PN-ABL-172

Guide to Management of Leucaena Seed Orchards

Robert A. Wheeler

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Winrock International sponsored this guide under the Forestry/Fuelwood Research and Development (F/FRED) Project, for which it is the primary implementing agency. Funded by the U.S. Agency for International Development, F/FRED is designed to help scientists address the needs of small-scale farmers in the developing world for fuelwood and other tree products. It provides a network through which scientists exchange research plans, methods, and results. Research and development activities center on the production and use of multipurpose trees that meet the several household needs of small farmers.

Wheeler, R.A. 1991. Guide to Management of Leucaena Seed Orchards. Winrock International-F/FRED: Bangkok, Thailand. v + 18 pp.

ISBN: 974-7315-37-8

Winrock International-F/FRED
c/o Faculty of Forestry
Kasetsart University
P.O. Box 1038
Kasetsart Post Office
Bangkok 10903
Thailand

Guide to Management of Leucaena Seed Orchards

For cooperators in the F/FRED Leucaena Seed Orchard program
for production of psyllid-resistant seed from selected parentage

Robert A. Wheeler

Nitrogen Fixing Tree Association
P.O. Box 680, 41-698 Ahiki Street
Waimanalo, Hawaii 96795, USA

1991

Winrock International

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Preface

This guide is intended primarily to give guidance for management of seed orchards established through the Leucaena Seed Orchard program, developed by the Nitrogen Fixing Tree Association (NFTA) with the support of the Forestry/Fuelwood Research & Development (F/FRED) Project. Since many of the orchard materials in the program will be out-crossing hybrid individuals, maintenance of identification of parentage is very important. It can be very difficult to identify hybrids from phenotypic traits. NFTA has been working for several years on the development and classification of interspecific hybrids, and can assist with classification of new hybrids. Currently, efforts are being made to use isozyme analysis to aid in clarification of parentage.

The first chapter gives a brief description of maintenance, cutting, and selection activities.

The second chapter provides a summary of the genus *Leucaena*, species hybridization within the genus, and production of potentially seedless varieties. More detailed descriptions of the species or hybrids should be directed to NFTA or other authorities working with the program.

The third chapter provides a detailed description of the procedures involved in hand pollination, seed collection, labelling, and storage. This section should be useful for training field personnel in hand pollination of Leucaena. It is based on procedures that have been used successfully for breeding Leucaena in Hawaii.

I would like to acknowledge Dr. James Brewbaker and Mr. Charles Sorensson for their work in the development of Leucaena breeding procedures and evaluation of interspecific hybrids at the Waimanalo Experiment Station. Special thanks also to Charles for his help with the photos used in this guide, and to Mr. Ken MacDicken, F/FRED, for assistance in preparing the guide. Finally, a thanks to the five cooperators in the program who have overseen the establishment and monitoring of the seed orchards: Dr. I.N. Oka (AARD, Indonesia), Mr. Narayan Hegde (BAIF, India), Dr. Fuh-Juinn Pan (TFRI, Taiwan), Mr. Panas Buranasilpin (TISTR, Thailand), and Dr. Rudolfo Escalada (ViSCA, Philippines).

Robert Wheeler
NFTA Research Associate

Introduction

The Leucaena Seed Production (LSP) project set out to establish foundation seed orchards in Asia. These facilities would provide aid for development and increase of selected Leucaena species and hybrids validated for high psyllid resistance/tolerance as well as for growth and form. Individual seed packets sent to the five LSP sites contained seed from selected trees in the field at the Waimanalo Research Station, Hawaii. This breeding program focuses on maintaining a broad genetic base while conducting intensive tree selection. The broad genetic base is maintained through use of composites of open pollinated seed from selected parents.

There are many types of seed orchards and several definitions. Zobel et al. (1958) defined a seed orchard as a "plantation of genetically superior trees, isolated to reduce pollination from genetically inferior outside sources and intensively managed to produce frequent, abundant, easily harvested seed crops."

Establishment of the seed orchards in the LSP program provides the following advantages as they influence the region-wide development of a Leucaena Psyllid Resistance program.

1. The higher genetic gain potential made available by using two select parents, rather than open-pollinated seed, in breeding for desired traits should result in major genetic gains over single-parent selection.

2. Production of hybrid seed is more efficient when both male and female flowers are in close proximity.

3. The orchards can be managed to maximize seed production on a frequent basis and permit careful monitoring for pod and seed damages that could adversely affect seed production.

4. Equipment and facilities for seed collection and processing are concentrated in one location to permit more efficient operation.

5. The orchards can serve as gene pool storage, allowing further protection of selected high-yielding individuals and rare or limited accessions.

6. Seed orchards provide further assurance of the development and careful identification of foundation seeds derived from select accessions. These accessions were obtained directly from field collections in the natural range of the genus in Mexico and Central America.

Chapter 1: Seed Orchard Maintenance

1.1 General Maintenance

A seed orchard should be well-maintained and fertilized to ensure healthy and vigorous seed trees, thereby assuring early and abundant seed production. Each entry in the seed orchard should be clearly labelled to prevent errors in breeding, seed collection, and distribution. Weed competition should be controlled by manual removal or mowing. Once trees have attained adequate height, controlled grazing may be permitted, but care must be taken to protect the seed trees from browsing damage. On sites with clayey soils, compaction should be minimized to prevent root diseases associated with poor water drainage.

