# Oil Mallee Breeding Strategy

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# SUMMARY

Investigation into cineole yield of the oil mallees in WA was initiated by Professor A. Barton of Murdoch University in 1983. Results of this study indicated significant gains in oil yields may be possible through selection and breeding. Economic studies have shown that the single most important character affecting industry viability is that of yield.

A breeding programme was initiated by J. Bartle in 1993 with the establishment of some small progeny trials of *Eucalyptus polybractea* and *E. horistes.* These were designed to yield seed for operational use in operational planting in 1997/98. As investigations into the viability of the industry progressed it became evident that a systematic strategy for breeding and genetic improvement of cineole yield was required.

The oil mallees provide a range of useful attributes such as ease of grafting and early flowering which lend themselves to rapid turnover of generations and maximum genetic gains.

The proposed strategy is based on recurrent selection and features the use of sublines to maximise flexibility in planning and to facilitate control of inbreeding in advance generation clonal seed orchards. Advance generation breeding populations are regenerated using controlled pollination. Seed for production purposes will ultimately be produced in open pollinated clonal seed orchards. During the lead up time to production from advanced generation clonal seed orchards, seed will initially come from select parent trees in the wild. The first improved seed from seedling seed orchards will come into use from 1998. Seed from first generation clonal seed orchards should become available from 1999/2000.

# Contents

## 1.0 Introduction

Rural land degredation in Western Australia has been a topic of serious concern for at least two decades and is the focus of attention for many land care groups throughout rural Australia. Tree planting strategies are widely regarded as having the greatest potential in ameliorating land degredation (Schofield et al 1989). Current attention is being focussed on the oil mallees as a farm crop suitable for planting in very low rainfall areas. Block and belt plantings on farms will yield the dual benefit of treating degredaded areas whilst providing an economic return from the harvest of eucalyptus oil.

Economic analyses (Bartle 1995) have shown leaf oil content to be the single most important factor influencing the viability of a commercial enterprise centered on eucalyptus oil production and harvest. Evidence to date indicates that significant gains in leaf oil content are possible through breeding. Barton et al (1991) estimated high family heritability $h^2_f = 0.83$ for *Eucalyptus kochii* maiden & Blakely ssp *kochii* and *Eucalyptus kochii* maiden & Blakely ssp *plenissima* (Gardener) Brooker. Milthorpe et al (1994) reports a 20% improvement in oil content in a selected line of *Eucalyptus polybractea* R.T. Baker at Condobolin in New South Wales.

CALM is presently investigating the 1,8-cineole oil production potential of five species of oil mallee and their associated subspecies. They include *E. kochii* ssp *kochii* and *plenissima*, *Eucalyptus horistes, Eucalyptus loxophleba* Benth. ssp *lissophloia,* and *E polybractea.* Implementation of a full scale breeding program for each species may be prohibitive in terms of available man power, cost and genetic resource and may necessitate the development of one or two species with the most potential.

This document outlines a strategy for breeding oil mallees. It is intended as a starting point for discussion and refinement. Every breeding program is dynamic and methodologies and tactics will change somewhat as new knowledge comes to light. Barnes (1984) notes that "no breeding program for any species can start from a position of completed knowledge and it is not possible to adopt an optimum breeding strategy at the outset. Therefore programs must be designed to be as flexible as possible".

# 2.0 The Biology of the Oil Mallees

Below is a summary of what is known of the five eucalypt species selected.

## 2.1 Classification

The classification of the selected species (Brooker et al 1990) indicates the relatedness of the different species and their potential for hybridising.

Eucalyptus

Symphyomyrtus

**Bisectaria** - deeply bisected cotyledons while remainder have bilobed or reniform cotyledons

***Loxophlebae***

*E.loxophleba* Benth. *subsp lissophloia* (*E.loxophleba* subsp. 'smooth bark'). York Gum.

***Oleosae***

*E.kochii* Maiden & Blakely subsp *kochii*

*E.kochii* Maiden & Blakely subsp *plenissima* (Gardener) Brooker

*E.horistes* Johnson & Hill (syn. *E.oleosa* F.Muell. ex Miq. *var. borealis* Gardener)

**Adnataria** - adnate anthers compared with versatile anthers

***Odoratae***

*E.polybractea* R.T.Baker

## 2.2 Common characteristics of the five oil mallee species

All the species listed above have a number of common characteristics.

- Foremost they all have a high oil content and within that oil content a high proportion of 1,8-cineole which can range from 65 -90% (Boland et al 1991). The remainder of the oils are made up by a number of other constituents which can also have commercial value. x-Terpineol is a bactericidal material which is the oil of interest in the melaleucas and 4-methyl pent-2yl-acetate is used as a vanilla flavouring.
- The species all form a lignotuber which is important for regular foliage harvesting and the re-coppicing of shoots.
- Additionally the species selected lend themselves to the lower rainfall zones of Western Australia between 400 and 600 mm. Western Australian species may be adaptable to saline conditions.

