6.6	7.6	102	110	127	136	149	100
MIN.	0.2	0.4	0.8	1.0	2.9	2.0	20	21	27	53	55	0
LSD .05**	0.1	0.5	0.7	0.6	1.2	1.0	19	19	23	30	33	28

* : Replicated accessions
**: LSD 0.05 based on twenty two replicated accession data

2PH1-1 and 2PH2-1 families from Kauai and 5K1-2 and 5K1-6 families from Maui.

Analysis of variance for tree height, DBH, and phyllode development showed significant differences ($P<0.01$) among the 22 replicated accessions (Table 10.3). Orthogonal analysis between different island accessions revealed that families and composites from Kauai (with the exception of four round-shaped seed families) and Oahu grew significantly ($P<0.01$) faster than the Hawaii accessions. Accessions from Hawaii showed significantly ($P<0.01$) earlier phyllode development than those from the other islands.

Correlation coefficients for average height and DBH of all koa families and composites in this study are presented in Table 10.4. Over the range of ages from 2 to 47 months, most of correlation coefficients between and within two growth traits: height and DBH, were significant ($P<0.01$) and ranged from 0.6 to 0.9.

Variance components estimates were presented in Table 10.5 for tree height, DBH, and phyllode development at various growth stages. Variance components for these traits were quite high. Variance components for tree height averaged 50%, ranging from 36.6 to 79.8% at various growth stages. Variance components for DBH averaged 64%, ranging from 58 to 69%.

Various disease symptoms of koa rust and sooty black were observed in this trial. Variation of disease symptoms

Table 10.3. Summary analysis of variance for replicated koa accession tree height and DBH after three and half year growth and phyllode development after one year growth at Hamakua, Hawaii (SET 91-1).

(a) Height		Growth period (month)					
Source	df	2	6	14	19	26	31
Replication	1	NS	**	NS	NS	NS	NS
Genotype	21	**	**	**	**	*	**
Kauai vs. Oahu, Hawaii, Maui	1	**	NS	**	*	**	**
Oahu vs Hawaii, Maui	1	*	*	*	*	**	**
Hawaii vs Maui	1	**	NS	NS	NS	NS	NS
Error	21						

(b) DBH		Growth period (month)				
Source	df	26	31	37	42	47
Replication	1	*	**	NS	NS	NS
Genotype	21	**	**	**	**	**
Kauai vs. Oahu, Hawaii, Maui	1	**	**	**	**	**
Oahu vs Hawaii, Maui	1	NS	**	**	**	**
Hawaii vs Maui	1	*	NS	NS	NS	**
Error	21					

(c) Phyllode development		
Source	df	
Replication	1	**
Genotype	21	**
Hawaii vs. Oahu, Kauai, Maui	1	**
Oahu vs. Kauai, Maui	1	**
Kauai vs. Maui	1	NS
Error	21	

**: Significantly different at $P < 0.01$, *: at $P < 0.05$
NS: not significantly different

Table 10.4. Correlation coefficients and observation numbers for average tree heights and DBHs of SET 91-1 including 48 koa families and composites over the indicated range of ages after planting at Hamakua, Hawaii.

	Heights at ages						DBH at ages				
Month	2	6	14	18	26	31	26	31	37	42	47
0	0.73	0.76	0.67	0.69	0.68	0.71	0.69	0.65	0.61	0.46	0.24
	**†	**	**	**	**	**	**	**	*‡		
	17	17	17	17	16	15	17	15	15	13	14
2		0.63	0.66	0.68	0.65	0.76	0.73	0.65	0.62	0.58	0.55
		**	**	**	**	**	**	**	**	**	**
		48	48	48	38	45	47	44	44	40	43
6			0.80	0.73	0.62	0.49	0.38	0.39	0.3	0.08	0.14
			**	**	**	**	**	**	*		
			48	48	38	45	47	44	44	40	43
14				0.87	0.74	0.66	0.51	0.44	0.36	0.28	0.22
				**	**	**	**	**	*		
				48	38	45	47	44	44	40	43
18					0.83	0.85	0.68	0.69	0.62	0.45	0.46
					**	**	**	**	**	**	**
					38	45	47	44	44	40	43
26						0.88	0.73	0.75	0.79	0.72	0.66
						**	**	**	**	**	**
						37	38	37	37	33	36
31							0.73	0.81	0.78	0.74	0.71
							**	**	**	**	**
							45	44	44	40	43
26								0.83	0.85	0.83	0.79
								**	**	**	**
								44	44	40	43
31									0.95	0.90	0.90
									**	**	**
									44	40	43
37										0.98	0.96
										**	**
										40	43
42											0.96
											**
											40

† : significant at P<0.01, and ‡: significant at P<0.05

Table 10.5. Variance components (%) of plant height, DBH, and phyllode development (PD) rate at various stages for 22 replicated koa accessions grown at Hamakua, Hawaii (SET 91-1).

Growth stages (month)	Plant Height	DBH	PD Rate
2	79.8		
6	52.4		
14	58.1		60.0
18	56.3		
26	36.6	58.0	
31	61.7	69.0	
37		69.0	
42		58.0	

between these koa families was noticed. Overall, the progenies from Hawaii showed more rust symptoms compared with the progenies from the other islands. However, tree growth performance was not affected by these disease symptoms.

After four years of growth, the fastest growing families in terms of DBH (>127mm) were 1M2-2, 1M6-1, and 1N2-5 from Oahu and 2NU2C and 2PK3-1 from Kauai, and 5K2-1 from Maui. Some of these families flowered and set seeds in the fourth year, and seeds were collected from these outstanding families. Testing of these advanced progenies will be carried out to study the potential genetic gains from this progeny testing and selection.

SET 92-1, Hamakua, Hawaii

The purpose of the koa and *Leucaena* mixed trial was to use high land *Leucaena* (var. K784) as a guide tree for koa to grow straight and fast. Due to vigorous growth of K784, koa trees were suppressed in the mixed tree plots. Therefore, K784 was cut at height of 0.5 m every half year. After one and half year planting, K784 was completely cut down.

Average tree heights, DBH, and survival rates among the five koa progenies after three year planting are summarized in Table 10.6. Overall, average koa tree height was about 4 m two years after planting, and DBH was 7.6 cm three years after planting. The best entry among these five koa accessions was 2PH2-1 from Kauai, which reached 4.7 m in height at two year's growth and 9.8 cm in DBH at three year's growth.

Analysis of variance revealed that tree height and DBH after two year's growth were significantly different among koa accessions (Table 10.7). No significant difference for tree survival rate was observed. Significant difference for koa tree DBH was observed between trees interplanted with *Leucaena* and planted alone, but not for tree height and tree survival rate, although *Leucaena* trees were completely cut down one and half years after planting. Koa trees in the mixed trees plots grew slower than koa trees grown alone. The results of this trial suggested that the idea of using

Table 10.6. Average plant height, DBH, and survival rate of five koa progenies interplanted with or without *Leucaena* (K784) after three years' growth at Hamakua, Hawaii (SET 92-2).

Accession	K784	Height		DBH		Survival rate	
		Y0.5†	Y2†	Y2	Y3†	Y2	Y3
		-m-	-m-	-mm-	-mm-	-%-	-%-
6-1188C	Yes	1.2	3.3	4.1	5.7	89	81
	No	1.1	3.0	4.2	5.5	83	81
6-0191C	Yes	1.0	3.1	3.5	4.7	72	64
	No	1.2	3.1	3.8	4.8	72	64
1PU1-1	Yes	1.2	3.5	6.0	8.3	83	69
	No	1.2	3.9	7.0	9.6	75	69
2ML1-1	Yes	1.3	4.7	6.3	8.1	100	100
	No	1.2	4.7	7.0	9.7	81	69
2PH2-1	Yes	1.2	4.3	7.0	8.5	94	94
	No	1.3	5.0	9.1	11.3	94	94
AVG.		1.2	3.7	5.8	7.2	84	79
LSD.05			0.5	1.1	1.4		

†: trees were one-half-, two-, and three-year's old respectively at the time of measurement

Table 10.7. Analysis of variance (Split-pot design) of SET 92-2 for plant height, DBH, and survival rate of five koa accessions interplanted with or without *Leucaena* (L) after three years' growth at Hamakua, Hawaii.

		Height		DBH		Survival rate	
Source	df	Y0.5†	Y2†	Y2	Y3†	Y2	Y3
Acc.	4	NS	**	**	**	NS	NS
Rep.	2	NS	NS	NS	NS	NS	NS
Error a	8						
L+ vs. L-	1	NS	NS	**	**	NS	NS
L x Acc.	4	NS	**	NS	*	NS	NS
Error b	10						
Total	29						

†: trees were one-half-, two-, and three-year's old respectively at the time of measurement,

Leucaena as a guide tree to push koa grow straight and tall was not practical.

SET 93-1, Hamakua, Hawaii

Two years after planting, survival rate averaged 59% and ranged from 19% of 1WAK1C and 1WAK4C from Oahu to 90% of 2MI1-1 from Kauai (Table 10.8). The accessions from West Oahu showed a lower survival rate than those from Hawaii. The 2MI1-1 from Kauai showed the highest survival rate. The low survival rate among West Oahu accessions was mainly attributed to susceptibility to coffee borer attacks, which caused tip dying of koa saplings.

Table 10.8. Average tree survival rate, height, DBH, phyllode development rate in percentage, and tree form (scale 1-9) of 14 koa accessions after two years' growth at Hamakua, Hawaii (SET 93-1).

Accession	Survival		Height		DBH	Phyllode percent	Tree form
	Y1†	Y2†	Y1	Y2	Y2	Y1	Y2
	-%-	-%-	-m-	-m-	-mm-	-%-	
1N2-1	91	75	3.4	4.3	25	43	4.2
1PU1-2	65	48	2.3	3.3	22	41	3.6
1WAK1	31	19	2.0	2.8	17	70	4.2
1WAK4	33	19	2.0	3.7	22	93	4.6
1WAK5	60	54	2.4	3.3	18	85	4.5
1WAI1	58	42	2.5	3.7	20	71	4.7
1WAI2	58	42	2.7	3.7	22	89	4.7
2MI1-1	94	90	3.0	3.9	22	24	4.4
6PUUC	85	77	2.9	3.7	18	95	3.8
6HAMC	62	62	3.1	3.9	23	96	5.2
6KEAC	81	81	3.1	3.8	19	80	4.5
6KUK1C	77	73	3.3	4.2	22	68	3.9
6KUK2C	79	71	3.1	3.8	21	67	3.0
6HONC	69	69	3.0	4.0	21	100	4.7
AVG	67	59	2.8	3.7	21	73	4.4
Max.	94	90	3.3	4.2	25	100	5.2
Min.	31	19	2	2.8	17	41	3.0
LSD 0.05	30	27	0.5	0.6		22	

†: trees were one- and two-year's old respectively at the time of measurement

The fastest growing accession in terms of DBH was 1N2-1 from Nuuanu area, Oahu, after two year's growth. The poorest growing accession was 1WAK1C from the Waianae Mountain region, Oahu. Overall, height for these accessions averaged 3.7 m and ranged from 2.8 m of 1WAK1C to 4.3 m of 1N2-1 from Oahu. DBH averaged 21 mm and ranged from 17 to 25 mm. Tree form averaged 4.4 and ranged from 3.0 of 6KUK2C to 5.2 of 6HAMC. Phyllode coverage averaged 73% and ranged from 24 of 2MI1-1 to 100% of 6HONC one year after planting.

Analysis of variance was significant ($P<0.01$) for tree survival rate, height, and phyllode development rate among these 14 accessions, but not for DBH and tree form (Table 10.9). More than 50% of the variation in survival rate, plant height, and coverage by phyllodes was due to differences in source of plants.

Early flowering of two accessions was observed two years after planting. These two accessions were 6PUUC of Puu Waawaa and 6HAMC of Hamakua, both from Hawaii.

The best accessions in terms of low mortality, height, DBH, and tree form were 1N2-1 from Nuuanu area of Oahu, 2MI1-1 from Milolii ridge of Kauai, and 6HAMC from UH Hamakua Research Station on Hawaii. The 1N2-1 and 2MI1-1 were also identified as fast growing accessions in the previous trial and proved to be good performers again. Five accessions from West Oahu and one from the north shore of

Table 10.9. Analysis of variance and percentage of variance components for tree survival, height, DBH, phyllode development rate, and tree form of 14 koa accessions at early growth stages after two years' growth at Hamakua, Hawaii (SET 93-1).

Source	df	Survival		Height		DBH	Phyllode coverage	Tree form
		Y1†	Y2†	Y1	Y2	Y2	Y1	Y2
Accession	11	**	**	**	**	NS	**	NS
Replication	2	NS	NS	NS	NS	NS	NS	**
Error	22							
Total	35							
percentage of variance components (%)								
Accession		57	73	72	48		78	
Error		43	27	28	52		22	

†: indicates trees were at one- and two-year's respectively old at the time of measurement

Oahu had very low survival rate due to susceptibility to the coffee borer attacks. These accessions also grew slowly.

SET 94-1, Hamakua, Hawaii and SET 94-3, Maunawili, Oahu

These trials were essentially duplicates, planted in May, 1994. At the Hamakua Station, three month old koa trees were heavily browsed by cows. As a result of the damage, the trees grew much slower than those at Maunawili Station on Oahu. Therefore, analysis of variance for the two trials was done separately.

Average survival rate, plant height, and phyllode development rate after one year's growth are summarized and presented in Table 10.10 for 43 accessions grown at Hamakua, Hawaii and 59 accessions grown at Maunawili, Oahu. For SET 94-1 at Hamakua, survival rate averaged 68%, and ranged from 20 to 100%. Tree height averaged 1.3 m, and ranged from 0.5 m of 2W3-2 to 1.9 m of 2MA2-2A.

For SET 94-3 at Maunawili, survival rate averaged 78%, and ranged from 40 to 100%. Tree height was 1.9 m, and ranged from 0.6 m of 2MA3-2 to 3.8 m of 1N2-1. Phyllode development rate averaged 34%, and ranged from 0 of many Kauai accessions to 87% of 6HAMC from Hawaii.

Analysis of variance for plant height and survival rate for SET 94-1 was significantly ($P<0.01$) different between 43 accessions (Table 10.11). More than 50% of the variation

Table 10.10. Average height (m), survival rate (%), and phyllode development rate (%) of 62 *Acacia koa* families after one year growth at Maunawili, Oahu (SET 94-3) and Hamakua, Hawaii (SET 94-1).