Spacing of seed trees after roguing will vary, but the methodology for trial roguing was designed to result in a 2 x 2 m spacing.

Selection of psyllid-resistant Leucaena varieties requires that the orchard be maintained in an insecticide-free environment.

Under conditions where psyllid control is desired, Applaud, Carbaryl (Sevin), Carbosulfan, Isoprocard, Monocrotophos, Diazanon, Azodrin, Lannate, Furadan, Neem oil, and Dimethoate (Perfecthion) have been effective.

1.2 Frequency of Harvest

Most leucaenas mature sexually in the first or second year after establishment. The production of seed from some LSP entries will begin during the first year. Since the first roguing is recommended at the end of the first year, be careful not to mix these seeds with later seeds developed after the intensive roguing has taken place. Many of the entries in the orchard will be self-incompatible, and use of seeds pollinated from unrogued pollen sources would contaminate the selected gains desired from the orchard.

In areas subject to monsoons, seed pods should be collected in the early dry season before the pods become overmature or badly damaged by insects. Information about collection and processing of pods is found in chapter 2 of this guide. In areas not subject to monsoons, pods will mature in a less cyclical pattern and careful monitoring will be necessary to determine when they should be picked.

The orchard manager should also be aware that pods of different Leucaena species mature at different rates, which are influenced by both genetic and environmental factors (Table 1).

1.3 Coppicing Methodology

After roguing has been completed, the selected trees should be coppiced to a height of 1 m. Selected trees should then be coppiced after each successive year's seed harvest. The advantages of this are: 1) ease of hand pollination and collection, and 2) easier monitoring of pod development and installation of pod protective bags if

needed. The seed orchard should have access to a large ladder (at least 3 m in height). The cutting of the stem should be made with a sharp instrument to make a straight and clean cut that does not shred the stump or excessively damage the bark or cambium layer at the point of cutting.

1.4 Tree Selection Intensity

The intensity of selection for individual seed orchard entries varies, depending on the species or hybrid. A summary of the selection instructions sent to each of the five seed orchards follows.

KX3 Maui Comp: This hybrid is self-fertile and segregates self-fertile progeny. These progeny should be tested to identify superior lines within the composite (pure line breeding). (Primary LSP plantings in the Philippines and Taiwan.)

Selection intensity: Conduct a 50% roguing at the end of the first year, based on psyllid resistance, cold tolerance, growth and form, and low flowering. Conduct a second 50% roguing at 1.5 years.

At the warm site (ViSCA): The KX3 Maui Comp is planted at a higher density and followed with a 75% roguing at one year, followed by a 50% roguing at 1.5 years.

K636 x K156: This hybrid is another KX3 type of cross, generating self-fertile progeny that will produce segregation for psyllid resistance and other desired traits. (Primary LSP planting in Taiwan.)

Selection intensity: Conduct a 75% roguing at the end of the first year, and then another 50% roguing at 1.5 years.

Div 4N Comp: These seeds were derived from self-fertile parents and will produce self-fertile progeny. (Primary LSP planting in the Philippines.)

Selection intensity: Conduct a 50% roguing at the end of 1 year, followed by another 50% roguing at 1.5 years.

Pallida Comp 1: These are self-sterile progeny and will produce out-crossed seeds. They are highly psyllid-resistant and should be selected highly for growth and form. (Primary LSP planting in Indonesia.)

Selection intensity: Conduct a 50% roguing at the end of the first year. These trees should be isolated at least 100 m from surrounding Leucaena pollen sources.

KX1 Comp 1: These hybrid, open-pollinated seeds from selected parents (K156 DIVE x K376 PALL) are variably fertile, with some being self-fertile and some self-sterile. (Primary LSP planting in Indonesia.)

Selection intensity: Conduct a 50% roguing at the end of the first year based on psyllid resistance, growth and form. This should be followed by another 50% roguing about 6 months later, with emphasis on selection for psyllid resistance.

KX2 Comp 1: This hybrid is a composite of F2 open-pollinated seeds from selected parents (K8 LEUC x K376), and will be quite variable for psyllid resistance and growth and form. It appears that there are both self-sterile and self-fertile progeny. Emasculation may be a concern with hand pollinations. (Primary LSP planting in India.)

Selection intensity: Conduct a 50% roguing at the end of one year, and follow with another 50% roguing at 1.5 years. Intense selection for psyllid resistance is required, because this hybrid as higher psyllid damage associated with the *L. leucocephala* in its parentage.

Collinsii Comp 1: These trees will be totally self-sterile, so outcrossing will be a concern. The stand should be isolated 100 m from surrounding sources of Leucaena pollen. (Primary LSP planting in India.)

Selection intensity: Conduct a 50% roguing at the end of the first year, based on psyllid resistance, growth and form.

