



An Australian Government Initiative



Breeding blue mallee for eucalypt oil

**A report for the RIRDC/Land & Water
Australia/FWPRDC/MDBC
Joint Venture Agroforestry Program**

by M. U. Slee

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Foreword

Selection and breeding programs are used to develop improved genetic material for enhancing the productivity of commercial plantations. Growth rates, wood and other key properties can be targeted for tree improvement.

Since the early 1990s, the Rural Industries Research and Development Corporation and the Joint Venture Agroforestry Program have been working with G.R. Davis Pty Ltd and the School of Resources, Environment and Society at The Australian National University to investigate a blue mallee (*Eucalyptus polybractea*) breeding program near West Wyalong in western New South Wales. The plantation has been producing eucalypt oil from blue mallee over two decades. The breeding program aims to improve eucalypt oil production, to increase commercial returns from future plantations. This is relevant to other low rainfall areas of Australia with prospects for growing oil mallee, including New South Wales where blue mallee originates.

The breeding research reported here has targeted oil quality and achieved excellent results for the selected traits (percentage of cineole present in the oil, and oil content per weight of leaf). While there was no overall production gain, this could be addressed in further selections (for overall yield and leaf quantity). A study of flowering behaviour of the trees in the orchards was also conducted. This knowledge assists in understanding the contribution of each tree to the orchard's seed production and is fundamental to breeding program management. The research also developed an effective procedure for bulk harvesting of experimental material, which has application to other eucalypt oil breeding programs.

This project was funded by the Joint Venture Agroforestry Program (JVAP), which is supported by three R&D Corporations — Rural Industries Research and Development Corporation (RIRDC), Land & Water Australia, and Forest and Wood Products Research and Development Corporation (FWPRDC), together with the Murray-Darling Basin Commission (MDBC). The R&D Corporations are funded principally by the Australian Government. Both State and Australian Governments contribute funds to the MDBC.

This report, a new addition to RIRDC's diverse range of over 1600 research publications, forms part of our Agroforestry and Farm Forestry R&D program, which aims to integrate sustainable and productive agroforestry within Australian farming systems.

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Peter O'Brien

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Executive Summary

What the report is about

This report compares the early gains achieved using three different breeding strategies (seed orchards) intended for improving blue mallee *Eucalyptus polybractea* oil production. The research also investigated the flowering behaviour of the trees in the orchards, which assists in understanding the contribution of individual trees to seed production, and has implications for breeding blue mallee.

Who is the report targeted at?

The report is for industry investors and researchers interested in the scope for improving blue mallee oil quality and yield, via improved genetic material.

Background

Eucalypt oil, of which cineole is a major constituent, is used widely for pharmaceutical purposes. Blue mallee is one of several species used commercially to produce eucalypt oil in Australia. Blue mallee originates from New South Wales and Victoria. In previous trials in West Wyalong, NSW, blue mallee proved superior to two other species (*E.kochii* and *E.plenissima*) which come from Western Australia. The research outlined in this report is centred on an active plantation program at Tallimbalong, West Wyalong (NSW) operated by G.R. Davis Pty Ltd, which grows blue mallee for eucalypt oil production. The plantation work is pioneering and has direct application in the low rainfall areas of Australia, including Western Australia, New South Wales and Victoria.

The Davis Company's breeding program aims to improve commercial eucalypt oil production. Previous work in the breeding program has used traditional forestry methods for i) selecting good quality trees as parents, (ii) collecting seed from these directly and (iii) developing orchards for the production of seed. In that work, two types of orchard were established – a clonal orchard using clones of the selected trees and a seedling orchard, with seedlings raised from seed of the selected trees. In 1999, both seed orchards produced good quantities of seed for the first time, so it became possible to conduct a detailed comparison of the three breeding strategies. This report describes new research to evaluate the genetic gain achieved so far.

Aims

The aims of this research were to:

- i) determine the oil quality and gains achieved by each of the three breeding methods (seed trees, clonal orchard and seedling orchard), compared with unimproved material
- ii) conduct a study of flowering behaviour of the trees in the orchards, to gain insight into the level of cross-fertilisation and impact on composition of the seed produced.

Methods

The trial tested the effect of four different levels of genetic improvement on tree height, oil production and oil quality. Four different seed sources were planted in the field in 2001, representing different levels of genetic improvement. These were (i) unselected control, (ii) selection from a single seed tree, (iii) a clonal seed orchard and (iv) a seedling seed orchard. The layout was a randomised block with five replications and the unit plots were single lines of approximately 80 trees. Trees were measured for height and oil samples taken in September 2003, and in November 2003 the trees were harvested using standard commercial procedure. The amount of cineole in the oil from each seed source, as well as oil yields per replicate line was analysed.