Accession	Height		Survival rate		Phyllode dev. rate
	Maunawili	Hamakua	Maunawili	Hamakua	Maunawili
1N2-1	3.8		100		57
1WAK1C	1.7		40		36
1WAK4C	1.2		40		36
2K3-1	1.3		90		4
2KU1-1	2.2	1.1	70	70	49
2KU1-1A	3.0	1.5	100	80	69
2KU1C1	2.5	1.7	85	60	55
2KU1C3	2.7	1.7	85	80	40
2MA1-1	2.6	1.3	95	95	54
2MA1-2	2.8	1.5	95	75	42
2MA1-3	2.0	1.7	70	95	59
2MA2-1	2.9	1.6	75	70	74
2MA2-2	1.5		80		0
2MA2-2A	2.4	1.9	75	70	54
2MA2-3	1.3		60		1
2MA3-1	1.4	0.7	85	45	3
2MA3-2	0.6		70		0
2MA3-3	1.1		85		5
2MI1-1	2.4	1.4	75	90	47
2MI1-2	1.6	1.4	100	65	31
2MI1-3	2.1	1.5	95	70	36
2MI2-1	1.1		95		2
2MI2-2	1.0		90		3
2MI2-3	1.8	1.4	85	70	34
2MI2-4	1.3	1.1	90	70	1
2MI2-5	1.0		70		1
2MI2-6	1.5		80		1
2NU1-2	1.4		95		0
2PH1-1	0.9	1.0	60	35	0
2PH1-2	2.4	1.4	70	100	14
2PH1-3	2.5	1.7	85	80	51
2PH2-1	1.1		80		8
2PH2-2	2.7	1.6	85	85	54
2PH2-3	1.3		75		1
2PK2-1	2.8	1.6	95	95	70
2PK2-1A	2.6	1.7	100	65	54
2PK2-2	2.2	1.5	90	85	51
2W1-1	0.9		100		3
2W2-1	2.2	1.5	80	65	72

Table 10.10.(Continued). Average height (m), survival rate (%), and phyllode development rate (%) of 62 *Acacia koa* families after one year growth at Maunawili, Oahu (SET 94-3) and Hamakua, Hawaii (SET 94-1).

	Height		Survival rate		Phyllode dev. rate
	Maunawili		Maunawili		
Accession		Hamakua		Hamakua	Maunawili
2W3-1	1.3		95		5
2W3-2	0.9	0.5	60	20	0
6HAMC	1.7		40		87
6KMC1-1	1.0	1.0	55	55	57
6KMC1-2	1.9	1.0	75	85	42
6KMC1-3	1.1	0.8	50	40	12
6KMC1-4	2.3	1.0	90	75	59
6KMC1-5	1.6	1.3	65	80	22
6KMC1C	2.3	0.9	95	70	55
6KMC2C	2.6	1.1	85	65	48
6KMC3C	2.6	1.4	75	75	52
6MLR1-1	1.9	1.2	75	85	20
6MLR2C	1.9	1.3	60	80	22
6MLR3C	1.4	1.2	45	75	26
6MLR4C	2.2	1.1	55	70	49
6MLR5-1	2.9	0.9	90	45	77
6MLR6C	2.1	1.5	70	50	40
6MLR7-1	2.2	1.1	50	55	32
6MLR7-2	2.0	0.8	70	45	41
6PUUC	2.2		83		49
6MLR8-1		1.0		65	
6MLR1-2		1.5		35	
2NU1-1A		0.9		45	
Avg.	1.9	1.3	78	68	34
Minimum	0.6	0.5	40	20	0
Maximum	3.8	1.9	100	100	87
LSD 0.05		0.3		17	

Table 10.11. Analysis of variance and percentage of variance components for plant height and survival of 43 *Acacia koa* accessions after one year growth at Hamakua, Hawaii (SET 94-1).

		Height		Survival Rate	
Source	df	Y0.5†	Y1†	Y0.5	Y1
Accession	42	**	**	**	**
Replication	1	**	NS	NS	NS
Error	42				
Total	85				
		Percentage of variance components (%)			
Accession		62	70	50	73
Error		38	30	50	27

†: trees were at one-half- and one-year old respectively at the time of measurement

for survival rate and tree height was due to difference in source of progeny.

No significant differences for these measured traits were found for 45 replicated accessions in SET 94-3 (Table 10.12). This unexpected result was attributed to the significant block effect in SET 94-3. Analysis of soil samples from two blocks revealed that Block I had a low pH of 4.5 and Block II had a pH of 5.5.

The best accessions among these 62 accessions with average height above 2.0 m across two sites were 1N2-1 from Oahu, 2MA1-2, 2MA2-1, 2MA2-2A, 2PH1-3, 2PH2-2, 2PK2-1,

Table 10.12. Analysis of variance for plant height, survival rate, and phyllode development of 46 replicated *Acacia koa* accessions after one year growth at Maunawili, Oahu (SET 94-3).

		Height		Survival rate		Phyllode development
Source	df	Y0.5†	Y1†	Y0.5	Y1	Y1
Accession	45	NS	NS	NS	NS	NS
Reps.	1	**	**	**	**	**
Error	45					
Total	91					

†: trees were at one-half- and one-year old respectively at the time of measurement

2KU1C1, and 2KU1C3 from Kauai, and 6KMC2C and 6PUUC from Hawaii.

SET 95-1. Hamakua, Hawaii and SET 95-2, Maunawili, Oahu

Average tree height, survival rate, and phyllode development rate are summarized in Table 10.13 for 58 accessions planted on 23 May 1995 at Hamakua station and 64 accessions planted on 1 June 1995 at Maunawili. Tree height across two sites averaged about 1.5 m, and ranged from 0.9 to 2.2 m after 5 month planting. The fastest growing accessions were 5CP1-2 and 5CP1-5 from Maui (Figure 10.1) and 2W2-2, 2PH2-3 and 2PH2-2(94) from Kauai. These five accessions attained more than 1.9 m in height in five months. The 5CP1-5 showed extraordinary growth and was suspected to be a hybrid between Hawaii and Maui koa, since

Table 10.13. Average tree height (m), survival rate (%), and phyllode development rate (%) of 72 *Acacia koa* accessions after 5 months' growth at Maunawili, Oahu (SET 95-2) and Hamakua, Hawaii (SET 95-1).

	Height		Survival rate		Phyllode dev.	
	Maunawili		Maunawili		Maunawili	
Accession		Hamakua		Hamakua		Hamakua
1N2-5(91)	2.0		100		22	
1PP1C	1.3	1.3	80	100	95	58
1PT1C	1.9	1.8	100	100	75	19
1WM1C(93)	1.7	1.4	85	75	77	41
2AA1-5	1.3	1.1	80	100	37	5
2AA2-5	1.2		85		55	
2HL1-1	2.0	1.4	100	100	70	16
2KH2-1		1.6		100		16
2K2-2	1.1		75		28	
2KH3-1	1.1		95		12	
2KU1C3(94)	1.8	1.5	80	95	43	17
2KW1-1	0.9		65		1	
2KW1-2	1.5	1.7	85	90	67	46
2KW1-4	1.6	1.8	100	100	77	30
2KW2-1	1.7	2.0	100	100	33	20
2KW2-2	1.7	2.0	100	100	54	23
2KW3-1	1.8	1.7	90	95	53	13
2MI2-1	1.2	1.5	85	95	1	3
2MI7-2	1.4		90		19	
2NU2-1(91)	1.9	1.6	100	100	67	27
2PH1-1	0.9		80		7	
2PH1-2	1.1		65		3	
2PH2-2(94)	1.9	1.9	80	75	73	17
2PH2-3	2.0	1.9	100	100	42	8
2PK1-2	1.5	1.5	85	100	24	6
2PK1-3	1.6	1.4	95	100	59	10
2PK2-1(94)	1.9	1.5	95	95	66	15
2PK2-1B	1.1	1.0	90	100	2	0
2PK2-2	1.4	1.4	100	95	52	26
2PK3-1(91)	1.7	1.7	100	85	60	22
2PK3-1B	1.8	1.6	100	100	72	6
2PK3-4	1.0		85		16	
2PO1-2	1.2	1.3	65	100	1	0
2W2-2	2.1	2.0	95	95	77	28
2W3-4	1.8	1.7	70	100	49	18
2W4-2	1.4	0.9	80	100	20	7
2W4-3	1.9	1.8	100	100	45	9
2WT1-1	1.2		85		6	
2WT1-4A	1.1	1.3	70	100	0	0

Table 10.13.(Continued). Average tree height (m), survival rate (%), and phyllode development rate (%) of 72 *Acacia koa* accessions after 5 months' growth at Maunawili, Oahu (SET 95-2) and Hamakua, Hawaii (SET 95-1).

	Height		Survival rate		Phyllode dev.	
	Maunawili		Maunawili		Maunawili	
Accession		Hamakua		Hamakua		Hamakua
5CP1-2	1.9	2.1	90	100	79	48
5CP1-3	1.8	1.5	95	100	21	2
5CP1-5	2.2	1.9	100	100	67	52
5GUL1C	1.5	2.1	80	90	100	68
5GUL2C	1.9	1.8	50	100	92	78
5KH1C	1.6	1.7	90	100	50	26
5KO1-1	1.2	0.9	65	80	100	64
5KP1C	1.8	2.0	85	90	96	49
5MA1-2	1.5	1.7	90	90	34	21
5SN1-2	1.5	1.6	90	100	23	15
6HAM1C(93)	1.5	1.4	70	95	92	71
6HK1C	1.2	1.2	100	95	29	6
6HK2C	1.7	1.4	90	100	40	26
6HUmC	1.4		75		99	
6KAmC	1.8		80		24	
6KA3C		1.5		100		29
6KH1C	1.4	1.8	60	95	80	87
6KH2C		1.3		95		18
6KK1C		1.3		100		20
6KM1C	1.1	1.1	30	95	87	59
6KP1C	1.7	2.0	70	100	85	64
6KU1C	1.3	1.3	90	100	48	25
6LPmC	1.7		90		47	
6LP2C		1.3		100		21
6ML1C		1.6		95		47
6ML2C						
6ML3C		1.8		100		66
6MLR5-1(94)	1.7	1.4	80	70	78	28
6MLmC	1.7		100		75	
6OF1C	1.2	1.4	50	85	87	63
6PUU1C	1.7	1.8	90	100	75	63
6SAD1C	1.3	1.3	40	75	95	55
6WV1-1	1.5	1.7	95	100	34	63
Means	1.5	1.6	83	96	51.4	31.1
Min.	0.9	0.9	30	70	0.0	0.0
Max.	2.2	2.1	100	100	100.0	86.6
LSD 0.05	0.3	0.5	26	20	19.5	26.8

Figure 10.1. The fast growing accession of 5CP1-5 from Maui after one year growth at Maunawili Research Station of Hawaii Agricultural Research Center, Oahu.

it was the only tree in the area with wide phyllodes, characteristic of koa from the Island of Hawaii. The slowest growing accessions were 2PH1-1, 2KW1-1, and 2PH2-1B from Kauai, 5KO1-1 from Maui, and 6KM1C from Hawaii. These slow growing accessions from Kauai were all the round-shaped seed phenotypes. Overall, Maui and Kauai progenies grew faster than those from Oahu and Hawaii.

Survival rate at Maunawili averaged 83%, and ranged from 30 to 100% at six months. Survival rate at Hamakua averaged 96%, and ranged from 70 to 100%. Phyllode development rate at Maunawili averaged 51%, and ranged from 0 to 100%. Phyllode development rate at Hamakua averaged 31%, and ranged from 0 to 87%. Overall, progenies from Oahu and Hawaii have an earlier phyllode development than those from Maui and Kauai.

Individual and combined analysis of variance for plant height, survival rate, and phyllode development rate over two sites are summarized in Table 10.14. The significant ($P<0.01$) differences for plant height and phyllode development rate after 5 months' growth between the 50 replicated accessions were observed. The genotype x environment interaction was significant ($P<0.05$) for koa phyllode development rate, but not for plant height and survival rate. Again, the high percentage of the variation for tree height and phyllode was due to seed sources.

Table 10.14. Individual and combined analysis of variance and percentage of variance components for tree height (TH), survival rate (SR), and phyllode development (PD) rate after 5 months' growth of the replicated koa accessions grown at Hamakua, Hawaii (SET 95-1) and Maunawili, Oahu (SET 95-2).

	Hamakua				Maunawili				Combined		
Source	df	TH	SR	PD	df	TH	SR	PD	df	TH	PD
Site									1	NS	**
Rep.	1	**	**	NS	1	NS	**	NS	2	**	**
Acc.	51	**	NS	**	49	**	*	**	49	**	**
P x S									48	NS	*
Err.	51				49				81		
Percentage of variance components (%)											
Acc.		97	26	71		76	37	90		46	63
Err.		3	74	29		26	63	10		54	37

**: Significantly different at P < 0.01, *: at P < 0.05
NS: not significantly different

Overall, Kauai and Maui progenies showed faster juvenile growth than those from Oahu and Hawaii, and Oahu and Hawaii progenies showed earlier phyllode development than those from Kauai and Maui. The round-shaped seed from Kauai grew significantly slower than the other progenies at all sites tested.

Discussion

It is clear from the results of these trials that *Acacia koa* is a fast-growing tree in the juvenile stage (Figure 10.2). The finding is in contrast with the general assumption that koa is a slow-growing tropical leguminous tree (NAS, 1979). The slow-growing conclusion may derive from the observation of koa trees grown in degraded marginal forest lands. There are some reports that koa grew very slowly in newly degraded plantations (Ching, 1980; Scowcroft and Adee, 1991). The slow-growing trees may also be due to seed source. Skolmen (1990) reported growth differences between koa progenies grown in the same location. However, an early study did record that koa was a fast-growing tree in juvenile stages in which it reached 9 meters in 5 years (Judd, 1919).