The oleosa species and *E.loxophleba* are endemic to WA, whereas *E.polybractea* comes from NSW and Victoria. Native collections from the full range of the native population can be made.

A tabular summary of the biology of the selected oil mallees can be seen in appendix 1

## 2.3 Flowering biology

There have been no investigations into the flowering biology of the oil mallees. However generally the eucalypts are preferentially outcrossing with a high outcrossing rates maintained by varying degrees of self-fertility, aided by protandry and reinforced by selection against the products of self-fertilization in later stages of the life cycle.

## 2.4 Self fertility

Most eucalypt species exhibit a marked reduction in seed yield following self-pollination compared to outcrossing, although within species there is a considerable variation in the level of self-fertility. In most of the species examined to date, the majority of individuals are partially self-fertile but individuals range from fully self-incompatible to fully self-sterile. Post mating barriers to self-fertilisation are thus rarely complete and Eldridge (1976) notes that "persistent attempts at artificial self-pollination have been successful to some degree on almost every tree tested".

## 2.5 Hybridisation

Natural and artificial hybridisation between species from the different subgenera does not occur although within subgenera hybridisation is relatively common. Within Symphyomyrtus, the section Bisectaria (*E.kochii, E.horistes and E.loxophleba)* is noted for its low hybridisation rates, average only 0.23% and the section Adnataria (*E.polybractea)* only 0.43%. This indicates that hybridisation of species within the section Bisectaria will be successful but between Adnataria and Bisectaria will not be possible.

## 2.6 Genetic parameters

Published estimates of genetic parameters in oil mallees is restricted to an estimate of family heritability ($h^2_f$=0.83, Barton et al 1991) for*E. Kochii* at age 2.5 years. The trial consisted of an unreplicated block planting of fifty families planted in six tree row plots. The data used consisted of means of forty three families represented by leaf samples from five trees in each family, correlated with leaf sample data from the parent trees in the field. Approximately equal numbers of *E.kochii spp kochii* and ssp *plenissima* were used in the trial.

## 2.7 Vegetative propagation

Three vegetative propagation methods have been experimented with to date with varying degrees of success.

### 2.7.1 Micropropagation

This is a technique that is capable of multiplying and producing large numbers of a single clone. It involves the sterilisation of stem tissue and using the axillary shoots for shoot multiplication. When required these shoots are isolated and rooted ready for hardening-off. This method has been used successfully on *E.kochii spp kochii* at CALM and McComb and de Fossard (pers.comm) have micropropagated *E.polybractea.* Considering other eucalypt micropropagation systems, these species are relatively easy. The question that still needs to be answered is their performance in the field. The problem is that lignotuber formation is critical for the harvesting system for oil extraction. Adventitious root formation in vegetative propagation may prevent the formation of the lignotuber. This aspect is still being investigated and if the lignotuber develops, there may be potential for this technique.

### 2.7.2 Rooted cuttings

Rooted cuttings have had varied success. When coppice shoots were taken from mature *E.kochii spp kochii* and *E.kochii spp plenissima* trees callus only formed at the base of the cutting with no normal root formation. Cuttings taken from seedlings rooted easily. The technique thus has potential to multiply young material. As with the stucklings, the formation of the lignotuber is a key consideration for the usefulness of this technique.

### 2.7.3 Grafting

The grafting of *E.kochii spp kochii, E.kochii spp plenissima, E.horistes* and *E.polybractea* can be successfully done. The rootstock must be young with no woodiness obvious in the stem. Incompatibility between the rootstock and the scion does occur and this needs to be further investigated. Nevertheless this is a reliable technique by which to multiply selections for seed orchard establishment.

## 2.8 Disease and pest problems

To date no serious disease or pest problems have been identified. The usual grasshopper attack at planting needs to be controlled and there is some insect leaf grazing.

# 3.0 Genetic Resource

## 3.1 Species Trials

To date some 27 species trials have been established on a wide range of sites encompassing as full a range of sites and soil types as possible (appendix 2 ). In addition to the trials listed, a further 5 trials are scheduled for planting in 1995.

## 3.2 Provenance/progeny trials

To date some 2 500 candidate trees across the 5 species have been screened with less than 15% meeting the 2.5% fresh weight 1,8 cineole content set as a minimum standard for parent trees. Random leaf samples are collected from candidate trees and stored in alcohol. The samples are then screened using gas chromatography for percentage wet weight of 1,8 cineole. Further screening of potential parent trees is in progress.

Provenance/progeny trials of *Eucalyptus horistes* and *Eucalyptus polybractea* have been established on three sites during 1993 (appendix 3). Trials of *E. Kochii ssp plenissima* were planted on four sites in 1994. A further set of three trials of *E. loxophleba* subsp *lissophloia* are scheduled for 1995.

At present the trials in the ground include relatively small numbers of families ie. 20-30. However, the current commitment to seed collection has resulted in an increase of the available numbers of species to between 40 and 60 families per species (J.Bartle pers

comm). Over the period 1995 to 1998 it is envisaged that the number of families collected will be increased to 300 families per species.