Preliminary flowering observations were made in 1998, followed by detailed studies in 1999 and 2001. As there were very few flowers in 2002 and 2003, no meaningful observations could be made in these years. Flowering was recorded for 57 trees in the clonal orchard and 23 trees in the seedling orchard flowered in 1999 and 2001. At each assessment, the proportion of flowers in each of 6 stages was recorded for each tree.

Results/key findings

The research demonstrated a clear improvement in oil quality, with a gain in cineole content of about 10% with all three breeding procedures. However, there was no gain in overall oil yield. This reflects the lack of selection for this characteristic. There was a gain in oil levels per weight of leaf with the seed tree and the clonal orchard, but not in the seedling orchard material. This may reflect the limited numbers of trees available for sampling in the seedling orchard, resulting in very low selection intensity.

Stage 3 flowering, defined as the visible presence of anthers, occurred from late February through until May. Some trees were clearly early flowering and others late, but none in the orchards were so isolated as to be unable to mate with those around.

However in 1998 some trees, which were later cut from the clonal orchard because of their poor oil qualities, were noted as flowering unusually late (July - August) and would have been unable to mate with others in the orchard. If some trees do flower out of synchronization with the bulk of the species they must be susceptible to self-pollination or to hybridisation with other species. This needs further study as it has evolutionary implications and is of direct practical importance for seed collection and conservation.

Implications for relevant stakeholders

The project has shown that improvements in oil quality can be obtained through breeding, and it is now important to develop a breeding procedure to select for overall oil yield (content per leaf and leaf quantity). Further study is also needed on the high levels of variation in wild populations and early orchard collections, and relationship to flowering synchrony which may affect seed production. The procedure for bulk harvesting of experimental material for oil analysis worked well and should be used in other eucalypt oil trials. The essential component is the planting of treatments in long lines so as to allow mechanical harvesting.

Recommendations

1. A procedure should be developed to select for gain in blue mallee oil yield (overall yield and leaf quantity). This should complement the improvements in oil quality (cineole percentage and oil content per leaf) gained in this study.
2. Prior to further breeding for oil quality and overall oil yield, larger-scale plantings should be established using a broader genetic base.
3. Tests of oil quality and yield should be large scale and based on commercial harvesting methods. The key recommendation for progeny trials is to plant each treatment as lines right through the experiment, so that the harvester can pass right through and collect the material in a container dedicated to that treatment, from which the oil content can be determined for each line. While organisation of the planting and distillation will be slower, the method will allow use of commercial procedures and reduce problems with extrapolation from conventional tree breeding experimental plots with few trees. The benefit of this approach is that it will (a) compensate for the substantial variation in tree vigour and total production in *E. polybractea* material collected from natural populations, (b) avoid cumbersome and expensive measurement of biomass and oil production per individual tree.
4. For seed production, larger plantings using a broader genetic base are recommended, to ensure adequate cross-pollination and seed set despite asynchronous flowering between individuals.
5. Research should explore the effect of site fertility on flowering time, and its impact on choosing sites for seed orchards.

Introduction

Eucalypt oil, of which cineole is a major constituent, is used widely for pharmaceutical purposes. Plantations for the production of eucalypt oil have potential in some low rainfall areas of Australia. Consequently the establishment of such plantations is under way or is being considered in various country areas, especially in Western Australia, but also in New South Wales.

Blue mallee (*Eucalyptus polybractea* R.T.Baker) is one of several species used commercially to produce eucalypt oil in Australia. Blue mallee originates from New South Wales and Victoria. In previous trials in West Wyalong, NSW, blue mallee proved superior to two other species (*E.kochii* and *E.plenissima*) which come from Western Australia (Slee 1996). Plantations for production of eucalypt oil, using blue mallee, have been developed over the last several decades at Tallimbalong, near West Wyalong in western NSW, Australia. The plantations supplement the long established production from natural forests.

The company engaged in oil production in the region is G.R.Davis Pty Ltd. This company has pioneered the development of the cultural techniques used in the plantations. An essential requirement of all plantations is a supply of seed of good genetic quality. G.R.Davis' breeding program to develop improved seed of *Eucalyptus polybractea* has been in progress since the late 1980s.

Improved seed has been produced in three different ways –

- (i) collecting from seed trees of high quality
- (ii) a clonal seed orchard
- (iii) a seedling seed orchard.