There appears to be major genetic differences for early plant growth, survival rate, and development among progenies collected from the islands. Consistent and significant differences in these traits from 1 to 5 year growth stages

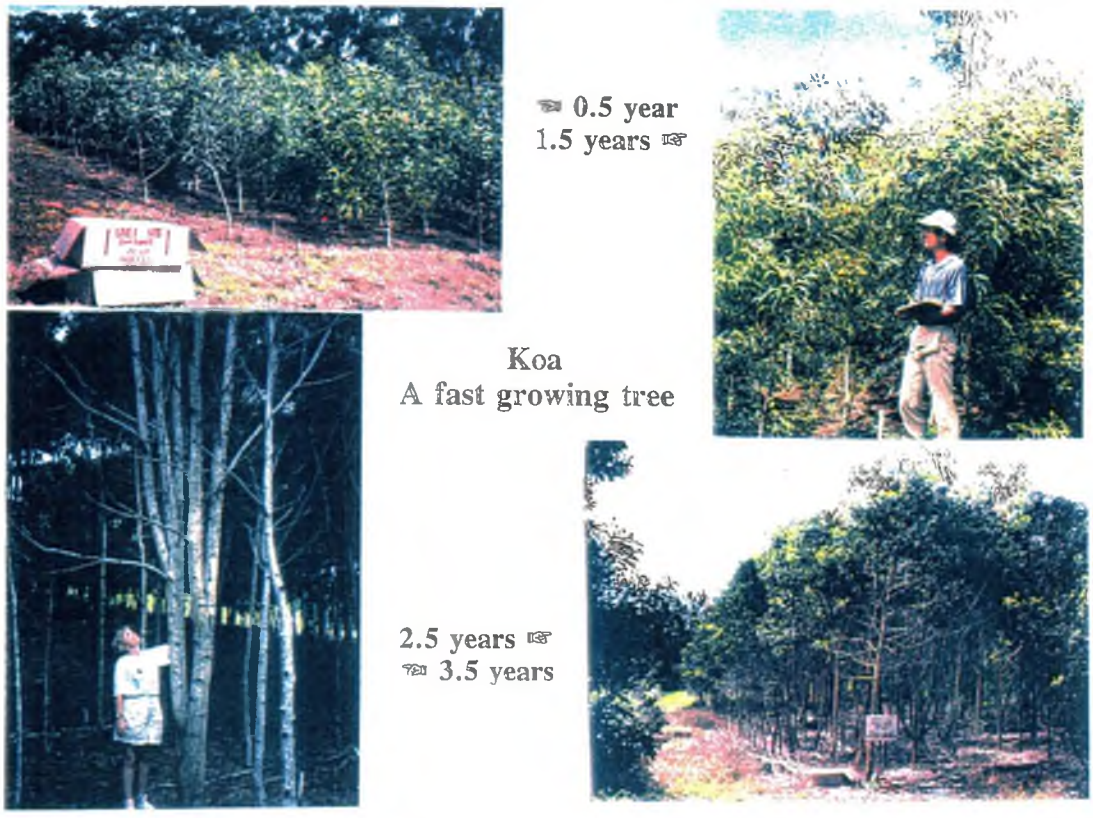


Figure 10.2. Koa trees at Hamakua Research Station of the College of Tropical Agriculture and Human Resources on the Island of Hawaii.

were largely due to seed source. Genetic differences were also observed in phyllode shapes and in seed and seedling characters (Brewbaker, 1977; Chapter 9). Significant differences in early growth were also reported for eight provenances from the Hawaiian islands (Conrad *et al.*, 1995). The presence of genetic variability in tree growth is very important and can be used immediately for rapid genetic advance at relatively low cost through tree improvement programs. The results clearly suggest that there is potential for genetic improvement of koa tree growth, especially, the fast-growing koa populations from some areas of Kauai and Maui.

Skolmen (1990) observed that only the big island koa with wide phyllodes could make a long, branch-free log for timber production. However, most of the fast-growing koa progenies with a single main stem identified from these trials were from Kauai, Oahu, and Maui. These koa populations, described as one koa group, are different from the koa from the Island of Hawaii with unique seedling characteristics (Chapter 8). The present findings suggest that more attention should be paid to selection and silviculture of these under-valued koa populations.

Selection for straight bole koa is possible, although leguminous trees including koa are notorious for their crooked growth and forking. Tree species in *Leguminosae* and *Fagaceae* have a tendancy to form numerous, quite small

metamorphs and reiterates, resulting in compound, unstable trunks and branches (Oldeman and Sieben-Binnekamp, 1994), especially when they are grown in an open area. Successful selection for straight stem trees in Leguminous species has been reported for *Acacia auriculiformis* from Springvale, Queensland, Australia, by Pinyopusarerk (1990). The great variation in tree form found in the natural forest suggests that there is the possibility that the straight koa trees can be selected. Generally, koa stands in Wood Valley and Honaunau area on Hawaii and in Puu Ka Pele area on Kauai had straighter and longer stems than other koas. The lack of significant differences for tree form in SET 93-1 could be due to the limited progeny tested and the high density planting.

Oldeman and Sieben-Binnekamp (1994) also suggested that silviculture practices be employed to straighten trees in plantations, as done for Oak (*Quercus*) and Beech (*Fagus*) in Europe. Straight and single stem koa trees can be produced, as in the present progeny testing trials, when koa was grown in a 1 x 1.5 meter tight spacing. The significant difference found for early growth among the koa families collected from the same area also suggests that tight spacing is necessary to weedout the slow growing trees in koa plantations.

The findings of these trials also can be applied to koa reforestation programs and advanced seed production for

further progeny testing. Immediate employment of the findings is to collect koa seeds from the regions with fast-growing families for koa reforestation. It is equally important to avoid collecting seeds from Waianae mountain region on Oahu and from Kauai with the round-shaped seed, due to susceptibility to coffee borer and slow growth characteristics, respectively.

Advanced progenies may be made within five to ten years though some koa populations flower as early as two years. The expected genetic gain for tree growth may only be realized if seeds are harvested from the selected and over-dominant trees, which usually will take more than five years in the progeny trials or in the plantations. One approach is to collect advanced progenies from the present trials. In order to facilitate this process, culling is necessary when data records for the trials are completed. Another approach is to collect seeds from the selected outstanding trees in koa plantations (e.g., Umikoa Ranch). Spacing in koa plantations is usually twice as wide as that of our trials. As observed in Umikoa Ranch, the outstanding trees were flowering and setting pods at the fifth and sixth year and the other trees were still at vegetative stages at this time.

In the long-term, the identified outstanding families should be tested over different environments to test the genotype x environment interaction. In order to exploit the

inter-provenance heterosis for tree growth and stability, hybrid breeding programs may also be initiated. Heterosis was reported in Australia *Acacias* species (Nikles and Griffin, 1991). Significant differences for DNA content among koa collections (Sun, unpubl. 1996) also suggests that intergenomic complementation for heterosis is found in koa.

Variation observed in koa early growth can also be used to aid classification of Hawaiian *Acacia* species. As discussed in Chapter 8, variations of koa seed and seedling traits supported the classification of the Kauai *acacia* population with the round-shaped seed type as *A. kauaiensis*. Evidence of slow early growth and low survival rate of the Kauai population further supports the treatment of *A. kauaiensis* by earlier botanists (Hillebrand, 1888; Lamoureux; 1971; St. John, 1979). *Acacia kauaiensis* with slow-growing and low survival rate traits in new environments may also relate to a plant-microorganism interaction, because healthy and large trees of *A. kauaiensis* can be seen on Kauai. Further study of the plant-microorganism interaction of *A. kauaiensis* is required if these trees are used for reforestation in former sugar land.

CHAPTER ELEVEN

SUMMARY OF SECTION TWO

The purpose of koa improvement in this study is to identify the quality koa seed source for reforestation through germplasm collection and evaluation and to select suporior koa as the basis for long-term genetic improvement.

A total of 334 koa accessions have been collected from across the Hawaiian Islands: Kauai, Oahu, Lanai, Maui, and Hawaii. Most of these accessions were derived as families from single trees. The collection represents majority of the koa growing area in Hawaii. They not only provide a broader genetic base for koa improvement, but also for taxonomic, physiological, and molecular genetics studies in the future.

During the past four years, seven koa progeny trials were established at the CTAHR Hamakua Research Station (2000′ elevation) on the Big Island and at the HARC Maunawili Research Station (600′ elevation), on Oahu. More than 178 accessions were evaluated for important seed and juvenile growth characteristics. These include seed size, seed shape, seed weight, seedling growth, juvenile growth, earliness of phyllode development, flowering pattern, duration of vegetative stage, tree form, and disease resistance.

The koa progenies clearly showed great variations for these traits. The variations observed between progenies are essentially genetic in origin and are useful in selecting superior progenies for tree improvement. For example, thirty months after planting of the SET 91-1 koa progeny trial, the average tree height was 6 m (ranging from 2 to 7.6), and the average DBH was 7.6 cm (ranging from 2 to 11). The fastest growing trees of a single family were 2PH1-1 and 2PK3-1 from Kauai, 1M6-2 and 1N2-5 from Oahu, and 5K1-2 from Maui with an average DBH of 15 cm in five years. The fast-growing koa families from these trials also included the collections from Puu Ka Pele areas of Kauai, Copp Road of Maui, and Kilauea Military Camp and Puu Waawaa of Hawaii. These areas are the important collecting sites of koa provenances for the reforestation programs. It is also equally important to avoid collecting the round-shaped seeds from Kauai since this Kauai genotype consistently showed slow growth in the trials.

Variations observed in these traits not only provide the bases for selection and tree improvement, but also provide information for the understanding of koa taxonomy and evolution. The three koa groups identified in the present study based on seed and seedling characteristics clearly support the taxonomy of Hillebrand (1888) and Lamoureux (1971) for the treatment of *A. kauaiensis* and Rock (1919) and St. John (1979) for the treatments of some koa

varieties. The grouping also seems to have some geographic partitioning in addition to morphological similarilities. This suggests that there may be some evolutionary significance in these groups. For this reason, it may be expected that other traits such as wood characters, phenology, and even molecular markers may also tend to be classified into the same groups.

After five years, most trees from the families in SET 91-1 have set seeds. More than ten of these families were selected for advanced progeny testing based on growth and tree form. The advanced progeny trials will be carried out at Maunawili of Oahu and Hamakua of Hawaii in 1997.

In the present study, silviculture practice of the pure and koa/leucaena mixed koa plots were also tested to see there is any beneficials for the mixed plots. The result showed that trees in the mixed plots grew slower than koa trees grown alone. It clearly suggests that the idea of using other fast-growing trees such as *Leucaena* as a guide tree is not practical.

Although symptoms of various disease and insect damages were noticed, they have not been fully evaluated among these trials. The koa rust was observed at the higher elevation site of Hamakua. The rust symptoms were often noticed on progenies collected from the Big Island. The 'koa blight' or 'koa sudden decline' was found at both lower and higher elevation sites, but it is more serious at the lower

elevation site of Maunawili, Oahu. The trees were first attacked by black twig borer beetles, and died with months. It was noticed that the trees in SET 94-3 at Maunawili began to show decline after one and half year of planting. After two year's growth, more than 50% of koa trees showed decline, most of them died. At Hamakua, beetles attack affected only the collections from Waianae and North shore area of Oahu in SET 93-1. The cause of this sudden decline has not yet been identified. It is suspected that the beetle attack may be related to the tree physiological status caused by root rot disease of *Fusarium spp.*, or by drought stress, or by fungi carried by the beetles. It will be important to determine the cause of this sudden decline disease and to select resistant or tolerant koa progenies.

APPENDIX A

Listed K number (or code), taxon, origin, latitude, logitude, PI number, ID number, and elevation of studied *Leucaena* accessions.

K No. Code†	TAXON	ORIGIN		LAT.	LONG.	PI No.	ID No.	ELEV.
L. collinsii ssp. collinsii (2n=52)								
456	coll	Chiapas	Mexico	16.45N	93.07W	443515	78-52c	750e
461	coll	Chiapas	Mexico	16.45N	93.07W	443516	78-57c	750e
905	coll	Chiapas	Mexico	16.45N	93.07W			
L. collinsii ssp. zacapana (2n=52)								
740	coll	El Progreso	Guatemala	15.02N	89.40W		CPI	480
914	coll	Zacapa	Guatemala	14.56N	89.31W		88-8	225
917	coll	Chiquimula	Guatemala	14.36N	89.40W		88-11	750
995	coll	Guatemala	Guatemala	14.54N	90.07W		P38	
L. cuspidata (2n=112)								
745		Puebla	Mexico	17.38N	97.37W			2400
89/92		Hidalgo	Mexico					
L. diversifolia ssp. trichandra (2n=52, diploids)								
399	div2		Cameroons					
749	div2		Australia				CPI46568	
907	div2	Quetzaltenango	Guatemala	14.43N	91.32W		88-1b	1600
909	div2	Guatemala	Guatemala	14.40N	90.26W		88-3	1200
919	div2	Jalapa	Guatemala	14.38N	89.48W		88-13	1500
926	div2	Jutiapa	Guatemala	14.17N	89.59W		88-20	1200
927	div2	Santa Rosa	Guatemala	14.24N	90.26W		88-22	1250
936	div2	Comayagua	Honduras	14.15N	87.28W		88-32	1000
964	div2	Guatemala	Guatemala	14.44N	90.21W		F3	
L. diversifolia ssp. diversifolia (2n=104, tetraploids)								
156	div4	Veracruz	Mexico	18.56N	97.00W	324356	67-35	1225e
783	div4	Veracruz	Mexico	18.52N	97.03W		85-14	1175e
785	div4	Veracruz	Mexico	18.52N	97.03W		85-16	1175e
946	div4	Veracruz	Mexico	19.26N	96.45W		45/87	800
947	div4	Veracruz	Mexico	19.32N	96.55W		46/87	1275
L. esculenta ssp. esculenta (2n=52)								
812	escu	Puebla	Mexico	18.20N	97.25W		85-45	1375e
948	escu	Guerrero	Mexico	18.18N	99.45W		47/87	1550
949	escu	Michoacan	Mexico	18.38N	100.48W		48/87	600
838	escu	Guerrero	Mexico	17.50N	99.35W		85-71	500e
950	escu	Guerrero	Mexico	17.51N	99.40W		49/87	650
L. greggii (2n=56)								
853	greg	Nuevo Leon	Mexico	24.40N	99.55W		VS85-7	1830e
855	greg	Nuevo Leon	Mexico	24.50N	100.05W		VS85-9c	1830e
856	greg	Nuevo Leon	Mexico	24.55N	100.05W		VS85-10c	1800e
859	greg	Coahuila	Mexico	24.50N	100.05W		VS85-13	1880
862	greg	Nuevo Leon	Mexico	25.45N	100.50W		VS85-16c	1830e
L. lanceolata ssp. lanceolata (2n=52)								
10	lanc	Nayarit	Mexico	21.50N	105.07W	286248		100e
385	lanc	Oaxaca	Mexico	16.36N	94.57W		77-43	150e
468	lanc	Chiapas	Mexico	16.15N	93.54W	443722	78-65c	275e
772	lanc	Veracruz	Mexico	19.15N	96.25W		JLB85-2C	100e
773	lanc	Veracruz	Mexico	19.15N	96.25W		85-3	100e
L. lanceolata ssp. sousae (2n=52)								
951	lanc	Oaxaca	Mexico	16.02N	97.35W		50/87	50
952	lanc	Oaxaca	Mexico	15.40N	96.30W		51/87	50