Salvadorensis Comp 1: This is a self-sterile composite of seeds of diploid (2n = 56) *L. salvadorensis*. Trees should be isolated 100m from surrounding sources of Leucaena pollen. (Primary LSP planting in Thailand.)

Selection intensity: Conduct a 50% roguing at one year, and another 50% roguing at about 1.5 years. Both roguings should be based on higher psyllid resistance, growth and form.

Leuc PR Comp 1: This is a composite of seeds from *L. leucocephala* parents that were 100% self-fertile. (Primary LSP planting in Thailand.)

Selection intensity: Conduct a 50% roguing at one year based on psyllid resistance, growth, form, and low flowering. Follow this with another 50% roguing after 6 months or more. These trees tend to be much more susceptible to psyllids than most of the other varieties, but NFTA has found varying degrees of resistance based on ability for regrowth after defoliation. Selection for this composite should not be made at the peak of the psyllid outbreak, but should be based on recovery rate following the outbreak.

1.5 Roguing Methodology

Evaluation of seedlings for psyllid resistance should be made at the peak of the psyllid population, when intensive selection can be made (likely within five to seven months). Inferior seedlings identified during the period of peak psyllid populations should be marked for removal and then cut. A cheap and convenient method of eliminating leucaena is to cut the tree off close to the ground (5 cm or less) and

immediately coat the freshly cut stump with diesel oil. Diesel should be applied either with a sprayer or poured onto the stump so that all exposed wood on the cut surface is covered. No branches with leaves should be left on the stump. Diesel should be handled with care, and gloves should be worn.

A rating system like the one used for the Leucaena Psyllid Trial program can aid in evaluation of psyllid damage. A copy of the damage rating, with examples, is shown below. Damage in the range of 7 to 9 is excessive, and such trees should be candidates for elimination. Consideration should be given, however, when the same damage level is displayed by the entire population. Plant damage can be worsened by factors such as excessive plant moisture stress. Trees that normally express good resistance can become more susceptible to damage under extremely stressful conditions. A high level of damage exhibited by the entire plantation (should this happen) would not necessarily indicate that all entries are highly susceptible to defoliation under reduced stress. Also, several species or varieties in the orchard may not be adapted to the orchard site, and so may be expressing more stress and psyllid damage than they would exhibit in areas for which they are better adapted.

Since many of these varieties or hybrids exhibit traits better suited to a specific product, such as fuelwood or fodder it is important to base the roguing selection on a representation of the management regime the trees will be grown under. Differential responses to management have been reported by Wheeler (1990). If the species is to be used for fodder production, then trees should be coppiced during the wet season and the regrowth allowed to reach 1.5 - 2 meters. Then an intensive selection of regrowth can be made based on quantity of leafy biomass and relative psyllid resistance.

Psyllid Damage Rating Scale

1 = No damage observed
2 = Slight curling of leaves
3 = Tips and leaves curling and yellow
4 = Tips and leaves badly curled, yellowish and covered with sap.
5 = Loss of up to 25% of young leaves
6 = Loss of up to 50% of young leaves
7 = Loss of up to 75% of young leaves
8 = 100% loss of leaves and blackening of lower leaves
9 = Blackened stem with total leaf loss

Chapter 2: The Genus Leucaena and Interspecific Hybrids

2.1 Description

The genus *Leucaena*, native to the Americas, has been described as having more than 50 species associated with it. Brewbaker (1987) proposed reducing this list to 12 widely recognized species, with the majority of the remaining purported species as synonyms. Hughes (1988) added *L. salvadorensis* to the list of recognized species. More may be added as the larger set of synonym taxa is further clarified. The 13 recognized species within the genus are listed in Table 1, which also includes a species code fertility classification, somatic chromosome counts (2n), and number of florets per head. The genus has been recognized internationally as having multiple uses and great variability. Species variability has been found also to include psyllid resistance, which has been the basis for development of the resistant/tolerant varieties used in the LSP program.

Table 1. Listing of 13 Leucaena species.

Species	Code	Fertility*	2n No.	No. of Florests	Pod days To mature
L. collinsii	COLL	SI	56	218	280
L. diversifolia	DIV4	SC	104	78	120
L. *diversifolia*	DIV2	SI	52	105	100, 160, 260
L. esculenta	ESCU	SI	52	107	110
L. greggii	GREG	SI	56	175	130
L. lanceolata	LANC	SI	52	484	80
L. leucocephala	LEUC	SC	104	180	90
L. macrohylla	MACR	SI	52	85	90
L. pallida	PALL	SI	104	134	110
L. pulverulenta	PULV	SI	56	42	90
L. retusa	RETU	SI	56	196	120
L. salvadorensis	SALV	SI	56	124	260
L. *shannoni*	SHAN	SI	52	172	130
L. trichodes	TRIC	SI	52	117	140

*SI = Self Incompatible SC = Self Compatible

2.2 Species Hybridization

A total of 171 of 182 possible interspecific crosses within the original 12 recognized species were made in Hawaii by Sorensson and colleagues (Brewbaker and Sorensson 1989). Of these 171 attempted crosses, 79 (46%) successfully produced viable F1 seeds. Due to the success rate for interspecific crosses, the genus is considered highly intercompatible and offers potential for cross-breeding desired traits from two or more species into high-yielding hybrids. Three-way crosses have been made in all directions for accessions of *L. diversifolia* 4n, *L. pallida*, and *L. leucocephala*. A list of peak damage ratings from a seven-month observation period

of 52 interspecific hybrids crossed and planted at Waimanalo, Hawaii appear in Table 2.