(These procedures are explained in detail in Chapters 1 and 2)

Both seed orchards produced good quantities of seed for the first time early in 1999 so it was possible to conduct a detailed comparison of the three strategies. This comparison forms the basis for the first part of this report. Unselected local material was also included to quantify the gains achieved.

The production of flowers in quantity in the orchards also allowed detailed study of the flowering patterns of individual trees. This knowledge is important for orchard management. The flowering patterns control the genetic composition of the seed produced. For example, any tree flowering at a different time to the others would not be able to mate and thus would not be useful contributor the orchard. Studies of the flowering are recorded in this report.

The report is structured as five chapters. The first outlines the structure of the breeding program and the second the composition and management of the seed orchards. The third chapter presents the field trial whilst the fourth describes the flowering observations. The fifth summarizes the conclusions from the project.

Chapter 1. Background

1.1 Tree Breeding Programs

1.1.1 Structure

The breeding of trees to improve production in plantations usually has three phases – (i) selection, (ii) testing and (iii) production of improved material.

1.1.1.1 Selection

Trees of good quality are chosen according to criteria appropriate for the plantations being established.

1.1.1.2 Testing

The selected trees are assessed for their ability to pass on their good characteristics to their offspring. Progeny of each individual tree can be assessed by collecting seed from that tree and then comparing the tree's offspring with those of other selected trees. The better parent trees can be determined from such tests, known as progeny tests.

1.1.1.3 Production

The production of large quantities of improved seed can be done in several ways. The simplest is to collect the seed from the selected trees. This procedure, however, has no control over the pollen parents so the trees will probably be pollinated by their neighbours, of unknown genetic quality.

One method of controlling pollination is to grow the selected trees in seed orchards. These are areas where the trees are isolated from other individuals of the same species. The isolation means that when the trees flower the mating will be with other nearby trees in the orchard. This effectively forces mating between the selected trees.

The use of seed orchards has practical advantages. Each orchard can be managed specifically for seed production using cultural procedures, which may not be appropriate in production plantations e.g. growing the trees at wide spacing or applying appropriate fertilizer. Having seed produced in central locations also has obvious logistic benefits.

1.1.2 Types of seed orchard

There are two types of seed orchard. One contains clones of the selected trees - a clonal seed orchard. The other contains seedlings raised from seed of the selected trees and is known as a seedling seed orchard. Seed in the clonal orchard is produced by mating of the clones of the selected trees whilst that from the seedling orchard is produced by crossing between their progeny.

An additional stage of selection is usual in a seedling orchard. This is done by establishing several seedling progeny of each tree and then retaining only the best.

1.1.3 Short cuts in the procedure

Any tree breeding program is essentially long term. Various procedures are used to shorten the time to seed production. One is to establish the orchard and conduct the progeny tests concurrently. Any selected trees, which underperform in the tests, can then be removed from the orchard.

Another short cut is to convert a completed progeny test to a seedling orchard. This is done by simply cutting out all except the very best trees and then managing the area as a seed

orchard. (n.b. to maintain genetic diversity a spread of parentage must be retained in the trees retained).

A similar conversion can also be effected with other material. For example it is usual to test genetic material of the species from other regions because material from other areas may be better than that from the local region. Tests to determine this, known as provenance trials, can be also be converted to seedling orchards.

1.2 The breeding program for blue mallee at Tallimbalong

Plantations of blue mallee (*Eucalyptus polybractea*) at Tallimbalong were originally established using seed from selected trees. The trees were chosen on the basis of oil quality and oil production. Oil quality was assessed as the percentage of cineole present in the oil and oil production as amount of oil per unit weight of leaf.

The vegetative propagation procedures necessary to establish clones were determined as part of RIRDC project ANU-10A (Slee 1996). A clonal orchard was established by G.R.Davis staff in early 1990's. This orchard came into production late in the decade. At the same time a seedling orchard was established by converting a provenance trial. This also came into production late in the 1990's.

Both orchards were established in the main plantation area. Pollen contamination was not a problem as the surrounding plantations are harvested well before any flowering occurs.

1.3 The project

By the late 1990's seed from three different sections of the overall programme was available – (i) seed from seed trees, (ii) seed from the clonal orchard and (iii) seed from the seedling orchard. This afforded the opportunity to compare the performance of the trees from each and also with unselected material, to demonstrate the gains achieved and consider future programs. The project has done this.

In addition the flowering of the trees in the orchards was examined in detail. It is important to understand the detail of the flowering behaviour of the species. Some trees never flower or flower only very rarely and such trees are not useful in the orchards. Also individual trees flower at different times during the year and it is important to ensure none are so isolated in time that they cannot effectively contribute to the orchard.