K No. Code	TAXON	ORIGIN		LAT.	LONG.	PI No.	ID No.	ELEV.
L. leucocephala ssp. leucocephala (2n=104)								
26	leuc	St. Croix	Virgin Islands			281605		
335	leuc	Yucatan	Mexico	20.27N	90.02W	342959		50e
400	leuc		Cameroons					
997	leuc	Oahu	USA	22.50N	157.45W			
L. leucocephala ssp. glabrata (2n=104)								
8	leuc	Zacatecas	Mexico	21.16N	103.10W	263695		1100e
397	leuc	Yantepec	Mexico				77-55	
420	leuc	Morazan	Salvador	13.37N	88.01W	443483	78-15c	275e
500	leuc		Australia					
565	leuc	Colima	Mexico	19.13N	103.42W	443631	3256	485e
584	leuc	Veracruz	Mexico	19.46N	96.25W	443643	3280	25e
608	leuc	Tamaulipas	Mexico	24.51N	98.10W	443664	3306	60e
612	leuc	Tamaulipas	Mexico	25.32N	97.43W	443668		15e
636	leuc	Coahuila	Mexico	25.25N	101.00W	443740	3336	1575e
638	leuc	Nuevo Leon	Mexico	25.40N	100.15W	443692	3338	500e
L. macrophylla (2n=52)								
158	macr	Veracruz	Mexico	18.25N	95.17W	324382	67-37	150e
836	macr	Morelos	Mexico	18.54N	99.58W		85-69c	1400e
902	macr	Oaxaca	Mexico	15.59N	97.16W		47/85C.H.	43
39/89	macr	Oaxaca	Mexico	15.58N	97.10W			
55/88	macr	Guerrero	Mexico	18.00N	101.18W			
L. multicapitula (2n=52)								
880	mult	Guanacaste	Costa Rica	11.05N	85.37W		BLSF#1758	10
955	mult	Los Santos/Hrre	Panama	7.55N	80.25W		81/87	30
959	mult	Penas Blancas	Costa Rica	11.10N	85.37W		86/81	
L. pallida (2n=104)								
178	pall	Oaxaca	Mexico	17.15N	96.51W	324367	67-58	1750e
376	pall	Oaxaca	Mexico	17.08N	96.46W		77-33c	1675e
748	pall	Oaxaca	Mexico				CPI84581	
804	pall	Puebla	Mexico	18.37N	97.24W		85-36	2000e
806	pall	Puebla	Mexico	18.37N	97.24W		85-38	2000e
819	pall	Oaxaca	Mexico	17.21N	96.50W		85-52	1925e
953	pall	Puebla	Mexico	18.38N	97.24W		52/87	2100
L. pulverulenta (2n=56)								
957	pulv	Tamaulipas	Mexico	23.36N	99.14W		83/87	1250
958	pulv	Texas	USA	27.35N	97.50W		84/87	
960	pulv	Texas	USA	27.45N	97.57W		TX1001	
L. retusa (2n=56)								
502	retu	Texas	USA	29.33N	103.05W	435920	79-2	915
506	retu	Texas	USA	29.51N	102.48W	435924	79-6	800
899	retu	Texas	USA	29.44N	102.43W			
900	retu	Texas	USA	30.37N	104.03W			
23/86		Coahuila	Mexico	28.44N	102.20W			
L. salvadorensis (2n=56)								
904	salv	Choluteca	Honduras	13.26N	87.11W		17/86	600
933	salv	Choluteca	Honduras	13.19N	87.03W		88-29	400
7/91	salv	Esteli	Nicaragua	13.12N	86.29W			
34/88	salv	Choluteca	Honduras	13.15N	87.06W			
L. shannonii (2n=52)								
441	shan	Campeche	Mexico	19.47N	90.30W	443728	78-36c	15e
916	shan	Chiquimula	Guatemala	14.36N	89.38W		88-10	770
941	shan	Chiquimula	Guatemala	14.36N	89.34W		88-37	800
924	shan	Jutiapa	Guatemala	14.22N	89.43W		88-18	500
925	shan	Jutiapa	Guatemala	14.16N	89.56W		88-19	900
930	shan		Honduras				88-26	
954	shan	Campeche	Mexico	19.20N	90.43W		53/87	10

K No. Code	TAXON	ORIGIN		LAT.	LONG.	PI No.	ID No.	ELEV.
L. sp. (2n=?)								
5/91	L. sp.	Olancho	Honduras	15.25N	86.50W			
87/92		Sonara	Mexico					
L. trichodes (2n=52)								
90	tric		Venezuela	10.35N	66.56W	317914		750e
738	tric	Cesar	Colombia	10.33N	73.12W		CIAT8814	200
751	tric		Australia				CPI86144	
903	tric	Trujillo	Venezuela	9.38N	70.18W		2/86C.H.	700

†: K number refers to accession number used by the University of Hawaii and code refers to accession number used by the Oxford Forestry Institute.

APPENDIX B

Designation of Acacia koa collection was based on Dr. Brewbaker's description. For example, 1LA2-3, the first numeric character 1 represents collection from Oahu (1=Oahu, 2=Kauai, 4=Lanai, 5=Maui, and 6=Hawaii); LA is abbreviation of area collected: LANIPO TRAIL; the fourth numeric character represents the provenance number; if it follows by a number, e.g., -3, which means the third individual tree or progeny collected from this provenance; if it follows by C that means a composite was collected. All koa collections were deposited at Hawaii Seed Foundation Facility.

Listed collection ID, 100 seed weight (g), location collected, island, collectors, year collected, and indication of collection planted in a series of SET trials.

Coll.	Wt. (g) 100 seeds	Location	Island	Collector	Year	Set Trials 91 1	92 1	93 1	94 1	94 3	95 1	95 2
1LA1-1	7.50	Lanipo Trail	Oahu	Brewbaker	1974							
1LA1-2	4.04	Lanipo Trail	Oahu	Brewbaker	1974							
1LA1-3	5.90	Lanipo Trail	Oahu	Brewbaker	1974							
1LA2-1	4.96	Lanipo Trail	Oahu	Brewbaker	1974							
1LA2-2	5.26	Lanipo Trail	Oahu	Brewbaker	1974							
1LA2-3	7.44	Lanipo Trail	Oahu	Brewbaker	1974							
1LA2-4	3.85	Lanipo Trail	Oahu	Brewbaker	1974							
1LA2-5		Lanipo Trail	Oahu	Brewbaker	1974							
1M1-1	6.68	Lanipo Trail	Oahu	Brewbaker	1974							
1M1-2	3.63	Round Top Drive	Oahu	Brewbaker	1974							
1M1-3	5.07	Round Top Drive	Oahu	Brewbaker	1974							
1M1-4	3.42	Round Top Drive	Oahu	Brewbaker	1974							
1M2-1	6.52	Round Top Drive	Oahu	Brewbaker	1974	1						
1M2-2	5.32	Round Top Drive	Oahu	Brewbaker	1974	1						
1M3-1		Round Top Drive	Oahu	Brewbaker	1974							
1M3-2		Round Top Drive	Oahu	Brewbaker	1974							
1M3-3		Round Top Drive	Oahu	Brewbaker	1974							
1M4-1		Round Top Drive	Oahu	Brewbaker	1974							
1M6-1	4.73	Round Top Drive	Oahu	Brewbaker	1974							
1M6-2	5.81	Round Top Drive	Oahu	Brewbaker	1974	1						
1M6-3	4.95	Round Top Drive	Oahu	Brewbaker	1974							
1M6-4	3.60	Round Top Drive	Oahu	Brewbaker	1974							
1M7-1	4.48	Round Top Drive	Oahu	Brewbaker	1974	1						
1M7-2		Round Top Drive	Oahu	Brewbaker	1974							
1M8-1	4.50	Round Top Drive	Oahu	Brewbaker	1974							
1M8-2	4.10	Round Top Drive	Oahu	Brewbaker	1974							
1M8-3	3.42	Round Top Drive	Oahu	Brewbaker	1974	1						
1M8-4	3.74	Round Top Drive	Oahu	Brewbaker	1974	1						
1MC	4.10	Round Top Drive	Oahu	Brewbaker	1974	1						
1MA1-1	4.26	Manana	Oahu	Brewbaker	1974							
1MA1-2	4.22	Manana	Oahu	Brewbaker	1974							
1MA2-1	4.26	Manana	Oahu	Brewbaker	1974							
1MO2-1		Moanalua Valley	Oahu	Brewbaker	1974							
1MO2-2		Moanalua Valley	Oahu	Brewbaker	1974							
1MO2-3		Moanalua Valley	Oahu	Brewbaker	1974							
1MO4-1		Moanalua Valley	Oahu	Brewbaker	1974							
1MO4-2		Moanalua Valley	Oahu	Brewbaker	1974							
1N1-1	5.17	Nuuanu	Oahu	Brewbaker	1974							
1N1-2	4.73	Nuuanu	Oahu	Brewbaker	1974	1						
1N1-3	6.98	Nuuanu	Oahu	Brewbaker	1974	1						
1N1-4	5.00	Nuuanu	Oahu	Brewbaker	1974	1						
1N1-5	5.20	Nuuanu	Oahu	Brewbaker	1974	1						
1N2-1	6.32	Nuuanu	Oahu	Brewbaker	1974	1		3	4	5		
1N2-2	3.60	Nuuanu	Oahu	Brewbaker	1974	1						
1N2-3	6.40	Nuuanu	Oahu	Brewbaker	1974							
1N2-4	6.10	Nuuanu	Oahu	Brewbaker	1974	1						
1N2-5	5.10	Nuuanu	Oahu	Brewbaker	1974	1						7
1N4-1	4.63	Nuuanu	Oahu	Brewbaker	1974							
1N4-2	6.12	Nuuanu	Oahu	Brewbaker	1974	1						
1PU1-1	2.20	Pupukea	Oahu	Brewbaker	1974							

Coll.	Wt. (g) 100 seeds	Location	Island	Collector	Year	Set Trials 91 1	92 1	93 1	94 1	94 3	95 1	95 2
1PU1-2	3.90	Pupukea	Oahu	Brewbaker	1974	1	2	3				
1PU2-1	4.05	Pupukea	Oahu	Brewbaker	1974	1						
1PU2-2	3.32	Pupukea	Oahu	Brewbaker	1974							
1SL1-1	3.30	St. Louie Hgts.	Oahu	Brewbaker	1974	1						
1SL1-2	4.30	St. Louis Hgts.	Oahu	Brewbaker	1974							
1SL2-1	5.22	St. Louis Hgts.	Oahu	Brewbaker	1974							
1SL2-2	3.49	St. Louie Hgts.	Oahu	Brewbaker	1974							
1SL3-1	5.55	St. Louis Hgts.	Oahu	Brewbaker	1974	1						
1SL3-2	5.87	St. Louie Hgts.	Oahu	Brewbaker	1974							
1SL3-3	5.64	St. Louie Hgts.	Oahu	Brewbaker	1974							
1SL3-4	4.59	St. Louis Hgts.	Oahu	Brewbaker	1974	1						
1SL3-5	6.00	St. Louie Hgts.	Oahu	Brewbaker	1974	1						
1SL4-1	4.94	St. Louis Hgts.	Oahu	Brewbaker	1974							
1SL5-1	2.42	St. Louis Hgts.	Oahu	Brewbaker	1974							
1SL5-2	2.46	St. Louis Hgts.	Oahu	Brewbaker	1974							
1SL6-1	5.61	St. Louis Hgts.	Oahu	Brewbaker	1974							
2AW1-1	10.90		Kauai	Brewbaker	1975							
2DT1-1	11.90	Ditch Trail	Kauai	Brewbaker	1975	1						
2DT2-1	8.50	Ditch Trail	Kauai	Brewbaker	1975							
2HA1C	5.82		Kauai	Brewbaker	1975	1						
2HA2-1	14.00		Kauai	Brewbaker	1975							
2K1-1	12.20	Above Kokee Park	Kauai	Brewbaker	1975							
2KU1-1	8.00	Kumuwela Trail	Kauai	Brewbaker	1975	1						
2KU2-1	6.60	Kumuwela Trail	Kauai	Brewbaker	1975	1						
2KU3-1	9.40	Kumuwela Trail	Kauai	Brewbaker	1975	1						
2MA1-1	10.00	Makaha Ridge Road	Kauai	Brewbaker	1975							
2MA2-1	10.00	Makaha Ridge Road	Kauai	Brewbaker	1975	1						
2MA3-1	10.00	Makaha Ridge Road	Kauai	Brewbaker	1975	1						
2ML1-1	7.20	Milolii Ridge Road	Kauai	Brewbaker	1975	1	2	3				
2NU1-1	8.80	Nualolo Trail	Kauai	Brewbaker	1975							
2NU2-1		Nualolo Trail	Kauai	Brewbaker	1975							
2NU2C	6.12	Nualolo Trail	Kauai	Brewbaker	1975	1					6	7
2OV1-1	10.00	Overlook Trail	Kauai	Brewbaker	1975	1						
2PH1-1	7.68	Puu Hinahina Area	Kauai	Brewbaker	1975	1						
2PH2-1	6.52	Puu Hinahina Area	Kauai	Brewbaker	1975	1	2					
2PK3-1	5.15	Puu Ka Pele Area	Kauai	Brewbaker	1975	1					6	7
4M1-1	6.26	Munro Trail	Lanai	Brewbaker	1975							
4M1-2	7.73	Munro Trail	Lanai	Brewbaker	1975							
4M1-3	6.21	Munro Trail	Lanai	Brewbaker	1975							
4M1-4	5.17	Munro Trail	Lanai	Brewbaker	1975							
4M1-5	3.91	Munro Trail	Lanai	Brewbaker	1975							
5H1-1	4.17	Gully 8 miles north of Hana	Maui	Brewbaker	1975							
5HM1-1	7.72	Haliimaile	Maui	Brewbaker	1975	1						
5K1-1	3.57	Kipahulu	Maui	Brewbaker	1975	1						
5K1-2	4.78	Kipahulu	Maui	Brewbaker	1975	1						
5K1-3	5.08	Kipahulu	Maui	Brewbaker	1975							
5K1-4	5.76	Kipahulu	Maui	Brewbaker	1975							
5K1-5	3.90	Kipahulu	Maui	Brewbaker	1975							
5K1-6	3.94	Kipahulu	Maui	Brewbaker	1975	1						
5L1-1		Lahaina	Maui	Brewbaker	1975							
5L1-2	3.46	Lahaina	Maui	Brewbaker	1975							
5L1-3	4.22	Lahaina	Maui	Brewbaker	1975							
5L1-4	5.69	Lahaina	Maui	Brewbaker	1975							
5L2-1	2.78	Lahaina	Maui	Brewbaker	1975							
5L2-2	2.24	Lahaina	Maui	Brewbaker	1975							
5L2-3	6.21	Lahaina	Maui	Brewbaker	1975							
5OL1-2	4.94	Olinda	Maui	Brewbaker	1975	1						
5SH1-1	6.49	Seabully Hall	Maui	Brewbaker	1975							
01-0191	12.20	Puu Waawaa	Hawaii	USDA FS	?	1	2					
01-1288b	6.70		Hawaii	USDA FS	?	1	2					
01-1288c	14.20	Waikea	Hawaii	USDA FS	?	1						
01-1288d	14.40	Umikoa Ranch	Hawaii	USDA FS	?	1						
6KA1-1	6.59	Kaumana City	Hawaii	Brewbaker	1975	1						
NFTA890C	9.20		Hawaii	NFTA	1990	1						
NFTA891C	7.40		Hawaii	NFTA	1990	1						
6WA1-1	15.14	Waipunalei	Hawaii	Brewbaker	1975	1						
1WAK1-1		Maunauna Area (Trail 8)	Oahu	Sun & Austin	1992			3		5		
1WAK1-4		Maunauna Area (Trail 8)	Oahu	Sun & Austin	1992			3		5		
1WAK1-5		Maunauna Area (Trail 7)	Oahu	Sun & Austin	1992			3				
1WAI1C		Waianae Valley	Oahu	Sun & Austin	1992			3			6	7
1WAI2C		Waianae Valley	Oahu	Sun & Austin	1992			3				
6PUU1		Puu Waawaa, 4500 ft.	Hawaii	Kevin Grace	1992			3		5	6	7
6HAM1C		Hamakua Station	Hawaii	Dr. B. & Sun	1992			3		5	6	7
6HON1		Honaunau, 2000 ft	Hawaii	USDA FS	1983			3				