Table 2. Damage ratings for interspecific hybrids observed at NFTA by Robert Wheeler on October 8, 1987.

Hybrid	Percentage	Rating	Hybrid	Parentage	Rating
Retu x Lanc	K280 x K10	2	Retu x Leuc	K280 x K500	6
Retu x Coll	K280 x K45	1	Leuc x Dive	K8 x K156	4
Leuc x Lanc	K42 x K264	3	Leuc x Escu	K8 x K138	1
Leuc x Pulv	K614 x K75	6	Leuc x Retu	K8 x K280	6
Leuc x pall	K636 x K376	2	Leuc x Tric	K8 x K738	3
Dive x Leuc	K156 x K500	3	Macr x Lanc	K158 x K393	1
Dive x Coll	K409 x K185	1	Dive x Lanc	K409 x K401	3
Dive x Lanc	K85 x K393	2	Dive x Pulv	K409 x K75	2
Dive x Shan	K409 x K401	1	Dive x Pall	K156 x K376	1
Coll self		1	Lanc x Shan	K393 x K445	3
Dive x Leuc	K11 x K8	1	Pulv x Leuc	K19 x K8	4
Pulv x Lanc	K19 x K385	7	Retu x Lanc	K280 x K10	3
Escu x Shan	K138 x K445	1	Shan x Retu	K445 x K502	3
Macr x Lanc	K158 x K566	1	Dive x Dive	K409 x K156	1
Lanc x Shan	K10 x K445	3	Shan x Lanc	K445 x K10	1
Macr x Dive		3	Lane x Coll	K393 x K450	1
Pulv x Lanc	K340 x K401	5	Retu x Escu	K280 x K138	3
Retu x Shan	K280 x K445	1	Retu x Dive	K280 x K156	2
Leuc x Shan	K8 x K405	1	Lanc x Dive	K10 x K385	4
Coll x Lanc	K185 x K264	3	Lanc x Lanc	K10 x K393	2
Retu x Greg	K280 x K795	1	Dive x Lanc	K156 x K10	4
Pulv x Dive	K19 x K156	1	Tric x Lanc	K739 x K10	1
Dive x Shan	K156 x K405	2	Dive x Greg	K399 x K79[illegible]	1
Retu x Pall	K280 x K376	3	Retu x Pulv	K280 x K881	4
Tric x lanc	K738 x K393	3	Leuc x Pall	K8 x K376	2

Recent interspecific hybrids developed at NFTA have focussed on crosses between accessions of *L. leucocephala* (LEUC), *L. diversifolia* (DIVE), and *L. pallida* (PALL). These crosses are generally categorized as KX1 for DIVE x PALL crosses, KX2 for LEUC x PALL crosses, and KX3 for LEUC x DIVE crosses. Seeds distributed from the seed orchard from KX1 and KX2 trees should be expected to exhibit some variability, and farmers receiving these seeds should be told that further selection of superior seedlings will be necessary to enhance the overall performance of delivered seeds.

Because KX3 is self-fertile, the care taken to assure good parentage of self-fertile species must also be taken when it is used in hybrid crosses. We have observed some diversity of phenotypic expression with the KX3 hybrids, which is likely due to the broad inheritance patterns expressed by these tetraploids. Selection of superior individuals of the KX3 will ensure continued success for seed development of relatively stable germplasm.

Four of the varieties included in the LSP program are self-infertile: *L. collinsii, L. salvadorensis*, KX1, and KX2. The management of these individuals in the orchard will require some isolation to prevent contamination of seed by external sources. This isolation should be at least 100 meters from any other sources of Leucaena pollen. If this is not possible, it will be important to inform users of the seed that some degree of contamination may have occurred. Seedling variability should be examined carefully and roguing undertaken to eliminate non-typical seedlings. For example, a KX2 hybrid with leaves similar to LEUC would likely be an outcross with possibly the common type (inferior) of LEUC. This would be undesirable. Plantations of seeds from the orchards should maintain the identity of their seed pedigree, and a follow-up visit by experts should be made to help identify desirable and undesirable traits. Undesirable traits would include seedlings exhibiting pollen contamination.

2.3 Production of Seedless Varieties

At the 1989 workshop Leucaena Psyllid: Problems and Management, procedures for development of seedless hybrids of Leucaena were outlined by Brewbaker et al. (1989). Seedless hybrids of Leucaena have been created primarily by cross-breeding self-infertile, diploid species with a self-fertile, tetraploid species, creating a sterile, triploid hybrid. Notable sterile hybrids planted in tea plantations in Indonesia were developed by crossing diploid *L. pulverulenta* x *L. leucocephala* and *L. diversifolia* (2n) x *L. leucocephala*. Analysis of pollen from these hybrids indicates that they are possibly aneuploid triploids, or contain an abnormal number of chromosomes from that expected of a triploid between these two species. Other potential seedless hybrids of importance would be crosses between (*L. diversifolia* x *L. esculenta*) x *L. leucocephala, L. diversifolia* (2n) x *L. leucocephala*, and *L. pallida* x *L. diversifolia* (2n). Production of these sterile triploids can be facilitated through open pollination by grafting a branch from the self-infertile diploid tree onto the self-fertile tetraploid tree. When doing this, be careful in order to assure adequate pollen per female flower.