For the 1996 planting season some 150 families per species, may be available for progeny testing.

## 4.0 Definitions

Prior to any discussion of breeding programs or strategies it is necessary to define some concepts common to tree breeding programs around the world. A tree breeding program will involve the use of at least 3 conceptual populations which may be represented by 3 physical populations of trees. More commonly one physical planting may serve the role of two or more conceptual population types. The following definitions are taken from White (1987).

| | |
|---|---|
| Base population - | All individuals available for selection in a given generation. |
| Selected population - | Members of the base population selected in any given generation of breeding. |
| Production Population - | Some or all of the members of the selected population set aside and managed to produce large quantities of genotypically superior propagules. The purpose of this population is to produce seed or clones for afforestation. |
| Breeding Population - | Some or all of the members of the selected population retained to be intermated to regenerate genotypic variability in the offspring.<br>Purpose: the highly variable offspring are planted as the next generations base population. |

## 5.0 Objectives

Tree breeding programs should aim to maximise gains per unit time in production populations whilst minimising the effects of inbreeding. Put more simply, a breeding program seeks to maximise the economic benefit of the production enterprise. A properly defined breeding objective is a critical part of any genetic improvement program as it sets the direction improvement will take (Woolaston and Jarvis 1995). Borralho *et al.* (1992) defined a breeding objective as the combination of characteristics or traits the breeder wishes to improve. Decisions about which traits to include in the breeding objective should be based on purely economic grounds.

The use of economic weights for each trait assessed can be incorporated into index selection (Cotterill and Dean 1990) to steer selection in the direction which will maximise profitability. An economic study of similar nature to that of Borralho and Cotterill (1992) should eventually be carried out when reliable estimates of genetic parameters become available.

Selection criteria for the oil mallee breeding program in perceived order of importance include:

1. High leaf oil content in early growth and in coppice,
2. Coppicing vigour,
3. Salinity tolerance,
4. Acid tolerance,
5. Form - ideal form is yet to be determined and will be influenced by harvesting methods.
6. Other oils

It should be noted that selection criteria ie. the things that are measured and therefore provide information on the breeding value are not necessarily the same as the traits that directly affect profitability and comprise the breeding objective.

## 6.0 Breeding populations

The success of a breeding program depends entirely on cumulative improvement of the breeding population. Control of inbreeding in advance generations of the breeding population and ultimately in the resulting production populations is a primary concern facing tree breeders. For these reasons some thought has to be given to the size and structure of the breeding population.

### 6.1 Size of a breeding population

A survey of the literature (Burdon and Shelbourne 1971, Burdon 1995, Cotterill 1986, Kang and Neinstadt 1987, Libby 1973, Nichols 1980, White 1992 & 1995, Zobel and Talbert 1984) suggest numbers ranging from 200 as a minimum up to 2000 effective breeding individuals. Cotterill (1984, 1986) and White (1992, 1995) suggest that a breeding population of size 300 effective breeding individuals should sustain an entire breeding program for many generations.

Advance generation base populations will require approximately 100 offspring per parent (Cotterill 1986), for example 35 offspring on each of three different sites.

It is of interest to note that it may be possible to narrow the genetic base for economically important traits whilst simultaneously broadening the base for adaptability to pest resistance because the two groups of characteristics are usually independent genetically (Brune and Zobel 1981).

### 6.2 Structure of the breeding population

There are three general options described in the literature for structuring a breeding population,
1. Maintaining and improving a single large population,
2. Sublining the main population or,
3. Dividing the main population into smaller subgroups or multiple populations (Burdon and Namkoong 1983).

For the first option detailed pedigrees for each family are required to ensure that the levels of inbreeding are minimised, although inbreeding will develop over several generations. Ultimately the concern is to be able to maintain production populations which contain only unrelated clones. This requires detailed records of pedigree. Maintenance of pedigree over successive generations is a large and critical task. The advent of TBIMS (Butcher 1993?) enhances record keeping capabilities. Problems may arise in open pollinated populations where paternal parentage is unknown. The difficulty being to ensure or at least minimise unrelated mating in advance generations of breeding and production populations.

Sublining as a strategy for structuring a breeding population requires division of the main population into small groups or sublines of equal size e.g. the *E. globulus* breeding population size is 300 individuals divided into 10 sub lines of 30 families each. Another example of sublining can be seen in McKeane and Beineke (1980). No out crossing between sublines is permitted. All crossing to regenerate the next generation occurs within a subline. Inbreeding within lines will occur quite quickly - particularly with open pollinated matings (McKeanal and Beineke 1980). In the production population, only the best single clone from each subline is incorporated into the production orchard in any given generation. This ensures that only unrelated mating occurs in the production population, and therefore maximum genetic gain is achieved.

The high levels of inbreeding within a subline may present problems with selection, particularly in the selection of the third and later breeding populations since some individuals or families may be more inbred than others and could be unfairly penalised (Van Buijtenen 1981) ie. some genotypes will produce poorer offspring when mated with close relatives than they would in unrelated matings. Hence true breeding values of individual parents may be difficult to obtain.