Chapter 2. The Seed Orchards

2.1. Introduction

The two seed orchards in this report provided the seed and contained the trees whose flowering was studied. The structure and composition of the orchards are described in this chapter.

2.2 The Seed Orchards

2.2.1 Clonal seed orchard

The clonal orchard seed was established in the early 1990's using cuttings of selected breeding trees. The production of the cuttings was by G.R.Davis staff and the procedures similar to that described in Slee (1996).

The site is high quality brown loam in the plantation area and the tree spacing is 4m x 1.5m. The nearest trees able to produce pollen that might contaminate the orchard are several hundred metres away.

There were originally about 90 trees representing 13 clones but in early 1999 this was reduced to 57 trees and 7 clones after oil quality assessment of the clones.

2.2.2 Seedling Seed Orchard

The seedling orchard was established by conversion of a provenance trial. This trial has been reported in detail by James (1991) and Grant (1997). The original aim was to compare the performances of trees originating from other areas with those being used at Tallimbalong. There were 168 trees originating from 11 different seed trees, one local Tallimbalong, five elsewhere in NSW (Blue Mallee State Forest, 33°55'S., 147°05'E) and five in Victoria (Whipstick Forest, 36°36'S., 144°19'E.)

The trees were planted in 1991. The layout is shown in Figure 2.1. The trial contained 42 plots in six rows. Each plot was a line of four trees. Tree spacing was 0.75m and row spacing 1.5m. The soil was a red brown clay loam with shale fragments.

Figure 2.1 The original layout of the provenance trial of *Eucalyptus polybractea* established at Tallimbalong in 1991.

Each plot was composed of 4 trees of the parent shown. The Tallimbalong parent was labelled Tall1, the NSW parents were numbered 2-6 and the Victorian 7-11 inclusive.

Row 1	NSW 4	VIC 6	NSW 2	NSW 5	NSW 3	VIC 10	NSW 4
Row 2	VIC 8	NSW 3	TALL 1	NSW 4	VIC 7	VIC 8	NSW 2
Row 3	NSW 5	VIC 11	VIC 7	VIC 10	VIC 9	TALL 1	VIC 7
Row 4	VIC 9	VIC 10	NSW 3	VIC 8	NSW 2	NSW 3	NSW 5
Row 5	NSW 6	VIC 8	VIC 11	NSW 6	TALL 1	VIC 7	VIC 10
Row 6	VIC 7	TALL 1	NSW 2	VIC 7	NSW 4	NSW 5	NSW 6

Trees were assessed (i) in the nursery and (ii) after two years in the field. The nursery observations were detailed in James (1991) and the field results in Grant (1997).

Following assessment in 1993 the plot was converted to a seed orchard. It was very heavily thinned to leave only 23 trees. The trees retained were selected primarily on their oil quality (% cineole) and oil production per unit weight of leaf. For the trees retained, the oil comprised 86% cineole, and the oil content per leaf dry weight 7.14%. In comparison, the figures for the original trial (all trees) were 72% and 5.46% respectively.

Trees were also chosen to ensure reasonable distribution through the orchard. However there was no effective selection for vigour – the average height of all trees before selection was 1.31m, and 1.26m after selection

Two progeny (NSW 3 and VIC 10) had trees with such poor oil quality that no representatives were retained.

2.2.3 Flowering

Flowers appeared in both orchards 3-4 years after establishment and by 1998 good crops were evident on many trees.

Chapter 3. Comparison of production from different breeding strategies

3.1 Introduction

The trial tested the effect of four different levels of genetic improvement, on tree height, oil production and oil quality. Four different seed sources were planted, representing different levels of genetic improvement. These were (i) unselected control, (ii) selection from a single seed tree, (iii) a clonal seed orchard and (iv) a seedling seed orchard.

3.2 Material

As noted, four seed sources provided the material for the trial.

3.2.1 Unselected

The material representing the local unselected seed source was provided by CSIRO Australian Tree Seed Centre. It was collected from 75 trees in the West Wyalong region and bulked.

3.2.2 Seed Tree

The seed tree material was collected from a local tree used at Tallimbalong to supply seed from the plantations – its progeny are known to perform well in the area.

3.2.3 Clonal seed orchard

Details of the clonal orchard have been presented in Chapter 2 – section 2.2.1

3.2.4 Seedling orchard

Details of the seedling orchard have been presented in Chapter 2 – section 2.2.2.

3.3 Nursery development

3.3.1 Sowing

The seed was sown into tubes at Tallimbalong in late December 2000 and set to germinate under mist sprays and high humidity conditions.