Chapter 3: Breeding Guide for Leucaena

This chapter provides a step-by-step procedure for effective hand pollination of Leucaena. In addition, recommendations for protection, processing, storage, and labelling of Leucaena accessions and hybrids are given.

Distributions of seeds from these seed orchards will need to be carefully identified to the farmers, along with the species/hybrid specific recommendations for roguing plantations due to anticipated segregation for desired traits.

3.1 The Leucaena Flower

A branch of Leucaena *shannonii with flowers in various stages of development.*

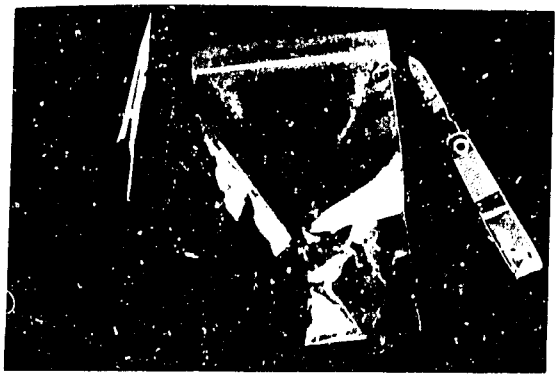
Brewbaker (1986) provides the following description of the Leucaena flower.

> FLOWERS - sessile in dense globose heads to 2 cm diameter, with 40-550 florets depending on species, color of inflorescence at anthesis white, reddish, or yellowish; heads borne on short (2 cm, occasionally to 8 cm) axillary peduncles in fascicles or occasionally in terminal leafless recemes; peduncles of the heads bear small bracts at or slightly below apex; flowers 5-parted; calyx tubular or capanulate, short (to 2 mm); sepals united and not overlapping, dentate at tip; corolla greenish and non-showy, with petals free, short (to 5 mm) and thin, variably villous; stamens 10, free, hairs, colorless or yellow or red pigmented lacking gleands; ovary with many (10-30) ovules, often pilose at tip, the style thin and white, yellow or reddish, normally longer than stamens; stigma terminal, minutely cupulate; pollen shed singly, not in polyads, binucleate, tricolporate (rare polyads are reported for the genus, and random pollen clumping can occur).

Flowers of Leucaena vary in color and aroma, but all occur as a multifloreted head. The incidence of color in both stamens and styles are important aids in identification of successful hybridization, such as between LEUC (white style/white another) and DIVE (reddish/reddish) or PALL (pinkish/pinkish).

3.2 Equipment for Hand Pollination

The following photos display key items used at Waimanalo for hand pollination.



- **Nail clipper**
  (about 55 mm long, file about 30 mm long)
- **Plastic bags**
  (dimensions can vary, ours are 75mm x 125mm) The bags can be sealed to prevent pollen loss.
- **Forceps**
  (120 mm, but can be most any size)

- **Apron**
  We find it invaluable to help carry the equipment, bags, and pollen.
- **Stapler**
  (Also a large supply of staples; we use Ace Clipper model 702)
- **Scissors**
  We use two types: a small surgical pair for fine snipping around the flower head (120 mm), and a larger pair for removing unwanted branches and leaves near the pollinating area.
- **Pollination bags**
  We use Lawson #217 corn pollination bags (5x16 cm) waxed paper construction (Lawson, 480 Central, Northfield, Illinois, U.S.A.).
- **Marker**
  For a number of uses, including marking the pollination bags, it is important to have a permanent, all-weather marker.

* **Flagging**
  We often use plastic flagging to help identify pollinated flowers and indicate what crosses were made at the flagged point on the branch.

* **Bottle**
  (small, containing 75% ethanol)

* **A supply of paper tissue**

* **Flashlight**

3.3 Preparation for Pollination

The male and female flowers intended for pollination should be bagged the day before pollination is to be done. Candidate inflorescence for bagging should appear as relatively larger-diameter heads that may have changed their color from a deep green to a greenish yellow. Another important clue to identify flowers likely to be opening is the change in appearance of the flower head's exterior from smooth to bumpy. This is caused by the underlying florets developing in preparation for opening, and is especially obvious in the Leucaena species with high numbers of florets per head, such as *L. lanceolata* or *L. macrophylla.*

Each pollination bag should be marked to indicate species and whether it is to be used as male or female application.