Two solutions exist:

1. Progeny testing of select parents in inbreeding populations using an unrelated mating from other sublines or external populations.
2. Introduction of fresh infusions of genetic material into the sublines (White 1995) from external sources.

A potential problem with 2. above is that as generations advance, it is probable that any new genotypes from unimproved populations will reduce the amount of gain that has already been achieved (Zobel and Talbert 1984). Progeny testing of potential infusions can overcome this concern (White 1987).

The third option of multiple populations can be used when there is a need to produce trees for different, possibly conflicting objectives. For example adaption to saline or acidic soil conditions. Negative correlations between desirable traits for different requirements may leave no option other than to use multiple populations (Cotterill 1984).

Sublining is probably suitable for the purpose of oil mallee breeding owing to its flexibility. The advantage of starting with sublines is that they can be amalgamated to form a main line population at any time whereas the reverse is much more difficult.

## 7.0 Advance generation Mating designs

To generate the next base population for selection, a range of options exist. The base population will require approximately 100 offspring per parent (Cotterill 1986), most probably in progeny trials replicated on 3 sites with 35 offspring per parent per site. Cotterill (1984) suggested that the most efficient ranking of families can be achieved with as few as 10 to 20 individuals per family. This conflicts with the goals of using the progeny tests as base populations for advanced generations where as many individuals as possible should be available for selection.

In a study of gains achievable from different mating designs Cotterill (1986) found that open pollinated (OP) mating with combined index selection produced reasonable gains. Examples of successful breeding programs employing OP matings include *E. robusta* in Florida which has reached its third generation (Cotterill 1986). The *E. grandis* breeding program in Florida has reached the fourth generation ( Reddy and Rockwood 1995). McKeane and Bieneke (1980) report on OP matings in Black Walnut.

If flowering times are synchronous amongst families in the breeding population there is reasonable expectation for good levels of outcrossing in a seed orchard. For example Moran *et al.* 1989 estimated the outcrossing mate in a first generation seed orchard as $t$ = 0.91 compared with an estimate of $t$ = 0.75 from a native stand. The disadvantage for production of advance generation breeding populations is that no control over paternal relatedness is exercised. Sublining circumvents this problem in the production population.

Possible options for control pollinated (CP) mating designs include single pair matings, OP, polycross and disconnected diallels.

## 8.0 Research required

### 8.1 Genetic parameters

8.1.1 Genetic architecture of species: At this point in time genetic architecture of the species in terms of oil production and its relationship to the various planting environments is critical. In order to construct a breeding population with sublines addressing environmental problems such as salt tolerance, soil acidity and drought it is essential that data on performance on these site types is collected.
Method: provenance/progeny testing on all sites.

8.1.2 Genotype by environment interactions. In construction of sublines it will be necessary to know if certain families are interactive with different site types. This may be determined by classical anova and the use of "Type B" genetic correlations (Burdon 1977).
Data Source: provenance /progeny trials.

8.1.3 Heritability. Estimates are required to aid in selection strategies and prediction of genetic gain.
Data Source: provenance /progeny trials.

**Note :** It is very important to establish a progeny trial including parents from across the spectrum of oil yield. This will give vital information on the efficiency of phenotypic selection in the wild. Present screening procedures rely on the assumption that good phenotype (high oil yield in the wild) is indicative of an individuals breeding value for this trait. We may be discarding valuable material unnecessarily.

8.1.4 Genetic and phenotypic correlations Necessary for selection and prediction of gain.
Data Source: provenance /progeny trials.

8.1.5 Age-Age correlations: i.e.Type A genetic correlations these are important in evaluating the efficacy of early selection (Burdon 1977).
Data Source: provenance /progeny trials.

8.1.6 Expression of inbreeding depression: This type of data can influence estimates of heritability and ultimately selection strategy.
Data Source: controlled crossing experiments.

8.1.7 Realised gains: demonstration plots.

**8.2 Biological constraints**

8.2.1 Phenology: Essential data for efficient construction of seed orchards.
Data Source: Monitor flowering times in field and in trials.

8.2.2 Crossing techniques: Necessary for efficient controled pollination.

8.2.3 Vegetative propagation: grafting, graft incompatibility.

8.2.4 Self fertility: overlaps with 8.1.6 above.

8.2.5 Hybridisation potential and performance: Significant gains in some traits can be achieved using hybrids of two species. Research in this area may yield very high gains in oil yield. Depending on seed yield which can be reduced in hybrid crosses, a hybridisation program may require the use of vegetative multiplication of propagules for deployment in the field- an area which still needs investigation.

8.2.6 Different species for different site types? i.e. Do /can we breed for site types within a species or is it more efficient to simply use different species for different sites?
Data Source: provenance /progeny trials.