3.3.2 Germination

The germination was quite poor especially in some of the clonal orchard material. A summary of the germination count is provided as Appendix 1. To obtain a suitable number of seedlings for trial an additional collection and sowing took place in February 2001.

3.3.3 Development

After germination seedlings were thinned to one per tube. Where possible the seedlings removed were pricked out to empty pots and remained in the trial. As seedlings became established tubes were moved to the shade house.

Seedlings in the shade house developed well and by October 2001 averaged 12-18 cm in height. The numbers available and used for planting were Unselected - 250, Seed Tree - 590, Clonal Orchard - 1119 and Seedling Orchard - 576.

3.4 Planting

Planting took place in October 2001 at Field 4 Glen Idle, Tallimbalong, using G.R.Davis' machines and planting direct into rip lines. Line spacing was 3m and the trees were at 0.75m spacing in the lines. The trees were irrigated at planting and over the subsequent months as necessary and with decreasing frequency.

The layout was a randomised block with five replications (see Figure 3.1). The unit plots were single lines. Each line contained approximately 80 trees of the one treatment. The unselected material had to be restricted to the first two blocks due to shortage of stock. The

other three treatments were represented in each block. Thus there were 17 lines in the main trial. Excess clonal orchard material was planted in three additional lines (plots 18-20, see Figure 3.1)

Figure 3.1 Diagrammatic detail of the planting layout.

Each plot was a single line approximately 80 trees in length. Block boundaries shown in bold.

Plot No	Treatment
1	Clonal Orchard
2	Seedling Orchard
3	Seed tree
4	Unselected local seed (CSIRO collection)
5	Seed tree
6	Clonal Orchard
7	Unselected local seed (CSIRO collection)
8	Seedling Orchard
9	Seed tree
10	Seedling Orchard
11	Clonal Orchard
12	Seed tree
13	Seedling Orchard
14	Clonal Orchard
15	Clonal Orchard
16	Seedling Orchard
17	Seed tree
18	Clonal Orchard
19	Clonal Orchard
20	Clonal Orchard

3.5 Field development, assessment and harvesting

Survival was excellent and development was good although slowed by the drought conditions.

Trees were measured for height and oil samples taken in September 2003. The samples were taken from five trees in each line and seven in the lines of unselected material (plots 4 and 7) because of the limited quantity of this material available. The trees selected were the first trees in each line over 140 cm in height. Lines 9 (seed tree) and 10 (seedling orchard) were not sampled.

In November 2003 the trees were harvested using the standard commercial procedure employed by GR Davis. Each tree was chopped by a tractor-drawn harvester and the chopped material collected into a trailer behind. The trailers are known as “pots”.

Harvesting was done line by line. As each line was a single treatment and extended through the experiment each pass of the tractor harvested one treatment. Separate pots were used for each treatment. The tractor passed down each line and the chopped material was collected in the relevant pot.

The pots have fittings, which allow connection to the oil extraction system. After harvest each filled pot is connected to steam pipes from the boiler. Steam is passed through the contents and the oil collected by distillation. Distillation is continued until no further oil comes off.

As there was insufficient material of each treatment to completely fill a pot, spent leaf from a previous distillation was used to ensure each pot had a full load. The pots were connected to the distillation system and the quantity and the quality (% cineole) of oil produced by each treatment determined.

3.6 Results

3.6.1 Tree Size

The average sizes of the trees of different origin are shown in Table 3.1 and by individual lines or plots in Figure 3.2.

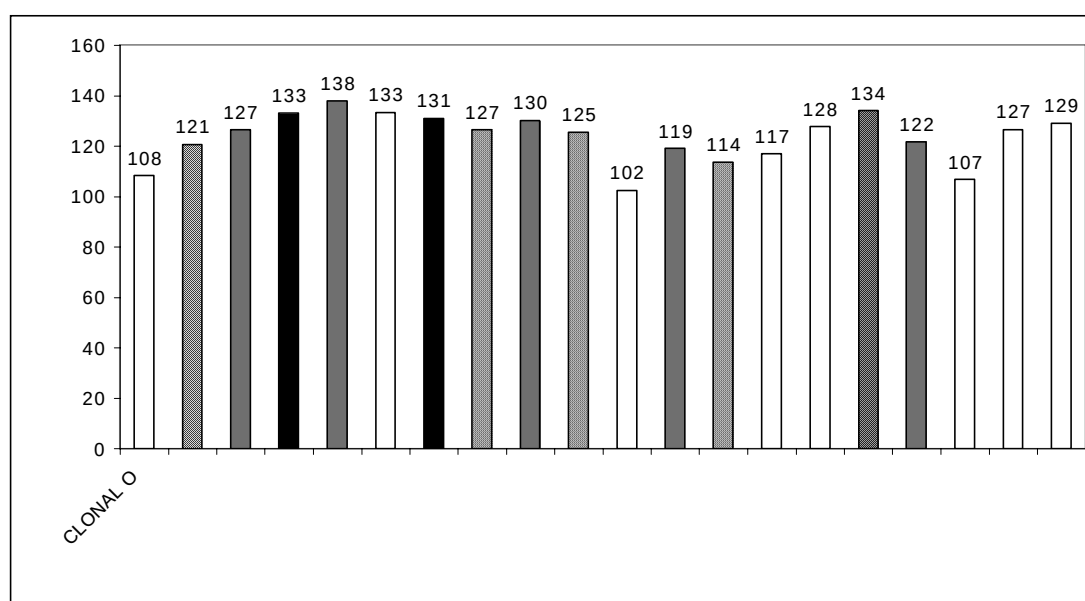
Table 3.1. The number of trees and average heights in each treatment.