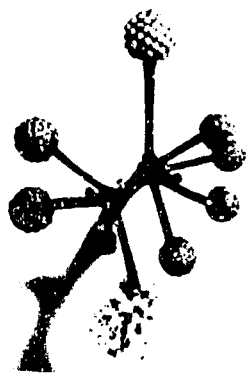


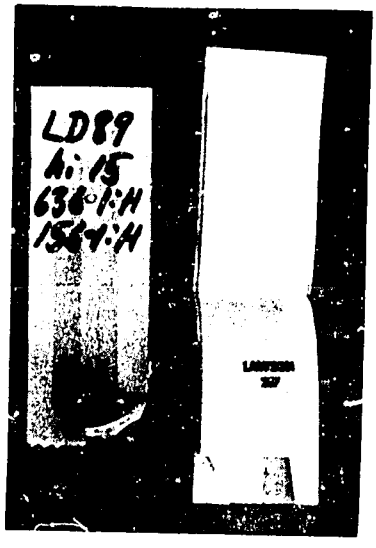
Photo showing flowers in various stages of maturity. The top flower is a good candidate for bagging, as it appears to be bumpy and has changed color to a yellowish green.

3.4 Bagging Procedures

The selected inflorescence must be handled carefully to prevent mechanical damage to the branch or peduncle that might cause the death of the flower. The same type of bag is applied to flowers intended for both male and female use. Surrounding leaves and flowers that might cause difficulty with accessing the selected inflorescence should be carefully removed. We have found scissors to be quite helpful for this. Often a subtended leaf will be present near the intended inflorescence. This should be cut.

The intended bag should be prepared as in the photo, by cutting off the open end of the bag so that the bag dimensions become 5x15cm. A slit should then be cut vertically, about half way between the open end sides of the bag. This vertical slit should be about 4cm long but can vary depending on the length of the flower peduncle.

Bag application can be difficult. Success of the pollination activity will depend on doing a good job of this. Open the trimmed bag and place it over the inflorescence so that the vertical slit in the bag fits snugly onto the supporting branch. The bottom



Bag preparation showing center vertical slit and labeling. The code reads, LD 89 (Leucocephala x Diversifolia *Cross 1989.*)

Bag attached to the branch. Label reads: Hi 15 (15 styles were pollinated and were good pollinations).
636 - Female parent 1-H plot location
156 - Male parent

edges of the bag are then tightly folded around the branch and the folds stapled securely to prevent insects from entering the bag.

3.5 Emasculation

Removal of anthers from self-fertile species. Photo taken during and after emasculation.

This is an important and tedious activity when dealing with pollination of self-fertile flowers (tetraploids). It is essential that all anthers are removed before pollen has dropped. This should be done 1-3 hours before dawn and varies by species and climatic conditions. As it gets warmer, the pollen drops earlier and the breeder should be aware that environmental factors such as daily temperature, morning humidity, precipitation, and plant moisture stress will influence the pollen dehiscence. The filaments are removed (plucked with forceps) just above the sepals in order to distinguish them from the styles. The flowers are then rebagged and await application of the pollen.

3.6 Processing Pollen

3.6.1 Pollen Collection

Sorensson (1988) found that pollen can be collected from most Leucaena species within a 60-to 90-minute period about 3.5 to 4.5 hours after dawn. He noted that pollen of the three diploid species of *L. diversifolia*, *L. macrophylla*, and *L. trichodes* can be shed within 30 minutes, and care should be taken to time the pollen collection accordingly. He also found that *L. diversifolia* will tend to drop its pollen about 0.5-1.0 hour before most of the other species.

The plastic bag for the collection of the pollen should be marked to indicate the species and tree identification of the pollen source. The bagged flower (pollen source) should be handled carefully to limit the amount of pollen lost before the pollen is collected in the plastic bag. The pollinating bag is cut off near the branch and the bag carefully removed from the flower. The inflorescences are slowly and carefully removed from the stem and quickly placed in the plastic bag already prepared and labeled for it. Twirling the flower quickly back and forth in the plastic bag will dislodge most of the pollen. The flower is then discarded.

3.6.2 Pollen Storage

Unpublished research by C.T. Sorensson has shown that *L. leucocephala* pollen can be kept alive for short periods of time if stored properly. Pollen stored in saran wrap packets over air-tight vials containing dessicant remained viable for over 30 days when kept at -20 degrees C, and over 100 days when stored at -75 degrees C.

Dislodging pollen into a labeled plastic bag by twirling the flower between fingers.

3.6.3 Pollen Analysis

A number of methods may be used to test pollen grain viability, including both germination and non-germination testing as reported by Stanley and Linskens (1974). Sorensson and Nagahara (1989) summarized the non-germination test as including the use of staining, such as with acridine orange and potassium iodide, redox dyes, inorganic acids, and nuclear magnetic resonance (NMR). One method, reported by Sorensson, is through *in vitro* pollen germination on agar substrate. The Agar medium was prepared using a mixture of 50 ml distilled water, .05g borate, .6g agar, and 4.0g sucrose at a pH of 5.5 poured on sterile microscope slides. The pollen is applied to the surface of the slide and allowed to incubate overnight at 24°C under moist conditions. After 12 hours, pollen tube elongation and frequency should be readily apparent.