Coll.	Wt. (g) 100 seeds	Location	Island	Collector	Year	Set Trials 91 1	92 1	93 1	94 1	94 3	95 1	95 2
6KAU1		Kaumana City	Hawaii	USDA FS	1983							
6KEA1		Keanakolu	Hawaii	USDA FS	1983			3				
6KUK1		Kukaiau	Hawaii	USDA FS	1983			3				
6KUK2		Kukaiau, 5200 ft.	Hawaii	USDA FS	1983			3				
2K3-1	12.20	Above Kokee Park	Kauai	Dr.B.,Key & Sun	1994					5		
2KU1-1		Kumuwela Trail	Kauai	Dr.B.,Key & Sun	1994				4	5		
2KU1-1.1		Kumuwela Trail	Kauai	Dr.B.,Key & Sun	1994				4	5		
2KU1-C1	9.00	Kumuwela Trail	Kauai	Dr.B.,Key & Sun	1994				4	5		
2KU1-C2		Kumuwela Trail	Kauai	Dr.B.,Key & Sun	1994							
2KU1C3	6.00	Kumuwela Trail	Kauai	Dr.B.,Key & Sun	1994				4	5	6	7
2KU1-C3	5.60	Kumuwela Trail	Kauai	Dr.B.,Key & Sun	1994							
2MA1-1	5.80	Makaha Ridge Road	Kauai	Dr.B.,Key & Sun	1994				4	5		
2MA1-2	7.60	Makaha Ridge Road	Kauai	Dr.B.,Key & Sun	1994				4	5		
2MA1-3	7.00	Makaha Ridge Road	Kauai	Dr.B.,Key & Sun	1994				4	5		
2MA2-1	7.60	Makaha Ridge Road	Kauai	Dr.B.,Key & Sun	1994				4	5		
2MA2-2	8.40	Makaha Ridge Road	Kauai	Dr.B.,Key & Sun	1994					5		
2MA2-2+	7.20	Makaha Ridge Road	Kauai	Dr.B.,Key & Sun	1994				4	5		
2MA2-3	12.20	Makaha Ridge Road	Kauai	Dr.B.,Key & Sun	1994					5		
2MA3-1	12.80	Makaha Ridge Road	Kauai	Dr.B.,Key & Sun	1994				4	5		
2MA3-2	9.20	Makaha Ridge Road	Kauai	Dr.B.,Key & Sun	1994					5		
2MA3-3	11.20	Makaha Ridge Road	Kauai	Dr.B.,Key & Sun	1994					5		
2MI1-1	4.80	Milolii Ridge Road	Kauai	Dr.B.,Key & Sun	1994				4	5		
2MI1-2	6.60	Milolii Ridge Road	Kauai	Dr.B.,Key & Sun	1994				4	5		
2MI1-3	8.60	Milolii Ridge Road	Kauai	Dr.B.,Key & Sun	1994				4	5		
2MI2-1	8.60	Milolii Ridge Road	Kauai	Dr.B.,Key & Sun	1994					5		
2MI2-2	15.40	Milolii Ridge Road	Kauai	Dr.B.,Key & Sun	1994					5		
2MI2-3	8.20	Milolii Ridge Road	Kauai	Dr.B.,Key & Sun	1994				4	5		
2MI2-4	12.80	Milolii Ridge Road	Kauai	Dr.B.,Key & Sun	1994				4	5		
2MI2-5	10.20	Milolii Ridge Road	Kauai	Dr.B.,Key & Sun	1994					5		
2MI2-6	10.00	Milolii Ridge Road	Kauai	Dr.B.,Key & Sun	1994					5		
2NU1-1	11.40	Nualolo Trail	Kauai	Dr.B.,Key & Sun	1994				4			
2NU1-2	10.80	Nualolo Trail	Kauai	Dr.B.,Key & Sun	1994					5		
2PH1-1	9.80	Puu Hinahina Area	Kauai	Dr.B.,Key & Sun	1994				4	5		
2PH1-2	5.20	Puu Hinahina Area	Kauai	Dr.B.,Key & Sun	1994				4	5		
2PH1-3	7.00	Puu Hinahina Area	Kauai	Dr.B.,Key & Sun	1994				4	5		
2PH2-1	10.60	Puu Hinahina Area	Kauai	Dr.B.,Key & Sun	1994					5		
2PH2-2	4.40	Puu Hinahina Area	Kauai	Dr.B.,Key & Sun	1994				4	5	6	7
2PH2-3		Puu Hinahina Area	Kauai	Dr.B.,Key & Sun	1994					5		
2PK2-1	5.40	Puu Ka Pele Area	Kauai	Dr.B.,Key & Sun	1994				4	5	6	7
2PK2-1.1	5.60	Puu Ka Pele Area	Kauai	Dr.B.,Key & Sun	1994				4	5		
2PK2-2	5.60	Puu Ka Pele Area	Kauai	Dr.B.,Key & Sun	1994				4	5		
2W1-1	4.20	Waimea Canyon Drive	Kauai	Dr.B.,Key & Sun	1994					5		
2W2-1	4.40	Waimea Canyon Drive	Kauai	Dr.B.,Key & Sun	1994				4	5		
2W2-2	1.72	Waimea Canyon Drive	Kauai	Dr. B. & Key	1994						6	7
2W3-1	10.00	Waimea Canyon Drive	Kauai	Dr.B.,Key & Sun	1994					5		
2W3-2	9.60	Waimea Canyon Drive	Kauai	Dr.B.,Key & Sun	1994				4	5		
6KMC1-1	7.80	Kilauea Mil. Camp	Hawaii	Dr. B. & Key	1994				4	5		
6KMC1-2	10.60	Kilauea Mil. Camp	Hawaii	Dr. B. & Key	1994				4	5		
6KMC1-3	9.60	Kilauea Mil. Camp	Hawaii	Dr. B. & Key	1994				4	5		
6KMC1-4	11.00	Kilauea Mil. Camp	Hawaii	Dr. B. & Key	1994				4	5		
6KMC1-5	6.80	Kilauea Mil. Camp	Hawaii	Dr. B. & Key	1994				4	5		
6KMC1C	15.40	Kilauea Mil. Camp	Hawaii	Dr. B. & Key	1994				4	5		
6KMC2C	8.40	Kilauea Mil. Camp	Hawaii	Dr. B. & Key	1994				4	5		
6KMC3C	10.00	Kilauea Mil. Camp	Hawaii	Dr. B. & Key	1994				4	5		
6MLR1-1	16.80	Mauna Loa Road	Hawaii	Dr. B. & Key	1994				4	5		
6MLR1-2	10.80	Mauna Loa Road	Hawaii	Dr. B. & Key	1994				4			
6MLR2C	13.00	Mauna Loa Road	Hawaii	Dr. B. & Key	1994				4	5		
6MLR3C	11.60	Mauna Loa Road	Hawaii	Dr. B. & Key	1994				4	5		
6MLR4C	13.20	Mauna Loa Road	Hawaii	Dr. B. & Key	1994				4	5		
6MLR5-1	14.20	Mauna Loa Road	Hawaii	Dr. B. & Key	1994				4	5	6	7
6MLR6C	11.60	Mauna Loa Road	Hawaii	Dr. B. & Key	1994				4	5		
6MLR7-1	7.80	Mauna Loa Road	Hawaii	Dr. B. & Key	1994				4	5		
6MLR7-2	12.60	Mauna Loa Road	Hawaii	Dr. B. & Key	1994				4	5		
6MLR8-1		Mauna Loa Road	Hawaii	Dr. B. & Key	1994							
1PP1C	4.40	Pacific Palisadee	Oahu	USDA FS	1990						6	7
1PT1C	5.60	Puupia Trail (Manoa Valley)	Oahu	Sun & Kamemoto	1995						6	7
2AA1-1		Awaawapuhi Trail	Kauai	Sun & Kamemoto	1995							
2AA1-2	11.80	Awaawapuhi Trail	Kauai	Sun & Kamemoto	1995							
2AA1-3		Awaawapuhi Trail	Kauai	Sun & Kamemoto	1995							
2AA1-4	11.40	Awaawapuhi Trail	Kauai	Sun & Kamemoto	1995							
2AA1-5	13.00	Awaawapuhi Trail	Kauai	Sun & Kamemoto	1995						6	7
2AA2-1	12.00	Awaawapuhi Trail	Kauai	Sun & Kamemoto	1995							
2AA2-2	9.60	Awaawapuhi Trail	Kauai	Sun & Kamemoto	1995							
2AA2-3	9.40	Awaawapuhi Trail	Kauai	Sun & Kamemoto	1995							

Coll.	Wt. (g) 100 seeds	Location	Island	Collector	Year	Set Trials 91 1	92 1	93 1	94 1	94 3	95 1	95 2
2AA2-4	7.40	Awaawapuhi Trail	Kauai	Sun & Kamemoto	1995							
2AA2-5	9.00	Awaawapuhi Trail	Kauai	Sun & Kamemoto	1995							7
2AA2-6	7.00	Awaawapuhi Trail	Kauai	Sun & Kamemoto	1995							
2AA3-1	9.20	Awaawapuhi Trail	Kauai	Sun & Kamemoto	1995							
2AA3-2	11.20	Awaawapuhi Trail	Kauai	Sun & Kamemoto	1995							
2AA3-3	8.40	Awaawapuhi Trail	Kauai	Sun & Kamemoto	1995							
2AA3-4	7.00	Awaawapuhi Trail	Kauai	Sun & Kamemoto	1995							
2AA3-5	9.80	Awaawapuhi Trail	Kauai	Sun & Kamemoto	1995							
2HL1-1	6.20	Haeleele Ridge	Kauai	Sun & Kamemoto	1995						6	7
2HL1-1a	6.60	Haeleele Ridge	Kauai	Sun & Kamemoto	1995							
2K2-1	12.20	Above Kokee Park	Kauai	Sun & Kamemoto	1995							
2K2-2	14.20	Above Kokee Park	Kauai	Sun & Kamemoto	1995							7
2KH3-1	12.20	Kauhao Ridge	Kauai	Sun & Kamemoto	1995						6	
2KH3-2	9.00	Kauhao Ridge	Kauai	Sun & Kamemoto	1995							
2KH2-1	5.20	Kauhao Ridge	Kauai	Sun & Kamemoto	1995							7
2KW1-1	11.00	Kaaweiki Ridge	Kauai	Sun & Kamemoto	1995							7
2KW1-2	7.60	Kaaweiki Ridge	Kauai	Sun & Kamemoto	1995						6	7
2KW1-3	5.00	Kaaweiki Ridge	Kauai	Sun & Kamemoto	1995							
2KW1-4	8.40	Kaaweiki Ridge	Kauai	Sun & Kamemoto	1995						6	7
2KW2-1	5.80	Kaaweiki Ridge	Kauai	Sun & Kamemoto	1995						6	7
2KW2-2	7.20	Kaaweiki Ridge	Kauai	Sun & Kamemoto	1995						6	7
2KW2-3		Kaaweiki Ridge	Kauai	Sun & Kamemoto	1995							
2KW2-4a		Kaaweiki Ridge	Kauai	Sun & Kamemoto	1995							
2KW2-4b	10.60	Kaaweiki Ridge	Kauai	Sun & Kamemoto	1995							
2KW3-1	7.20	Kaaweiki Ridge	Kauai	Sun & Kamemoto	1995						6	7
2MI1-1	10.80	Mililii Ridge Road	Kauai	Sun & Kamemoto	1995							
2MI1-2	8.60	Mililii Ridge Road	Kauai	Sun & Kamemoto	1995							
2MI2-1	15.40	Mililii Ridge Road	Kauai	Sun & Kamemoto	1995						6	7
2MI2-2	10.80	Mililii Ridge Road	Kauai	Sun & Kamemoto	1995							
2MI2-3	11.00	Mililii Ridge Road	Kauai	Sun & Kamemoto	1995							
2MI3-1	9.40	Mililii Ridge Road	Kauai	Sun & Kamemoto	1995							
2MI3-2	10.20	Mililii Ridge Road	Kauai	Sun & Kamemoto	1995							
2MI3-3	12.80	Mililii Ridge Road	Kauai	Sun & Kamemoto	1995							
2MI4-1	14.20	Mililii Ridge Road	Kauai	Sun & Kamemoto	1995							
2MI4-2	13.60	Mililii Ridge Road	Kauai	Sun & Kamemoto	1995							
2MI4-3	11.00	Mililii Ridge Road	Kauai	Sun & Kamemoto	1995							
2MI4-4	10.40	Mililii Ridge Road	Kauai	Sun & Kamemoto	1995							
2MI5-1	12.60	Mililii Ridge Road	Kauai	Sun & Kamemoto	1995							
2MI5-2	10.40	Mililii Ridge Road	Kauai	Sun & Kamemoto	1995							
2MI5-3	9.80	Mililii Ridge Road	Kauai	Sun & Kamemoto	1995							
2MI5-4	8.60	Mililii Ridge Road	Kauai	Sun & Kamemoto	1995							
2MI5-5	10.20	Mililii Ridge Road	Kauai	Sun & Kamemoto	1995							
2MI6-1	11.00	Mililii Ridge Road	Kauai	Sun & Kamemoto	1995							
2MI7-1	11.40	Mililii Ridge Road	Kauai	Sun & Kamemoto	1995							
2MI7-2	11.20	Mililii Ridge Road	Kauai	Sun & Kamemoto	1995							7
2PH1-1	12.40	Puu Hinahina Area	Kauai	Sun & Kamemoto	1995							7
2PH1-2	15.60	Puu Hinahina Area	Kauai	Sun & Kamemoto	1995							7
2PH2-1	10.80	Puu Hinahina Area	Kauai	Sun & Kamemoto	1995							
2PH2-2	11.60	Puu Hinahina Area	Kauai	Sun & Kamemoto	1995							
2PH2-3	7.80	Puu Hinahina Area	Kauai	Sun & Kamemoto	1995						6	7
2PH2-4	11.60	Puu Hinahina Area	Kauai	Sun & Kamemoto	1995							
2PK1-1	12.80	Puu Ka Pele Area	Kauai	Sun & Kamemoto	1995							
2PK1-2	6.60	Puu Ka Pele Area	Kauai	Sun & Kamemoto	1995						6	7
2PK1-3	7.40	Puu Ka Pele Area	Kauai	Sun & Kamemoto	1995						6	7
2PK1-4	13.00	Puu Ka Pele Area	Kauai	Sun & Kamemoto	1995							
2PK2-1a	13.00	Puu Ka Pele Area	Kauai	Sun & Kamemoto	1995							
2PK2-1b	13.00	Puu Ka Pele Area	Kauai	Sun & Kamemoto	1995						6	7
2PK2-2	7.80	Puu Ka Pele Area	Kauai	Sun & Kamemoto	1995						6	7
2PK2-3	6.80	Puu Ka Pele Area	Kauai	Sun & Kamemoto	1995							
2PK3-1a	11.00	Puu Ka Pele Area	Kauai	Sun & Kamemoto	1995							
2PK3-1b	7.00	Puu Ka Pele Area	Kauai	Sun & Kamemoto	1995						6	7
2PK3-2	6.40	Puu Ka Pele Area	Kauai	Sun & Kamemoto	1995							
2PK3-3	6.40	Puu Ka Pele Area	Kauai	Sun & Kamemoto	1995							
2PK3-4	12.20	Puu Ka Pele Area	Kauai	Sun & Kamemoto	1995							7
2PO1-1	10.20	Polihale Ridge	Kauai	Sun & Kamemoto	1995							
2PO1-2	14.40	Polihale Ridge	Kauai	Sun & Kamemoto	1995						6	7
2PO2-1a	9.00	Polihale Ridge	Kauai	Sun & Kamemoto	1995							
2PO2-1b	9.80	Polihale Ridge	Kauai	Sun & Kamemoto	1995							
2PO2-2	11.60	Polihale Ridge	Kauai	Sun & Kamemoto	1995							
2PO2-3	11.00	Polihale Ridge	Kauai	Sun & Kamemoto	1995							
2PO3-1	4.60	Polihale Ridge	Kauai	Sun & Kamemoto	1995							
2PO3-2	12.60	Polihale Ridge	Kauai	Sun & Kamemoto	1995							
2W3-1	8.80	Waimea Canyon Drive	Kauai	Sun & Kamemoto	1995							
2W3-2	9.20	Waimea Canyon Drive	Kauai	Sun & Kamemoto	1995							