TREATMENT	MEAN HEIGHT (CM)	STANDARD DEVIATION	NO. TREES
UNSELECT	132	44.98	170
SEED TREE	127	45.03	397
CLONAL ORCHARD	115	41.11	586
SEEDLING ORCHARD	124	43.91	403

The overall figures for mean height per treatment (Table 3.1) are misleading, as there were some site differences. Plots 3-10 generally had bigger trees than the others (Figure 3.1) probably associated with a shallow watercourse. The only two plots with unselected material (4 and 7) were both in the favoured area and are no larger than the trees alongside. Also there was considerable variation in the average heights of the material from the clonal orchard (Figure 3.2). Some of the trees originating from the clonal orchard are smaller than surrounding trees (Plots 1, 11, 14 and 18) whilst others (Plots 6, 15, 19 and 20) are as big or bigger than plots alongside.

This suggests there are no real differences in the heights of trees from the different seed sources. Certainly any differences in height would be quite small.

Figure 3.2 The average height (cm) of the trees measured in each row.



3.6.2 Oil Quality

The amount of cineole in the oil from each seed source is shown in Table 3.2. Here there is a very definite advantage to the improved material. The quality of oil from the unselected material is clearly inferior with 76% cineole compared to over 83 % in the other plots. The progeny of the seed tree has produced oil slightly superior to that from the two seed orchards (85% vs 83%).

The quality of the oil from the sampled trees is shown in Table 3.3. The results are similar to those from the overall measures, although the values are lower (ranging from 73% in the unselected material to 79% in that from the seed tree and clonal orchard). There was a very high degree of tree to tree variation, as shown in the standard deviation figures (Table 3.3).

The standard deviation for the oil quality of the unselected material is higher than for the other treatments (Table 3.3). This indicates more variation in the unselected material. In other words the breeding programs have reduced the amount of tree to tree variation in oil quality.

3.6.3 Oil Yield (overall)

The oil yields from the distillation are shown in Table 3.2. As the gross yields were from different numbers of lines and different numbers of trees the data have been adjusted to show the yield per 100 trees. Also some trees were very small and presumably very low yielding so an additional adjustment was made using only 'effective' trees, defined as those over 60cm in height. The values for overall yield, yield per 100 trees and yield per 100 effective trees are shown in Table 3.2.

Table 3.2 The oil quality and yields for each treatment.

The oil yields per 100 trees and per 100 effective trees (i.e. those over 60cm height) are shown.

TREATMENT	Oil quality (% cineole)	Total Oil (kg)	Oil yield (kg per 100 trees)	No. of effective trees	Oil yield (kg per 100 effective trees)
UNSELECTED	76.02	5.9	3.47	161	3.66
SEED TREE	85.04	11.5	2.85	370	3.11
CLONAL ORCHARD	83.93	20.0	3.41	545	3.67
SEEDLING ORCHARD	83.25	12.6	3.17	366	3.44

Unselected material produced as much oil as any other treatment and generally appreciably more. Table 3.2 shows that overall the unselected material gave 3.47 kg per 100 trees, the seed tree 2.85, the clonal orchard 3.41 and the seedling orchard 3.17. However, as noted above the lines of the unselected material only occurred on the better quality site and this probably favoured the unselected material. This is supported when the yields per 100 effective trees are compared. The unselected material and the clonal orchard then have very similar figures at 3.66kg and 3.67kg. The yield from the seedling orchard and the seed tree progeny are both clearly poorer than from the other two sources.