3.7 Pollination - (Controlled hand crosses with selected parents)

Before beginning pollination procedures, clean the nail clipper file with the ethanol solution and dry with the tissue. There should be sufficient pollen in the bag so that it is readily visible as a film or powder mass inside the plastic. As shown in photo (1), open the plastic bag, gather pollen on the side of the nail file, and press it out as a uniform smear on the file surface.

Holding the inflorescence carefully, move the nail file with the pollen smear across the head of the style at a 45-degree angle, depositing the pollen into the small depression of the stigma at the end of the style (photo 2). Sliding the stigma through the pollen smear on the nail file should leave a small, visible trace as in the photo (3).

Photo 1 - Obtaining a pollen film on the file surface.

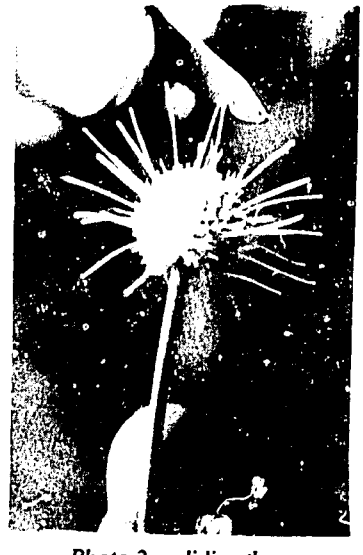


Photo 2 - sliding the stigma through the pollen, film on the file.

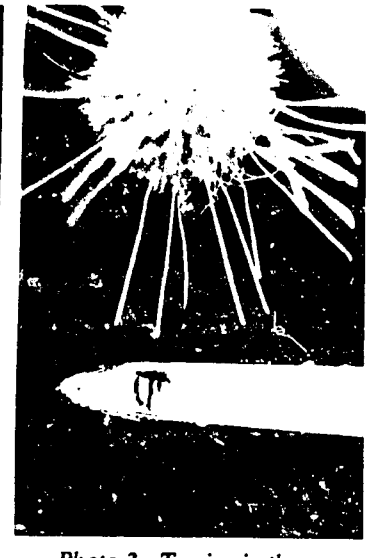


Photo 3 - Tracing in the pollen film from stigma pollination. Notice pollen mass on the stigma on lower style

3.8 Evaluation of Pollination Success

Failure with hand pollination can be caused by a number of factors. These include old or bad pollen, poor bagging and pollinating technique, female sterile inflorescence (Table 1), mechanical damage to the flower or peduncle, pollen/pistil incompatibility, and insect and disease problems. In Hawaii, flower head damage by moth caterpillars (possibly *Ithome lassula* has been a major

Photo showing a successful pollination with several young pods developing.

problem. It is important that breeders determine if they are having at least initial success with their crosses.

The breeder should check several of the initial crosses to determine what percentage were successful. This can be done by carefully opening the pollination bag 3-7 days after pollination to determine if seed pods are developing. These will appear as tiny, bright green lumps on the flower head.

Unsuccessfully pollinated flowers will normally have dried or dehisced. Unsuccessful crosses should be noted in the records by recording the date of the cross, found on the pollinating bag from the failed pollination, and marking it failed. These bags can then be used as references for failed crosses in the record's for the orchards pollination activities. In Hawaii, if a cross has been successful, we place a length of polyethylene ribbon (20 cm or more) toward the end of the branch from the successful cross. On this ribbon we indicate the date and parentage of the cross, and number of pods observed. This information should also be entered into the orchard's breeding records.

Cloth bag protects developing seeds from damage by insects.

3.9 Bagging Crosses for Successful Seed Collection

In Hawaii, we have encountered severe seed losses from hand pollinations during the final phases of seed development by seed insects, such as *Araccerus levipennis* Jordan. These insects are especially damaging to species such as *L. pallida* and hybrids utilizing *L. pallida* parentage. Recent use of larger cloth or paper bags to protect developing pods from insect damage has been successful and we recommend this as an alternative to control seed losses. Sorensson has found that attempts to control insect damage with insecticides have not been as effective as the use of cloth bagging.

3.10 Pod Collection and Processing

Seed pod maturation varies among species within the genus, as shown in Table 1. A general rule is that pods are ripe and ready for picking when they lose their leathery feel and appearance (generally are flexible and rubbery), become dry and brittle, and change color from green or reddish brown to brown or dark brown. If controlled crosses are involved, care should be taken that the fragile pods are kept in separate closed bags, so that if the pods should break open the seeds will not be lost or mixed with other seed.

Once the pods have been collected, it is necessary to dry them to a low moisture content level. Drying

CAUTION

* **Green pods** have low seed germination
* **Always keep labels** with seeds during extraction process
* **Moisture and mold** can destroy seeds
* **Insect damaged seeds** generally give only 10 to 40% germination

time can vary from 2 to 4 weeks, depending on the pod and storage conditions. A seed dryer can also be used to prepare the pods, but open air drying, although slower, can be equally effective. Once the seed pods are dried they should be prepared for processing. Make sure that all pod samples are adequately labelled so that there they are correctly identified during seed extraction.

3.11 Seed Extraction

Seeds can be removed from the pods in many ways. The slowest but most exacting way is to simply open each pod by hand. This approach is especially useful when processing small lots of hand-crossed seeds. The seeds are usually placed in a pan or enclosure and then opened individually.