## 9.0 Breeding strategy - *E. horistes, E.polybractea, ,E. kochii ssp kochii and ssp plenissima, E. loxophleba*

9.1 Minimum breeding population size of 300 unrelated families per species.

9.2 Where sufficient numbers of families become available, main breeding population to be sublined - 10 sub lines consisting of 30 parents. This strategy maximises gain in trees used for afforestation.

9.3 Recurrent selection within sublines to produce an advanced generation base population and breeding population.

9.4 Production populations: First generation to consist of seedling seed orchards (SSO) and clonal seed orchards (CSO) containing 20 - 30 clones. These will constitute an interim seed supply until clonal orchards from the mainstream breeding program begin production. Include a large number in an attempt to circumvent the problems with phenology. CSOs to be established at Como SID, wheatbelt and Wellington catchment.

9.5 Open pollination (OP) in production orchards.

9.6 Breeding populations: First generation breeding populations to be built up over time as resources permit for each species. Selections from progeny trials to be stored in a clonal archive located at Como and replicated at one other location (Narrogin? Location to be determined) for security.
**Note:** Progeny tests of wild parents will not (can not) be established on the basis of sublines. Rather, sublines of the first generation breeding population will be constructed using either backward or forward selection or a combination of the two, depending on the breeding values of the individuals involved.

9.7 Control pollination in breeding population using a mating design to maximise number of an unrelated full sib parents eg. single pair mating or a nested polycross (Burdon and Shelbourne 1971) type design (to be decided).

9.8 Second generation base population consists of progeny tests of offspring produced in 9.7 above. Note: Advance generations of base population will be established as progeny trials within the subline structure.

9.9 Index selection with restrictions on numbers of parents selected per family to select the 2nd generation breeding population. Possible use of BLUP breeding values.

9.10 Selections to be stored in a clonal archive to form the second generation breeding populations.

9.11 Best individual from each subline into second generation CSO for production.

9.12 Steps 9.7 to 9.11 repeated to form advance generations of breeding and production populations.

9.13 Infusions of new families can be incorporated into sublines e.g. 3-5 families in each generation to further control inbreeding.

9.14 Hybridisation: For species with very small numbers eg. *E. kochii* ssp *kochii*. It may be possible to hybridise with another more plentiful species eg. ssp *plenissima* and therefore treat *E. kochii* as a subline of ssp *plenissima*.

## 10.0 Seed Production Strategy

The primary goal in every breeding program is that of producing improved propagules for deployment in operational planting. Seed collected from trees in the wild may produce off spring with varying degrees of inbreeding depression as a result of neighbourhood mating patterns between close relatives (Zobel and Talbert 1984). Seed orchards provide a means for maximising heterosis by establishing outcrossing opportunities between non-relatives,.

**10.1 Interim seed supplies - Seedling seed orchards - current status.**
At present all seed for production purposes is collected from select parent trees located in the field. It is anticipated that orchard seed will become available in 1997 once the current trials begin producing.

The provenance/progeny trials established for *E. horsites* and *E. polybractea*, *E. kochii* ssp *plenissima* are destined for use as seedling seed orchards. Following assessment the trials will be thinned to single tree plots to maximise outcrossing potential.

**10.1.1 Trial design:**
Twenty to thirty families planted in three tree row plots replicated 14 to 20 times (Table 1) within a trial. Each trial was replicated on at least three sites.

**10.1.2 Within family selection:**
Assess all trees in trial for oil yield at age 3 years. Cull the two poorest trees from each family plot. Culling should be done prior to the initial seed set to prevent matings between members of the same family.

**10.1.3 Mating strategy:** Open pollinated mating.

**10.1.4 Research value:**
These trials are the first replicated trials of oil mallees with family identities in

Western Australia. Every effort should be made to collect genetic data from them prior to culling.

### 10.1.5 Measurement program:

1995 - assess trials for oil content - age 2 years on all sites. Assess for vigour ie. height, crown diameter, crown density.

Crown density may be assessed subjectively using a six point scale (Cotterill and Dean 1990). In this instance a score of six would represent dense foliage for the site and a score of 1 would represent poor or very sparse foliage for the site
1996/7 Reassess trials for above traits, immediately prior to culling. Data collection ideally would coincide with the age of first harvest to give the data a more practical application.

Data obtained will yield heritability of traits assessed, phenotype and genetic correlations between traits, age-age correlations for all traits and an indication of genotype x environment interaction, parent offspring correlations. All these parameters are necessary to fine tune the breeding strategy.

Subsequent to culling and harvesting of the seedcrop, further experimentation with coppicing ability may be possible.

A potential problem with open pollinated seedling seed orchards (OPSSO's) is that of flowering. Synchrony of flowering in the OPSSO's established to date remains to be seen.

## 10.2 Interim seed supplies - Clonal seed orchards (CSO)

The bulk of the proposed seed production strategy is based on the use of clonal orchards and open pollination. Advantages of the CSO are that the most outstanding genotypes are included since they can be replicated many times. By comparison an outstanding genotype may occur only once in an OPSSO. Where asynchronous flowering occurs, the opportunity for control pollination exists to allow crossings to which may otherwise not occur.