3.6.4 Oil yield (per weight of leaf)

The oil yields per weight of leaf obtained from the sample trees are shown in Table 3.3. There has been selection for this characteristic in the breeding programs and the result is the clonal orchard and the seed tree are better than the unselected material with 3.64, 3.15 and 3.07% respectively. However, the yield from leaves in the seedling orchard material

(2.91%) is clearly lower, possibly reflecting the low selection intensity used in selecting the trees in this orchard. Again, there was a very large variation in all treatments, shown by the maximum and minimum values recorded.

Table 3.3 Results of the oil analyses conducted on the trees sampled within the trial.

(Data are the number of trees sampled, the quality of oil and the amount of oil per fresh weight of leaf, with the maximum, minimum and standard deviation values shown.)

TREATMENT	no trees	Oil quality (% cineole)				oil % of leaf wt (fresh wt)			
		average	max	min	sdev	average	max	min	sdev
UNSELECTED	14	73.46	93.20	25.30	22.35	3.07	5.50	1.55	0.95
SEED TREE	20	79.43	92.50	43.50	13.87	3.15	4.88	1.76	0.87
CLONAL ORCHARD	44	79.11	93.40	22.40	16.34	3.64	7.19	1.23	1.12
SEEDLING ORCHARD	19	76.69	93.10	28.30	19.77	2.91	4.69	1.18	1.09

3.7 Discussion

This study has shown that all three breeding methods used for genetic improvement have produced a gain over the wild (unselected) material in one characteristic, oil quality, which was deliberately selected for. The gain has been a substantial increase in cineole percentage of the order of 10% on average, i.e. from about 75% to 85%.

There has also been an improvement in the production of oil per weight of leaf especially in the clonal orchard and seed tree material. This characteristic was also selected for. Unfortunately this gain did not translate into an overall gain in production. Overall production from the three breeding procedures is similar or poorer than that from the unselected material. This reflects the fact that neither overall yield nor leaf quantity could be directly targeted as a selection criterion without progeny testing.

Consequently, the most important result from this study is its demonstration of a technique for assessing the yield from progeny of selected trees. It would be relatively simple to establish lines of material from selected trees and then to harvest and extract the oil separately by parents. In this way one could determine the high yielding parents.

The absence of any gain in production makes it very difficult to quantify economic gains from the operation. The substantial improvement in quality is obviously extremely important but its actual value depends on its use in final products or possibly the quality of alternative supplies. One can conceive of situations in which oil quality might be all-important and could mean the difference between having a market and having no market.

It is interesting that the seed tree and the seedling orchard have given good results. The seedling orchard is especially interesting given the very low selection level used – 23 trees from 216. So it is possible to achieve good gains with low levels of selection and simple procedures. This is a common practice when new species are introduced to plantation programmes both in Australia and overseas. The practice results in the development of a reliable local seed source with some selection for better quality seed whilst maintaining genetic diversity.

Chapter 4. Flowering Observations

4.1 Introduction

The trees in the seed orchards produced flowers when they were between one and two metres high. This is a convenient height to study the flowers. Also by having all the trees together in the two locations allowed detailed and frequent study of the flowering of individual trees. The study is reported in this chapter.

4.2 Material

The flowering patterns of individual trees were recorded in the two seed orchards at Tallimbalong.

4.3 Flowering Observations

4.3.1. Procedure

In both orchards preliminary flowering observations were made in 1998 followed by detailed studies in 1999 and 2001. There were very few flowers in 2002 and 2003 and no meaningful observations could be made in these years.

The eucalypt flowers were considered to pass through six stages as follows –

- Stage 1 - Clusters of small reproductive buds
- Stage 2 - Swollen reproductive buds
- Stage 3 - Operculum shed – bright coloured white anthers present
- Stage 4 - Yellow and dying anthers present
- Stage 5 - Green capsules with style evident
- Stage 6 - Green capsules

At each assessment each tree was examined and the proportion of flowers in each stage estimated. The flowering period for each tree was defined as when there were flowers with white or yellow anthers present on the tree - stages 3 and 4.

4.3.2 Results

The flowering periods for each tree are shown in Figures 4.1 and 4.2. The results for 1999 (Figure 4.1) show the period when each tree was carrying either white anthers or yellow withered anthers. Those for 2001 (Figure 4.2) are more detailed and indicate when each tree carried (i) only white anthers, (ii) both types and (iii) yellow anthers.

Three of the 23 trees in the seedling orchard did not produce flowers and in the clonal orchard, all five trees from one clone did not flower. All other trees in the clonal orchard did flower.

The flowering periods generally commenced in late February/March and were complete by mid May (Figures 4.1 and 4.2). Some trees commenced in February but others were much later, i.e. mid March. One tree (a member of the N2 family) did not commence until late April in 2001 (Figure 4.2).