Coll.	Wt. (g) 100 seeds	Location	Island	Collector	Year	Set Trials 91 1	92 1	93 1	94 1	94 3	95 1	95 2
2W3-3	10.20	Waimea Canyon Drive	Kauai	Sun & Kamemoto	1995							
2W3-4	6.00	Waimea Canyon Drive	Kauai	Sun & Kamemoto	1995						6	7
2W4-1	11.80	Waimea Canyon Drive	Kauai	Sun & Kamemoto	1995							
2W4-2	7.60	Waimea Canyon Drive	Kauai	Sun & Kamemoto	1995						6	7
2W4-3	7.40	Waimea Canyon Drive	Kauai	Sun & Kamemoto	1995						6	7
2W4-4	9.20	Waimea Canyon Drive	Kauai	Sun & Kamemoto	1995							
2WT1-1	15.40	Waimea Trail	Kauai	Sun & Kamemoto	1995							7
2WT1-2	12.60	Waimea Trail	Kauai	Sun & Kamemoto	1995							
2WT1-3	13.20	Waimea Trail	Kauai	Sun & Kamemoto	1995							
2WT1-4a	14.46	Waimea Trail	Kauai	Sun & Kamemoto	1995						6	7
2WT1-4b	10.20	Waimea Trail	Kauai	Sun & Kamemoto	1995							
2WT1-5	10.20	Waimea Trail	Kauai	Sun & Kamemoto	1995							
5CP1-1	7.20	Copp Road	Maui	Sun & Kamemoto	1995							
5CP1-2	8.00	Copp Road	Maui	Sun & Kamemoto	1995						6	7
5CP1-3	7.60	Copp Road	Maui	Sun & Kamemoto	1995						6	7
5CP1-4		Copp Road	Maui	Sun & Kamemoto	1995							
5CP1-5	9.80	Copp Road	Maui	Sun & Kamemoto	1995						6	7
5GUL1C		Honomanu Bay	Maui	Dr. B., Sun & Kam	1995						6	7
5GUL2C		Honomanu Bay	Maui	Dr. B., Sun & Kam	1995						6	7
5KH1C	5.70	Kuiaha	Maui	USDA FS	1990						6	7
5KO1-1	6.40	Kokomo Road (Hill side)	Maui	Sun & Kamemoto	1995						6	7
5KP1C	5.70	Kaupo Forest, 1400 ft	Maui	USDA FS	1990						6	7
5MA1-1	8.20	Hamana Road (below Olinda)	Maui	Dr. B., Sun & Kam	1995							
5MA1-2	5.80	Hamana Road (below Olinda)	Maui	Dr. B., Sun & Kam	1995						6	7
5MA1-3	7.00	Hamana Road (below Olinda)	Maui	Dr. B., Sun & Kam	1995							
5SN1-1		Sanitorium	Maui	Sun, Kamemoto & R	1995							
5SN1-2	5.00	Sanitorium	Maui	Sun, Kamemoto & R	1995						6	7
5SN1-3		Sanitorium	Maui	Sun, Kamemoto & R	1995							
5SN1-4	4.32	Sanitorium	Maui	Sun, Kamemoto & R	1995							
6HK1C	11.50	Hakalau (Triangle)	Hawaii	USDA FS	1991						6	7
6HK2C	11.10	Hakalau (Woodland))	Hawaii	USDA FS	1993						6	7
6HU1C	5.80	Honaunau, 2700 ft	Hawaii	USDA FS	1983							
6HU2C	4.60	Honaunau, 2800 ft	Hawaii	USDA FS	1983							
6HU3C	6.10	Honaunau, 3700 ft	Hawaii	USDA FS	1983							
6HU4C	11.30	Honaunau, 4200 ft	Hawaii	USDA FS	1983							
6HU5C	10.00	Honaunau, 4500 ft	Hawaii	USDA FS	1983							
6HU6C	16.70	Honaunau, 4500 ft	Hawaii	USDA FS	1983							7
6KA1C	12.80	Kukaiau, 3800 ft	Hawaii	USDA FS	1983							
6KA2C	10.00	Kukaiau, 4500 ft	Hawaii	USDA FS	1983							
6KA4C	13.30	Kukaiau, 5200 ft	Hawaii	USDA FS	1983							
6KA5C	17.70	Kukaiau	Hawaii	USDA FS	1983							7
6KA3C	12.70	Kukaiau, 4500 ft	Hawaii	USDA FS	1983						7	
6KC1C	5.00	Kaumana City, 2500 ft	Hawaii	USDA FS	1990							
6KH1C	15.70	Keahou 4600 ft	Hawaii	USDA FS	1983						6	7
6KH2C	7.40	Keahou, 5450 ft	Hawaii	USDA FS	1983						6	
6KK1C	12.60	Keanakolu, 5600 ft	Hawaii	USDA FS	1983						6	
6KL1C	5.90	Koa for Kulani	Hawaii	USDA FS								
6KM1C	5.80	Kaumana City, 2500 ft	Hawaii	USDA FS	1990						6	7
6KP1C	8.65	Kapapala	Hawaii	USDA FS	1990						6	7
6KR1C	12.70	KK Ranch	Hawaii	USDA FS	1977							
6KU1C	17.30	Ka'u, 6820 ft	Hawaii	USDA FS	1991						6	7
6LP1C	10.35	Laupohoehoe, 4600 ft	Hawaii	USDA FS	1983							
6LP3C	13.10	Laupohoehoe, 5500 ft	Hawaii	USDA FS	1983							7
6LP2C	11.00	Laupohoehoe, 5000 ft	Hawaii	USDA FS	1983						6	
6MH1C	13.70	Magnetic Hill	Hawaii	USDA FS	1986							
6ML1C	13.90	Mauna Loa Road, 4300 ft	Hawaii	USDA FS	1983						6	
6ML2C	10.30	Mauna Loa Road, 4500 ft	Hawaii	USDA FS	1983						6	
6ML3C	14.60	Mauna Loa Road, 5200 ft	Hawaii	USDA FS	1983						6	
6ML4C	16.60	Mauna Loa Road	Hawaii	USDA FS	1983							7
6OF1C	6.50	Kaumana City, 2500 ft	Hawaii	Sun & Nick	1995						6	7
6PH1C	16.60	Piha, 7000 ft	Hawaii	USDA FS	1983							
6SAD1C		Saddle Road (9 mile)	Hawaii	USDA FS	1983						6	7
6WV1-1	10.20	Wood Valley	Hawaii	USDA FS	1991						6	7
No. of accessions collected: 234; No. of accessions planted: 178.						48	5	14	43	59	58	64

APPENDIX C

Average seed width (W), length (L), L/W ratio,and seed shape of 92 koa accessions.

Accession	Seed shape	Seed Traits (mm) Width	Length	L/W	Accession	Seed shape	Seed Traits (mm) Width	Length	L/W
1PP1C	O*	4.66	8.16	1.75	2W4-2	R*	5.84	8.98	1.54
1PT1C	O	4.54	9.08	2.00	2W4-3	O	5.24	10.44	1.99
2AA1-5	R	7.96	10.38	1.30	2WT1-1	R	7.98	9.72	1.22
2AA2-3	R	7.08	8.69	1.23	2WT1-4a	R	8.30	10.44	1.26
2AA2-5	R	7.53	8.56	1.14	2WT1-5	R	6.98	8.82	1.27
2AA2-6	R	6.93	8.42	1.21	5CP1-2	O	5.56	11.10	2.00
2AA3-3	R	6.08	8.40	1.38	5CP1-3	O	4.86	11.86	2.45
2AA3-5	R	7.01	9.83	1.41	5CP1-5	O	5.80	10.06	1.74
2HL1-1	O	4.91	9.87	2.01	5KH1C	O	5.00	9.00	1.81
2K2-2	R	8.60	10.40	1.21	5KO1-1	O	5.50	9.38	1.71
2KH2-1	O	4.64	10.04	2.17	5KP1C	O	5.34	9.72	1.82
2KH3-1	R	8.20	9.42	1.15	5MA1-2	O	4.76	10.68	2.25
2KW1-1	R	7.06	9.48	1.34	5SN1-2	O	4.48	9.12	2.04
2KW1-2	O	5.38	9.76	1.81	6HK1C	O	7.18	11.84	1.67
2KW1-4	O	5.14	10.96	2.14	6HK2C	O	7.72	11.92	1.57
2KW2-1	O	4.76	8.58	1.80	6HU1C	O	5.22	9.24	1.77
2KW2-2	O	4.58	10.98	2.42	6HU2C	O	4.60	8.24	1.78
2KW2-4b	R	7.01	9.28	1.32	6HU3C	O	5.52	9.86	1.79
2KW3-1	O	4.76	11.16	2.35	6HU4C	O	6.74	11.22	1.66
2MI1-1	R	7.44	10.28	1.39	6HU5C	O	7.08	10.00	1.42
2MI1-2	R	7.18	9.64	1.36	6HU6C	O	7.88	12.70	1.61
2MI2-1	R	8.66	11.14	1.29	6KA1C	O	7.42	11.46	1.55
2MI3-2	R	7.36	9.80	1.33	6KA2C	O	6.62	9.96	1.51
2MI4-2	R	7.36	11.26	1.54	6KA3C	O	6.60	11.02	1.68
2MI5-4	R	7.08	9.50	1.35	6KA4C	O	6.74	10.92	1.62
2MI6-1	R	6.98	10.76	1.56	6KA5C	O	7.86	13.40	1.71
2MI7-2	R	7.82	11.04	1.42	6KC1C	O	4.32	9.14	2.13
2PH1-1	R	8.35	9.32	1.12	6KH1C	O	7.12	11.42	1.60
2PH1-2	R	9.28	10.70	1.15	6KH2C	O	6.10	8.64	1.42
2PH2-2	R	7.40	10.00	1.36	6KK1C	O	6.70	9.92	1.48
2PH2-3	O	4.90	10.36	2.12	6KO1C	O	3.94	9.70	2.53
2PK1-1	R	8.08	10.14	1.26	6KM1C	O	4.42	9.95	2.25
2PK1-2	O	5.10	9.88	1.95	6KP1C	O	6.02	9.44	1.57
2PK1-3	O	4.96	11.60	2.35	6KR1C	O	7.04	10.84	1.55
2PK2-1b	R	8.04	11.30	1.41	6KU1C	O	7.48	12.34	1.65
2PK2-2	O	5.26	10.91	2.08	6OP1C	O	6.00	11.62	1.94
2PK3-1b	O	4.68	9.74	2.08	6OP2C	O	6.36	10.36	1.63
2PK3-4	R	7.48	9.14	1.22	6OP3C	O	7.34	10.64	1.45
2PO1-2	R	7.48	10.54	1.41	6MH1C	O	6.90	10.62	1.55
2PO2-1a	R	7.04	9.68	1.38	6MO1C	O	6.46	11.14	1.72
2PO2-2	R	7.60	10.54	1.39	6MO2C	O	6.54	10.10	1.55
2PO2-3	R	7.58	9.50	1.26	6MO3C	O	7.10	11.06	1.56
2W2-2	O	3.38	6.68	1.98	6MO4C	O	7.65	10.94	1.43
2W3-2	R	7.38	8.56	1.17	6OF1C	O	4.90	10.56	2.16
2W3-4	O	4.86	9.74	2.01	6PH1C	O	6.90	11.78	1.71
2W4-1	R	8.08	9.54	1.18	6WV1-1	O	6.48	11.10	1.71
Means							6.44	10.16	1.64
Min.							3.38	6.68	1.12
Max.							9.28	13.40	2.53
LSD 0.05							0.54		1.27

*: R and O represent the round-shaped seed and the oblong-shaped seed, respectively.