### 10.2.1 CSO Strategy.

A first generation CSO of the best 20 clones for each species should be established as quickly as possible, to augment SSO seed. At this stage it is prudent to maximise the number of clones in a clonal orchard to circumvent problems with asynchronous flowering

Following assessment in 1996/97, a second generation (interim) clonal seed orchard can be constructed using the best selections from the Prov/progeny trials. As a result of crossing, flowering times may become less of a problem, although this is not guarranteed. Depending on how many clones are used, gains in oil production should be greater than those achieved through OPSSO's

### 10.3 Main stream CSO

Establishment of CSO's derived from sublining will be established as soon as the main breeding population has been constructed and breeding values estimated for all parents.

## 11.0 Provenance/Progeny testing

It is anticipated that 80 select parents per year can be added to the progeny testing program using current resources(J.Bartle pers. comm. 1995)). On this basis it will take 4 years including P1996 through to 1999 to establish progeny test of the target 300 select parent trees in the field and a further 3 years to collect sufficient data and construct the full size breeding population. Screening and collection of seed from parent trees is at present the slowest part of establishing the breeding program. devotion of more resources to this process will significantly reduce the time required to reach the second generation of breeding.

Progeny testing of the field selections is essential to obtain genecological data as well as estimating breeding values to aid in the selection of the very best trees.

In order to enable efficient use of BLUP for estimating breeding values, 30% linkage or connectiveness between trial is required (Sue Jarvis STBA pers. comm) it is therefore recommended that a minimum of 28 families be treated as standards to be planted on all future provenance/progeny trials. This will necessitate extra collections of the standard families. These could comprise the families presently used in the progeny/OPPSO trials for each species. The qualification being that standard families must show stable performance across sites so that standard x environment interaction will be small in all future progeny tests and thus not confound family comparisons across trials (White et al 1986). Inclusion of the same families in all trials will maximise precision of family comparisons across tests.

**11.1 Trial design:** 12 x 9 alpha lattice with 7 replications. Trees to be planted in 5 tree row plots to give a total of 35 trees/family/site. Spacing could go as low as 2m x 2m to allow for thinning down to seed orchard status. Although an OPSSO is desirable end use for the trials, spacing chosen must not compromise the applicability of data to commercial situations. Trials should be replicated on a minimum of 3 sites.

**11.2. Structure of Seed Collection:** Where possible, if provenance effects are considered likely, balanced representation of families from each provenance is desirable (Land et al 1986).
An estimated time table for trial establishment and seed production in tabular form is presented in appendix 2.

## 12.0 Working plan Action 1995-96

12.1 Seed collection to advance as rapidly as possible.

12.2 Collate all data from field to identify possible geographic variation. Geographic variation will be an important factor in strucuring breeding population.

12.3 Assessment of early performance of special trials on different sites. Will yield information on the suitability of species for different site types. May give an early indication of provenance variation

12.4 Establish clonal production orchards - all species.

12.5 Estimate of seed/yield per tree. Therefore number of replicates of basic seed orchard unit required to meet projected demands. Estimate of time to CSO seed production 2-3 years?

12.6 Progeny test of 60 parents using 20 progeny from the low,mid and high range of oil yield. This will give important data on the effectiveness of phenotypic selection and the validity of using 2.5% as a cut off point in selecting parents.

12.7 Progeny testing of all selections in field - to be established beginning P1996 in sublines of 30 families. Trial design 6x5 alpha lattice designs. Seedlings to be raised at Narrogin nursery.

12.8 Initiate research on hybridisation potential between appropriate species . Data provided will enable more detailed structuring of breeding populations and strategy. Starting date depends when flowering of species in clonal orchards begin.

12.9 Initiate screening of trials in ground for oil production - to provide preliminary estimates of genetic parameters as well as provide data for genetic correlations with subsequent measurements ie. juvenile-mature correlations.

12.10 Penology - Initiate observations in trials and field to observe timing of flowering. Very important data for design of seed orchards.

12.11 Security of land tenure to be organised.

12.12 Collation, centralisation and backup of all available data on computer preferably in a format completely compatible with Dbase. 1995
Ultimately all data should be stored in TBIMS format. TBIMS is 'almost' completed (T. Butcher pers. comm.)

## 13.0 References

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## **Appendix 1.** Biology of the selected oil mallee species

Below are a set of tables outlining the main biological criteria of each of the species. Data is summarised from Boland, D.J, Brophy, J.J., and House APN. , (1991), Boland, D.J., Brooker, M.I.H. and Turnbull, J.W., (1980) and Brooker, M.I.H. and Kleinig, D.A., 1990.