When dealing with larger quantities, we have had success in using a soy bean thrasher, into which the pods are dropped. By the thrashing process the pods are opened and seeds are blown out. These seeds will include a number of bad seeds, so it is helpful to use a seed sorter that sifts or removes bad or damaged seeds. Our mechanical seed sorter uses blown air to remove bad seed. Another method for cleaning seeds is to place the seeds in a bucket and run water through them; bad seeds float to the surface and can be removed. Seeds should be dried after being subjected to the flotation treatment. It is important that equipment used for seed processing is carefully cleaned when changing seed lots to prevent contamination of seed lots with the wrong seed. The seed label should be kept with each seed lot at all phases of seed processing.

Another method of seed extraction is to place the pods in a heavy cloth bag and crush the bag and pods to release the seeds. It is important that seeds be carefully labelled for storage once they have been extracted, if they are not going to be immediately cleaned.

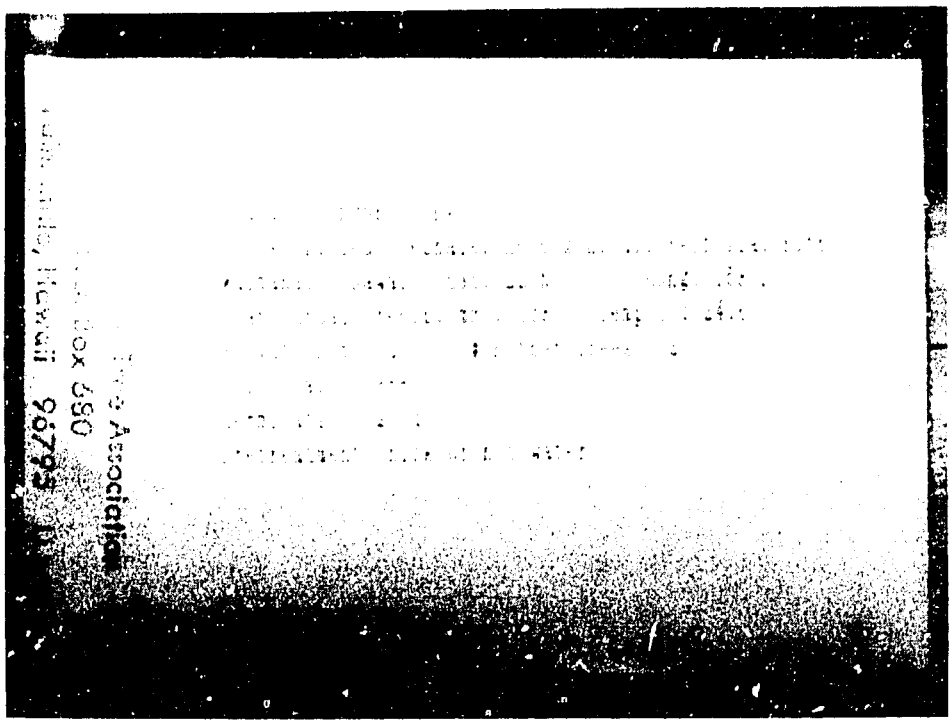
3.12 Seed Labelling and Storage

Once the seeds have been cleaned, they should be stored in a cool, dry environment. Warmth and moisture are very detrimental to seed viability. If insects are likely to be a problem, then the seed should be treated with Seven or Isotox. If molds are a problem, a fungicide should be used. Once the seeds have been cleaned and dried they should be stored in sealed containers, such as plastic bottles or sealed plastic bags. The container should be able to seal out moisture, and should be properly labelled.

Proper seed labelling is essential if gains from seed distributed by the orchard are to be maintained. Chaos will surely follow if careful identification of species (or hybrids) and sources are not maintained. After the seeds are distributed to the field for planting, it is nearly impossible to determine the true parentage of individuals without an extensive background of the source materials used in the seeds distributed to the seed orchard. If a farmer finds that a certain variety is working well, it would be very difficult to follow this up with further breeding and improvement without knowledge of that variety's breeding history and species/hybrid status.

Strict attention must be given to labeling all materials collected and processed at the seed orchard. Records of the species/hybrid pedigree should always be on the storage label. Seeds sent out to villages should always be identified so farmers know the variety provided. Then, if some grow better than others, the farmer can report which ones are bad and which are the best. An example of labelling used on the seed distribution packets at Waimanalo is shown in the photo below.

Labels should always include seed source, species, origin, and date.



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Institutions Participating in the Leucaena Seed Production Project

BAIF Research Foundation
Senapati Bapat Mar
Pune, Maharashtra 411016
India

Bogor Research Institute for Food Crops
Jl. Cimanggu No. 3A
Bogor
Indonesia

Taiwan Forestry Research Institute
Pan. 53 Nan-Hai Road
Taipei, Taiwan
Republic of China

Thailand Institute of Scientific and
Technological Research
196 Phahonyothin Road
Bangkhen, Bangkok 10900
Thailand

Visayas State College of Agriculture
Baybay, Leyte
Philippines