APPENDIX D

Average seedling height (Ht) of 81 *Acacia koa* collections from Oahu, Kauai, Maui, and Hawaii measured at two-, five-, and eleven-week-old ages in greenhouse at Waimanalo Research Station in 1995.

Acc.	Seed shape	Sdling color	Ht (cm) in weeks			Acc.	Seed shape	Sdling color	Ht (cm) in weeks		
			Two	Five	Eleven				Two	Five	Eleven
1PP1C	O*	P*	4.7	8.7	22.6	2PO2-3	R*	G*	4.6	8.1	14.7
1PT1C	O	P	4.0	9.5	26.0	2W2-2	O	P	3.0	8.3	30.9
2AA1-5	R	G	4.5	8.0	18.7	2W3-2	R	G	4.1	6.7	14.7
2AA2-3	R	G	4.1	8.7	16.1	2W3-4	O	P	4.7	9.1	31.2
2AA2-5	R	G	3.9	8.6	15.2	2W4-1	R	G	4.5	7.2	21.8
2AA2-6	R	G	4.0	8.3	12.0	2W4-2	R	G	4.2	7.6	23.5
2AA3-3	R	G	4.0	6.6	14.8	2W4-3	O	P	4.2	14.0	34.2
2AA3-5	R	G	3.7	6.8	12.1	2WT1-1	R	G	4.4	9.5	20.0
2HL1-1	O	P	3.9	9.0	30.3	2WT1-4a	R	G	4.6	11.3	23.2
2K2-2	R	G	4.0	7.0	16.3	2WT1-5	R	G	3.0	5.5	12.2
2KH2-1	O	P	3.3	7.2	22.4	5CP1-2	O	P	3.7	10.9	34.9
2KH3-1	R	G	4.0	7.5	14.8	5CP1-3	O	P	2.5	7.9	33.7
2KW1-1	R	G	3.8	6.0	13.0	5CP1-5	O	P	4.9	10.8	32.0
2KW1-2	O	P	2.9	8.5	30.4	5KH1C	O	P	3.4	6.9	28.5
2KW1-4	O	P	3.3	7.9	28.5	5KO1-1	O	PP	6.2	9.5	26.6
2KW2-1	O	P	2.4	7.1	32.3	5KP1C	O	P	4.1	13.5	37.1
2KW2-2	O	P	3.8	11.5	36.5	5MA1-2	O	P	3.7	10.1	32.9
2KW2-4b	R	G	3.9	8.9	17.1	5SN1-2	O	P	4.0	10.4	30.5
2KW3-1	O	P	4.2	9.5	32.3	6HK1C	O	G	5.7	13.3	29.2
2MI1-1	R	G	3.8	7.3	13.0	6HK2C	O	G	5.7	15.2	29.9
2MI1-2	R	G	3.7	6.7	12.9	6HU5C	O	G	4.4	10.8	
2MI2-1	R	G	4.3	9.1	18.9	6KA2C	O	G	2.6	10.6	
2MI3-2	R	G	3.4	7.8	18.3	6KA3C	O	G	5.2	13.4	
2MI4-2	R	G	3.9	6.8	14.5	6KA4C	O	G	3.0	10.8	
2MI5-4	R	G	4.1	6.0	11.8	6KH1C	O	G	4.8	13.6	39.9
2MI6-1	R	G	4.8	7.7	12.9	6KH2C	O	G	4.8	13.4	35.8
2MI7-2	R	G	4.1	8.0	21.0	6KK1C	O	G	4.8	13.0	29.0
2PH1-1	R	G	4.3	8.2	16.5	6KM1C	O	G	4.9	12.5	29.7
2PH1-2	R	G	4.0	8.1	20.2	6KP1C	O	G	6.0	18.0	36.6
2PH2-2	R	G	3.7	5.6	14.3	6KU1C	O	G	4.4	14.3	
2PH2-3	O	P	4.1	9.3	28.6	6LP1C	O	G	4.8	9.5	25.1
2PK1-1	R	G	4.0	6.6	23.0	6LP2C	O	G	4.2	10.6	
2PK1-2	O	P	3.6	7.2	29.6	6MH1C	O	G	4.6	9.3	
2PK1-3	O	P	4.0	7.6	26.2	6ML1C	O	G	4.4	12.6	25.7
2PK2-1b	R	G	4.0	8.2	14.6	6ML2C	O	G	5.0	11.3	24.0
2PK2-2	O	P	2.6	6.4	28.4	6ML3C	O	G	3.8	10.2	
2PK3-1b	O	P	3.4	9.9	30.1	6ML4C	O	G	3.6		
2PK3-4	R	G	4.0	6.5	20.6	6OF1C	O	G	5.1	13.8	35.5
2PO1-2	R	G	3.7	9.0	24.0	6PH1C	O	G	4.2	10.6	23.6
2PO2-1a	R	G	3.8	7.2	16.6	6WV1-1	O	G	6.1	15.4	37.9
2PO2-2	R	G	3.7	7.3	15.1						
Means									4.1	9.4	24.0
Min.									2.4	5.5	11.8
Max.									6.2	18.0	39.9
LSD 0.05									0.9	1.5	4.4

*: O indicates the oblong-shaped seeds, R indicates the round-shaped seeds, P indicates the purple seedlings, and G indicates the green seedlings

APPENDIX E

E.1

SET 91-1 *Acacia koa* Progeny Trial

Four month old seedlings of Acacia koa were raised at Waimanalo Station on Oahu from Jan. to May, 1991. The seedlings were transported to Hamakua Station on the Island of Hawaii on 22 May 1991 and transplanted on 23 May 1991. A total of 48 progenies and composites of koa were tested. An augment design of randomized complete block with two replications was employed. Twenty two progenies and composites were replicated. Spacing was 1 x 1.5 meters. Plot size was 3 x 5 sq. meters and ten seedlings were planted in each plot. Whole trial occupied the area of 18 x 85 sq. meters. Due to the limited seedlings of some progenies, only five trees were planted. Field plots were arranged as following:

PLOT LAYOUT

```
|--------------------|
| x  x  x  x  x | 3 m
| x  x  x  x  x |
|--------------------|
        5 m
```

TREE NO. ORDER

```
|--------------------|
| 5  4  3  2  1 | 3 m
| 6  7  8  9  10|
|--------------------|
        5 m
```

FIELD TRIAL LAYOUT

```
                Bob wheeler's trial      Up. slope
-------------------------------------------------------------------
101 112 113 124 125 136 137 148 149 160 201 212 213 224 225 236 237 |
102 111 114 123 126 135 138 147 150 159 202 211 214 223 226 235 238 |
103 110 115 122 127 134 139 146 151 158 203 210 215 222 227 234 239 18
104 109 116 121 128 133 140 145 152 157 204 209 216 221 228 233 239 m
105 108 117 120 129 132 141 144 153 156 205 208 217 220 229 232 239 |
106 107 118 119 130 131 142 143 154 155 206 207 218 219 230 231 239 |
-------------------------------------------------------------------
|-- --- --- --- --- --- --- --- 85 m--- --- --- --- --- --- --- ---|
                ▼ Facing sea      Bottom slope
```

Field plot No. and corresponding progeny or composite

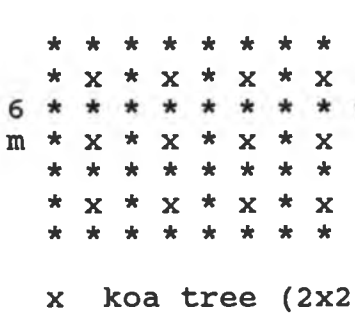
101 1M6-1	121 2KU1-1	141 6-0191	156 2DT2-1 L	212 2ML1-1	232 2KU1-1
102 1M8-3	122 2KU1-1	142 6-0191	2KU3-1 R	213 1SL3-1	233 6-1288b
103 1M8-4	123 2KU1-1	143 61299b	157 2MA2-1 L	214 6KA1-1	234 1SL3-5
104 1Mc	124 2KU2-1	144 61299b	5HM1-1 R	215 5K1-1	235 2KU2-1
105 1Mc	125 2MA3-1	145 6-1288c	158 6WA1-1 L	216 1PU1-2	236 2PH1-1
106 1N1-3	126 2ML1-1	146 6-1288c	N890C R	217 2ML1-1	237 2PK3-1
107 1N1-3	127 2ML1-1	147 6-1288d	159 N891C L	218 2HA1c	238 5OL1-2
108 1N1-4	128 2ML1-1	148 6-1288d	1PU2-1 R	219 1PU1-2	239 Mi.40 Ts.
109 1N1-5	129 2ML1-1	149 6KA1-1	160 mixed trees	220 2ML1-1	
110 1N2-1	130 2NU2c	150 6KA1-1	201 6-1288b	221 1N1-5	
111 1N4-2	131 2NU2c	151 1M2-1 L	202 1N1-3	222 2HA1C	
112 1PU1-2	132 2OV1-1	1M2-2 R	203 1Mc	223 2ML1-1	
113 1PU1-2	133 2PH1-1	152 1M7-1 L	204 2KU1-1	224 2KU1-1	
114 1PU1-2	134 2PH2-1	1N1-2 R	205 6-0191	225 6-1288d	
115 1S13-1	135 2PH2-1	153 1N2-2 L	206 6KA1-1	226 1PU1-2	
116 1S13-1	136 2PK3-1	1N2-4 R	207 6-1288d	227 1SL3-1	
117 1SL3-5	137 5K1-1	154 1N2-5 L	208 1Mc	228 2NU2c	
118 2HA1c	138 5K1-1	1SL1-1 R	209 2KU1-1	229 6-0191	
119 2HA1c	139 5K1-2	155 1SL3-4 L	210 1N1-4	230 2PH2-1	
120 2KU1-1	140 5OL1-2	5K1-6 R	211 6-1288c	231 6-1288c	

E.2

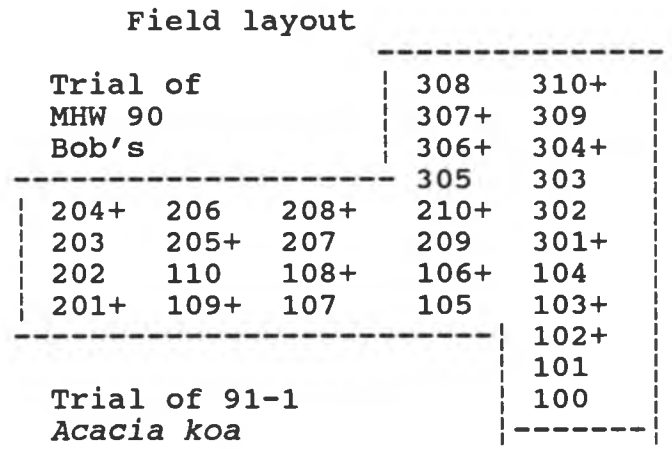
SET 92-2 *Acacia koa* & *Leucaena* Mixed Trial

Five koa progenies based on the evaluation of SET 91-1 trial on Hamakua Sation were selected for high value hardwood trial using a high land fast-growing *Leucaena* (K784) as a guide tree. Split-plot design was employed. Main plots were five koa progenies, and subplots were K784 interplanted with koa vs. the control. The main plot size was 6 x 8 meters and spacing for koa was 2 x 2 meters. The subpplot size was 4 x 6 meters and spacing for the mixed trees was 1 x 1 meter. Koa seedlings were three and K784 seedlings were four and half month old. Seedlings were raised at Waimanalo Research Station of UH on Oahu and transfered to Hamakua Station on the Island of Hawaii 27 May, 1992.

Plot layout



Field layout



>>> ROAD <<< Facing sea

Field plot No. and corresponding progeny

100 01-1188b + 1Pu1-2 + 2ML1 (1x2 9 trees each)

101 01-0191	201 1Pu1-2	301 01-1188b
102 01-0191	202 1Pu1-2	302 01-1188b
103 01-1188b	203 2ML1	303 2PH2
104 01-1188b	204 2ML1	304 2PH2
105 1Pu1-2	205 01-0191	305 01-0191
106 1Pu1-2	206 01-0191	306 01-0191
107 2ML1	207 2PH2	307 1Pu1-2
108 2ML1	208 2PH2	308 1Pu1-2
109 2PH2	209 01-1188b	309 2ML1
110 2PH2	210 01-1188b	310 2ML1

+ indicates that the plot was planted with *Leucaena* trees

E.3

SET 93-1 *Acacia koa* Progeny Trial

Forteen progenies and composites of *Acacia koa* collected from Oahu, Kauai and Hawaii were planted at Hamakua Station on 27 May 1993. Three month and half old seedlings were raised at Waimanalo Station on Oahu and transported to Hamakua Station on the Island of Hawaii. An augment design of randomized complete block with three replications was employed. Plot size was 3 x 8 sq. meters and 16 seedlings were painted in each plot. Field plots were arranged as following:

PLOT LAYOUT

```
--------    8 m    --------------
|  x  x  x  x  x  x  x  x
3
m  x  x  x  x  x  x  x  x
```

TREE NO. ORDER

```
9  10 11 12 13 14 15 16
8   7  6  5  4  3  2  1
```

ID	Source
1	1N2-1
2	1PU1-2
3	1WAK-1
4	1WAK-4
5	1WAK-5
6	1WAI-1
7	1WAI-2
8	2ML1
9	6PUUC
10	6HAM1
11	6HON1
12	6KAN1
13	6KEA1
14	6KUK1
15	6KUK2

FACING KAWAIHAE

Corresponding plot No. and ID

		312	305	304	
		3	5	4	
		311	306	303	
		13	15	10	REP III
		310	307	302	
		14	7	1	
	313	309	308	301	
	8	6	2	9	
	210	209	204	203	
	4	5	8	15	
	211	208	205	202	REP II
	14	9	3	2	
	212	207	206	201	
	6	13	10	7	
	110	109	104	103	
	10	9	4	3	
114	111	108	105	102	REP I
15	11	8	5	2	
113	112	107	106	101	
14	13	7	6	1	

FARTHEST FROM KAWAIHAE

<<< ROAD >>>

E.4

SET 94-1 *Acacia koa* Progeny Trial

Fourty three progenies and composites of *Acacia koa* collected from Kauai and Hawaii were planted at Hamakua Station on 26 May 1994. Three month old seedlings were raised at Waimanalo Station on Oahu and transported to Hamakua Sation on the Island of Hawaii. An augment design of randomized complete block with two replications was employed. Plot size was 3 x 5 sq. meters and ten seedlings were planted in each plot. Field plots were arranged as following:

PLOT LAYOUT

```
----  5 m  ----
|   x   x   x   x   x
3
m   x   x   x   x   x
```

TREE NO. ORDER

```
0  9  8  7  6

1  2  3  4  5
```

FIELD LAYOUT **(UP HILL)**

55 m.