***E.loxophleba* Benth. *subsp. lissophloia*** (*E.loxophleba* subsp. 'smooth bark').

| | |
|---|---|
| Appearance | Mallee 5-15m. Bark smooth, grey over rich coppery. |
| Oil yield | 2.4% with 67% 1,8-cineole + x-pinene, trans-pinocarveol, x-terpineol, 4-methyl pent-2yl acetate. |
| Distribution | Widespread but sporadic distribution in the eastern wheatbelt and goldfields, often in depressions; endemic to WA. |
| Soil type | Loams to sandy loams |
| Age to flowering | 3-4 years |
| Inflorescence appearance | Inflorescences axillary, unbranched 7 to 11 flowers. Stamens all fertile, white. |
| Flowering period | September - February (December) |
| Capsule appearance | Pedicellate, obconical |
| Seed collection | Dec - April. Seed does not hold on well. Should be collected during this time for maximum harvest. |
| Seed per capsule | |
| Viable seed per g | 631 $\pm$ 350 viable seeds per 1g seed and chaff. Highest 1470. 29 seedlots were tested with 0.1g/replicate at 25'C and counted between 5 and 21 days. 6000 pure seeds per g |

***E.kochii* Maiden & Blakely subsp *kochii***

| | |
|---|---|
| Appearance | Mallee tree with bark rough and grey |
| Oil yield | |
| Distribution | Restricted to Wongan Hills, Watheroo - Dalwallingu area . Endemic to WA. |
| Soil type | Usually on yellow sand |
| Age to flowering | 3-4 years |
| Inflorescence appearance | Inflorescence axillary with (7)9 - 13 flowers. Stamens fertile and white. |
| Flowering period | December to February (May) |
| Capsule appearance | Short pedicel, globose to urceolate shape. |
| Seed collection | Dec - Feb with good retention of seed. |
| Seed per capsule | |
| Viable seed per g | 228 viable seed and chaff per 1g. Only one seedlot tested with 0.2g replication at 15'C and counted between 7 and 21 days. 1800 pure seed/g. |

## *E.kochii* Maiden & Blakely subsp *plenissima* (Gardner) Brooker.

| | |
|---|---|
| Appearance | Mallee tree with bark rough and grey. Differing from kochii in the broader juvenile leaves, larger adult leaves and more robust buds and fruit. |
| Oil yield | |
| Distribution | Widespread in the north-eastern wheatbelt of Western Australia, north of Murchison River to between Mount Magnet and Sandstone, extending east to Southern cross. |
| Soil type | Usually on loam to sandy loams |
| Age to flowering | 3 - 4 years |
| Inflorescence appearance | Inflorescence axillary with (7)9 - 13 flowers. Stamens fertile and white. |
| Flowering period | December to February |
| Capsule appearance | Short pedicel, globose to urceolate shape. |
| Seed collection | Dec - Feb with good retention of seed. |
| Seed per capsule | |
| Viable seed per g | 228 viable seed and chaff per 1g. Only one seedlot tested with 0.2g replication at 15'C and counted between 7 and 21 days.<br>1800 pure seed/g |

## *E.horistes* Johnson & Hill

(syn. *E.oleosa* F.Muell. ex Miq. *var. borealis* Gardner)

| | |
|---|---|
| Appearance | Mallee with grey rough bark. Differing in appearance from kochii and plenissima by having glossier leaves, longer beaked opercula and north-westerly distribution. |
| Oil yield | 2.1 - 4.7% oil yield dry wt with 73 - 83% being 1,8-cineole. |
| Distribution | Widespread in the far northern wheatbelt of WA, particularly Morawa, Mullewa and Canna districts and to the east thereof, also north-west towards Yuna. |
| Soil type | Usually on red loams. |
| Age to flowering | |
| Inflorescence appearance | Inflorescence axillary with (7)9 - 13 flowers. Buds have beaked operculum. Stamens fertile and white. |
| Flowering period | December to February |
| Capsule appearance | Short pedicel, globose to urceolate shape. |
| Seed collection | Dec - Feb with good retention of seed. |
| Seed per capsule | |
| Viable seed per g | 60-440 seed and chaff per 1g with 68 - 97% viability. Tested on 15 seedlots at 15'C over 21 - 28 days.<br>1800 pure seed/g |

## *E.polybractea* R.T.Baker

| | |
|---|---|
| Appearance | Bark rough grey, persistent on the lower half of the stems becoming papery or ribbony and then smooth grey to pink on upper half.. |
| Oil yield | 0.7 - 5% with a cineole content of 92%. Other oils are terpinen-4-ol (0.5%), x-terpineol (0.4%) and carvone (0.2%). |
| Distribution' | Two disjunct populations, near Wyalong in western NSW (altitude 250 - 350m, mean annual rainfall 470mm) and in Bendigo area of NVictoria (altitude 140 - 200m, mean annual rainfall 540mm). |
| Soil type | Commonly red-brown loams often with quartz or sandy and shale soils. |
| Age to flowering | |
| Inflorescence appearance | Axillary with 7 to11 flowers with buds diamond shaped, |
| Flowering period | March to June |
| Capsule appearance | Pedicellate cupular to barrel shaped. |
| Seed collection | |
| Seed per capsule | |
| Viable seed per g | 846 viable seed and chaff per 1g. Highest 1260. $ seedlots tested with replicate 0.05g at 20/15'C and counted between 10 and 28 days.<br>6000 pure seed/g. |

**Appendix 2.** Species Trial Information

| Year | Location | Farmer | # Species | # Reps | Length of species hedge |
|---|---|---|---|---|---|
| 1993 | Canna | C. Croot | 7 | 3 | 30m |
| | Eneabba | B. Betts | | | |
| | Kalannie | W. Campbell | | | |
| | " | R. Martin | | | |
| | Wongon Hills | M. Davey | | | |
| | Beverly | C. Ayres | | | |
| | Tammin | P. Chatfield | | | |
| | Trayning | C. Riley | | | |
| | Merriden | Ag. Dept. | | | |
| | Jerramungup | R. Edminson | | | |
| | " | " | | | |
| | Newdegate | Ag. Dept. | | | |
| | Esperance | Agnews | | | |
| | " | Stewarts | | | |
| | " | Freemans | | | |
| | Mt Barker | Ag. Dept. | 7 | 1 | ? |
| | Bonnawarrup Crk | ? Tim Mitchell | | | |
| | Kalgan River | " | ? | | |
| | Wellington | D. Nile | | | |
| | | | | | |
| 1994 | Yuna | K. Williamson | 5 | 4 | 30 |
| | Perenjori | G. Smith | | | |
| | Beverly | C. Aures | | | |
| | Lake Towerining | I. Pearce | | | |
| | Brookton | Doorman | | | |
| | Corrigin | L. Pitman | | | |
| | Mekering | | | | |
| | Nth Stirlings | B. Whitham | | | |
| | " | B. Hamms | | | |
| | " | D. Hancock | | | |

**Appendix 3.** Current number of progeny trials for different oil Mallee species

| SPECIES | PDATE | SITES | FAMILIES | DESIGN |
|---|---|---|---|---|
| *E. horsites* | 93 | 2 | 20 | 20 reps x 3 tree plot |
| | | 1 | | 14 reps x 3 tree plot |
| | | | | |
| | | | | |
| | | | | |
| *E. polybractea* | 93 | 2 | 30 | 20 reps x 3 tree plot |
| | | 1 | | 14 reps x 3 tree plot |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| *E. plenissima* | 94 | 4 | 20 | 20 reps x 3 tree plot |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| *E. lissophloia* | 95 | 3 | 25 | 20 reps x 3 tree plot |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| *E. polybractea* | 96 | 3 | 30 | |
| | | | | |
| | | | | |
| *E. kochii* | 96 | 3 | 20 | |
| | | | | |

**Appendix 4**

Potential timing of trials and seed production

| **Species X** | | **1993** | **94** | **95** | **96** | **97** | **98** | **99** | **2000** | **01** | **02** | **03** | **04** | **05** | **06** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Progeny trials 20 families : | site 1 | P | | | OC | S-> | | | | | | | | | |
| | site 2 | P | | | OC | S-> | | | | | | | | | |
| | site 3 | P | | | OC | S-> | | | | | | | | | |
| | | | | | | | | | | | | | | | |
| Interim CSO 10-20 clones: | site 1 | | | | G | P | | *S->* | | | | | | | |
| | site 2 | | | | G | P | | *S->* | | | | | | | |
| | | | | | | | | | | | | | | | |
| Progeny trials 300 families in 4 stages of 80 families | | | | | | | | | | | | | | | |
| Series 1 | site 1 | | | | P | | | OC | *S->* | | | | | | |
| | site 2 | | | | P | | | OC | *S->* | | | | | | |
| | site 3 | | | | P | | | OC | *S->* | | | | | | |
| | | | | | | | | | | | | | | | |
| Series 2 | site 1 | | | | | P | | | OC | *S->* | | | | | |
| | site 2 | | | | | P | | | OC | *S->* | | | | | |
| | site 3 | | | | | P | | | OC | *S->* | | | | | |
| | | | | | | | | | | | | | | | |
| Series 3 | site 1 | | | | | | P | | | OC | *S->* | | | | |
| | site 2 | | | | | | P | | | OC | *S->* | | | | |
| | site 3 | | | | | | P | | | OC | *S->* | | | | |
| | | | | | | | | | | | | | | | |
| Series 4 | site 1 | | | | | | | P | | | OC | *S->* | | | |
| | site 2 | | | | | | | P | | | OC | *S->* | | | |
| | site 3 | | | | | | | P | | | OC | *S->* | | | |
| Total of 300 families in progeny trials | | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | |
| Construct sub lines in clonal archive Breeding popn | | | | | | | | | | | G | P | | CpS-> | |
| | | | | | | | | | | | | | | | |
| CSO | | | | | | | | | | | G | P | | S-> | |
| Advance generation base population | | | | | | | | | | | | | | | P |
| Next series of progeny trials repeat cycle | | | | | | | | | | | | | | | |

C = cull, Cp = control pollinate, G = graft, O = test oil content, P = plant, S = seed production.

Letters in italics are options to augment seed production if required.