108	109	124	125	140	141	211	212	227	228	243	¦
107	110	123	126	139	142	210	213	226	229	242	¦
106	111	122	127	138	143	209	214	225	230	241	24
105	112	121	128	137		208	215	224	231	240	m
104	113	120	129	136		207	216	223	232	239	¦
103	114	119	130	135	201	206	217	222	233	238	¦
102	115	118	131	134	202	205	218	221	234	237	¦
101	116	117	132	133	203	204	219	220	235	236	¦

Facing SET 93-1 koa trial

Field plot No. and corresponding progeny

101 2MI1-1	123 6MLR3C	201 2NU1-1a	223 2PH2-2
102 2PH1-2	124 6KMC1-1	202 2MA3-1	224 6KMC1-4
103 2KU1C3	125 6MLR1-2	203 2MI2-4	225 2MI1-1
104 2KU1-1a	126 6MLR2C	204 2PH1-1	226 6KMC1-2
105 2MA2-2a	127 6KMC3C	205 2W3-2	227 2MA2-1
106 2PK2-2	128 6KMC1C	206 6MLR8-1	228 2KU1-1
107 2MA1-3	129 6KMC2C	207 2PK2-2	229 2MA1-2
108 2MI1-2	130 6KMC1-2	208 6MLR2C	230 6MLR4C
109 2MA1-2	131 6KMC1-4	209 2MA1-3	231 2PH1-3
110 2MI2-3	132 6MLR1-1	210 6KMC2C	232 2MI2-3
111 2PK2-1	133 6MLR4C	211 2KU1C1	233 2MA1-1
112 2MA1-1	134 6KMC1-5	212 6KMC1-5	234 6KMC1-1
113 2W2-1	135 6MLR7-2	213 2PK2-1a	235 2PK2-1
114 2PH1-3	136 6MLR8-1	214 2KU1-1a	236 2MI1-3
115 2PH2-2	137 6KMC1-3	215 6MLR1-1	237 6MLR3C
116 2KU1-1	138 2PK2-1a	216 2PH1-2	238 6MLR1-2
117 2MI1-3	139 2NU1-1a	217 6KMC3C	239 6MLR6C
118 2MA2-1	140 2MA3-1	218 2MA2-2a	240 2W2-1
119 2KU1C1	141 2MI2-4	219 6KMC1-3	241 6KMC1C
120 6MLR7-1	142 2PH1-1	220 6MLR5-1	242 2MI1-2
121 6MLR6C	143 2W3-2	221 2KU1C3	243 6MLR7-1
122 6MLR5-1		222 6MLR7-2	

E.5

SET 94-3 *Acacia koa* Progeny Trial

Seedlings of fifty nine progenies and composites of *Acacia koa* from Hawaii, Kauai amd Oahu were raised at Waimanalo Station and transplanted at Maunawili Station of HARC on June 22, 1994. An augment design of randomized complete block with two replication was employed. Plot size was 3 x 5 sq. meters and ten seedlings were planted in each plot. Field plot layout was arranged as following:

	REP II				REP I					
F	237	236	201							
I	238	235	202							
E	239	234	203		101	132	133	152		
L	240	233	204	R	102	131	134	151	153	
D	241	232	205	O	103	130	135	150	154	
	242	231	206	A	104	129	136	149	155	
P	243	230	207	D	105	128	137	148	P	
L	244	229	208		106	127	138	147	O	
O	245	228	209		107	126	139	146	w	
T	246	227	210		108	125	140	145	e	
	247	226	211		109	124	141		r (Power lines)	
L	248	225	212		110	123	142			
A	249	224	213		111	122	143	l		
Y	250	223	214		112	121	144	i		
O	251	222	215		113	120	n			
U	252	221	216		114	119	e			
T	253	220	217		115	118	s			
	254	219	218		116	117				

Corresponding progeny for each plot

201	2MI2-3	228	6PUUC	101	2MI2-3	128	2MI1-1
202	6PUUC	229	2PK2-1A	102	6KMC3C	129	6KMC2C
203	6MLR1-1	230	2MA2-1	103	2KU1C1	130	2MA1-3
204	6MLR7-2	231	2PK2-1	104	6MLR1-1	131	6KMC1C
205	6KMC1-5	232	6MLR5-1	105	6MLR5-1	132	6MLR2C
206	2MA2-2a	233	6KMC1-4	106	2PH1-3	133	2KU1C3
207	6MLR7-1	234	2MA1-2	107	6MLR6C	134	6MLR7-1
208	2KU1C1	235	6KMC1-2	108	6PUUC	135	6KMC1-4
209	6MLR7-2	236	6KMC1C	109	6HAMC	136	2MI1-3
210	2MI1-3	237	6MLR2C	110	2PH1-1	137	2PK2-1
211	2MI1-1	238	6KMC2C	111	2NU1-2	138	2PH2-2
212	2MA2-2	239	6MLR4C	112	2MA3-1	139	6KMC1-2
213	2W1-1	240	6KMC3C	113	6KMC1-1	140	1WAK4C
214	2K3-1	241	2KU1C3	114	2MA2-3	141	2MI1-2
215	2MI2-5	242	6PUUC1-1	115	2MI2-2	142	2W3-2
216	2MI2-6	243	6MLR3C	116	2MI2-1	143	2MA3-2
217	2MI2-1	244	2MA1-1	117	2MI2-6	144	2MI2-5
218	2MI2-2	245	2KU1-1A	118	2MI2-4	145	1WAK1C
219	2MI2-4	246	2PH2-2	119	2PH2-3	146	2W2-1
220	2W3-2	247	2PH1-3	120	2W3-1	147	6MLR3C
221	2NU1-2	248	2KU1-1	121	2K3-1	148	2MA2-1
222	2PH2-3	249	2MA3-1	122	2MA3-3	149	2PK2-1A
223	6KMC1-1	250	2MA3-2	123	2PH2-1	150	6KMC1-5
224	2MA3-3	251	2W3-1	124	2PK2-2	151	6PUUC
225	2PH1-2	252	2PH2-1	125	6KMC1-3	152	6MLR4C
226	2W2-1	253	2MA2-3	126	2MA1-2	153	2MA2-2
227	1N2-1	254	2PH1-1	127	6MLR7-2	154	6PUUC1-1
						155	2MA1-1

E.6

SET 95-1 *Acacia koa* Progeny Trial

Fifty eight progenies and composites of *Acacia koa* collected from Kauai, Maui, Oahu, and Hawaii were planted at Hamakua Station on 19 May 1995. Three month old seedlings were raised at Waimanalo Station and transported to Hamakua. An augment design of randomized complete block with two replications was employed. Plot size was 3 x 5 sq. meters and ten seedlings were planted in each plot. Field plots were arranged as following:

FIELD TRIAL LAYOUT

70 m

108	109	124	125	140	141	156	201	216	217	232	233	248	249	¦
107	110	123	126	139	142	155	202	215	218	231	234	247	250	¦
106	111	122	127	138	143	154	203	214	219	230	235	246	251	24
105	112	121	128	137	144	153	204	213	220	229	236	245	252	m
104	113	120	129	136	145	152	205	212	221	228	237	244	253	¦
103	114	119	130	135	146	151	206	211	222	227	238	243	254	¦
102	115	118	131	134	147	150	207	210	223	226	239	242	255	¦
101	116	117	132	133	148	149	208	209	224	225	240	241	256	¦

Escuxleuc(8) **div2xleuc(8)** **16 Leucaena triploid clones**

<<<ROAD>>>

Field plot No. and corresponding progeny

101 2PH2-3	129 2W3-4	201 2KW1-4	229 6ML2C
102 6OF1C	130 2PH2-2(94)	202 2KW2-1	230 2HL1-1
103 2PK2-2	131 1PP1C	203 2AA1-5	231 6HK2C
104 6ML1C	132 2KW3-1	204 2MI2-1	232 2PK1-2
105 6ML2C	133 2PK3-1(91)	205 2WT1-4A	233 5SN1-2
106 2PK1-3	134 1WM1C(93)	206 2W4-2	234 6HK1C
107 5KO1-1	135 6WV1-1	207 2PO1-2	235 2W2-2
108 6HK1C	136 5KP1C	208 2PK2-1B	236 1PT1C
109 2PK2-1(94)	137 6HK2C	209 6HAM1C(93)	237 2PK3-1B
110 6SAD1C	138 5MA1-2	210 2KW2-2	238 1PP1C
111 5CP1-5	139 2NU2-1(91)	211 5CP1-2	239 2PK1-3
112 2KH2-1	140 1PT1C	212 6PUU1C	240 6WV1-1
113 5CP1-3	141 6PUU1C	213 2NU2-1(91)	241 6MLR5-1(94)
114 6KU1C	142 5GUL1C	214 2PK2-1(94)	242 2PH2-2(94)
115 2W4-3	143 2KW2-2	215 6ML1C	243 1WM1C(93)
116 6KH2C	144 2KW1-4	216 2W3-4	244 2W4-3
117 6KK1C	145 2KW2-1	217 5CP1-5	245 6KH1C
118 6MLR5-1(94)	146 6KP1C	218 5KH1C	246 2PK2-2
119 2PK3-1B	147 2KW1-2	219 6KU1C	247 5CP1-3
120 6LP2C	148 5SN1-2	220 2KU1C3(94)	248 6OF1C
121 6KH1C	149 2PO1-2	221 2PH2-3	249 2KH2-1
122 5CP1-2	150 2AA1-5	222 6KM1C	250 2KW1-2
123 2PK1-2	151 2MI2-1	223 5GUL2C	251 6ML3C
124 5KH1C	152 2WT1-4A	224 6KP1C	252 5KP1C
125 2KU1C3(94)	153 2W4-2	225 6KH2C	253 5KO1-1
126 2HL1-1	154 2PK2-1B	226 6SAD1C	254 5MA1-2
127 6HAM1C(93)	155 2W2-2	227 6KA3C	255 2PK3-1(91)
128 6KM1C	156 6WV1-1	228 2KW3-1	256 6WV1-1

E.7

SET 95-2 *Acacia koa* Progeny Trial

Sixty four progenies and composites of Acacia koa collected from Kauai, Maui, Oahu, and Hawaii were planted at Maunawili HSPA Station on 1 June 1995. Three month and half old seedlings were raised at Waimanalo Station. An augment design of randomized complete block with two replications was employed. Plot size was 3 x 5 sq. meters and ten seedlings were planted in each plot. Field plot layout was arranged as following:

FIELD PLOT LAYOUT <<<Road & up hill>>>

45 m

	101	102	103	104	105	106	107	108	
R	116	115	114	113	112	111	110	109	
E	117	118	119	120	121	122	123	124	
P	132	131	130	129	128	127	126	125	
	133	134	135	136	137	138	139	140	
I	149	148	147	146	145	144	143	142	141
	150	151	152	153	154	155	201	202	203
R	212	211	210	209	208	207	206	205	204
E	213	214	215	216	217	218	219	220	221
P	230	229	228	227	226	225	224	223	222
	231	232	233	234	235	236	237	238	239
II	248	247	246	245	244	243	242	241	240
	249	250	251	252	253	254	255	256	257
	266	265	264	263	262	261	260	259	258

Field plot No. and corresponding provenance

101 5KH1C	129 2PK3-1(91)	203 2WT1-1	235 5MA1-2
102 2PK1-3	130 6MLR5-1(94)	204 2PH1-1	236 6OF1C
103 2KW3-1	131 2W4-3	205 2KW1-1	237 2MI7-2
104 2KW1-2	132 6WV1-1	206 2PH1-2	238 2AA1-5
105 2PK2-2	133 6HK1C	207 5GUL1C	239 2KH3-1
106 2KW2-1	134 5CP1-2	208 6KH1C	240 2KU1C3(94)
107 2KW1-1	135 2HL1-1	209 5CP1-2	241 2KU1C3(94)
108 2WT1-1	136 6SAD1C	210 2KW2-1	242 6HUmC
109 2KH3-1	137 6KAmC	211 5KO1-1	243 6HAM1C(93)
110 2PH1-1	138 6OF1C	212 6PH1C	244 5KP1C
111 2KW2-2	139 2MI2-1	213 5KH1C	245 6KU1C
112 2NU2-1(91)	140 2K2-2	214 2PK1-3	246 6SAD1C
113 6KP1C	141 2PK3-4	215 2PK3-1B	247 6WV1-1
114 6KU1C	142 2AA1-5	216 1PT1C	248 2W4-3
115 2PK3-1B	143 2AA2-5	217 6MLR5-1(94)	249 2KW3-1
116 2PK1-2	144 5KP1C	218 2PK1-2	250 2PH2-3
117 2KW1-4	145 6KM1C	219 2PK3-4	251 2HL1-1
118 5KO1-1	146 2PH2-2(94)	220 2K2-2	252 5GUL2C
119 1WM1C(93)	147 2W2-2	221 2MI2-1	253 1WM1C(93)
120 2KU1C3(94)	148 1PT1C	222 2PO1-2	254 2PH2-2(94)
121 6HAM1C(93)	149 5CP1-5	223 2W4-2	255 5SN1-2
122 5SN1-2	150 5CP1-3	224 2AA2-5	256 2KU1C3(94)
123 2PO1-2	151 2PH2-3	225 1PP1C	257 2KU1C3(94)
124 2MI7-2	152 1PP1C	226 2W3-4	258 2PK2-1(94)
125 2PH1-2	153 6PUU1C	227 6PUU1C	259 2PK2-1(94)
126 2W4-2	154 2PK2-1(94)	228 2KW1-2	260 1N2-5(91)
127 5MA1-2	155 6HUmC	229 2KW1-4	261 6KP1C
128 2W3-4		230 5CP1-3	262 2KU1C3(94)
	201 2PK2-1B	231 5CP1-5	263 2PK2-1(94)
	202 2WT1-4A	232 2KW2-2	264 2W2-2
		233 2NU2-1(91)	265 6HK2C
		234 6MLmC	266 6LPmC

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