Generally trees which flowered early in 1999, also did so in 2001. Similarly late flowering trees tended to be so in both years. Trees in clone E were especially obvious as being late flowering in both years (Figures 4.1 and 4.2)

Despite the different flowering times there was high degree of overlap of flowering periods. This is evident in the detail of the patterns shown in Figure 4.2. Even the trees in the late flowering clone E overlapped with other clones.

Figure 4.1 The flowering period for each tree which produced flowers in 1999, in the seedling and clonal orchards.

The trees in the seedling orchard are labelled N1, V6 etc indicating the source (either NSW or Victoria respectively) and the identity of the family. The trees in the clonal orchard are identified by clone (A-E).

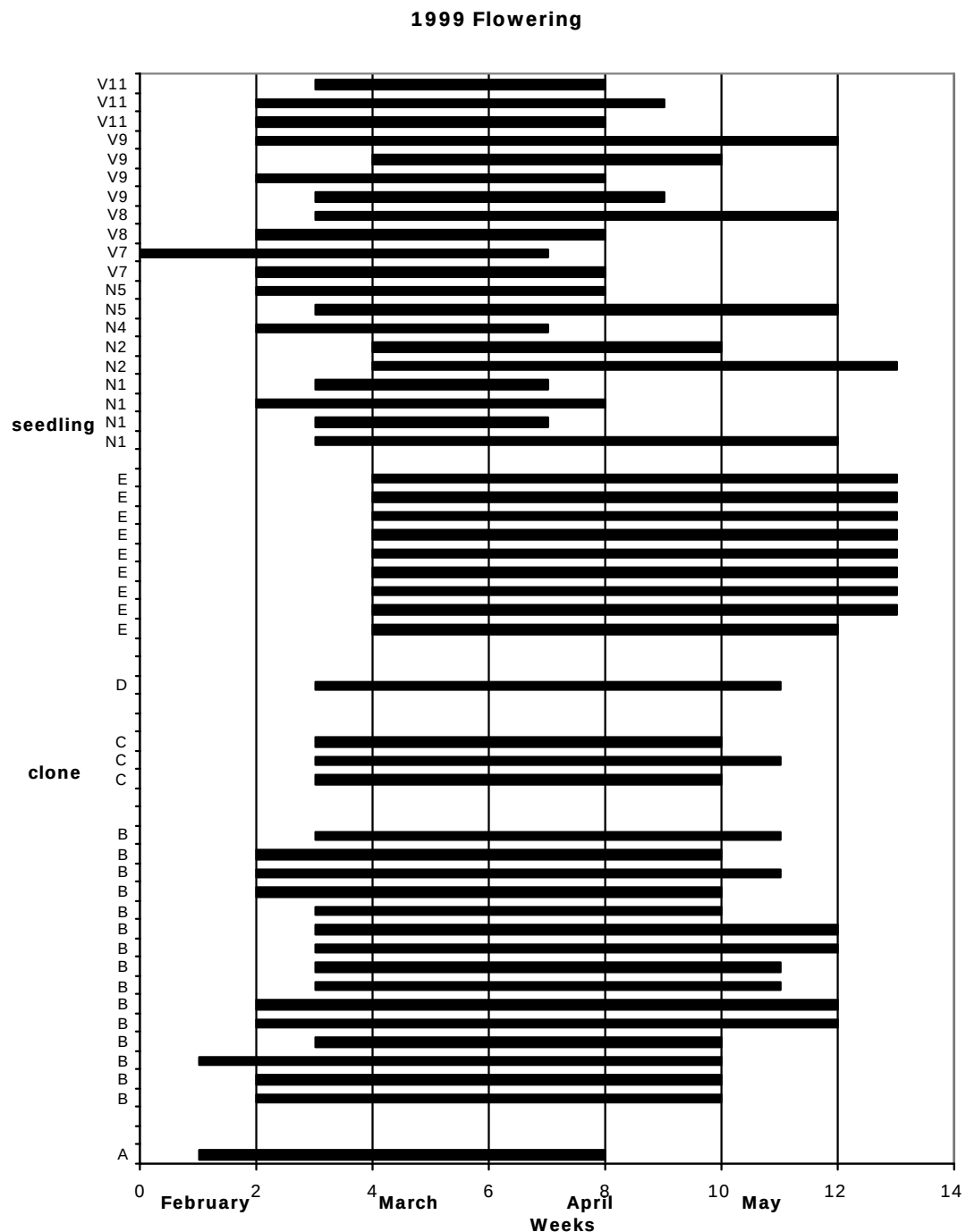
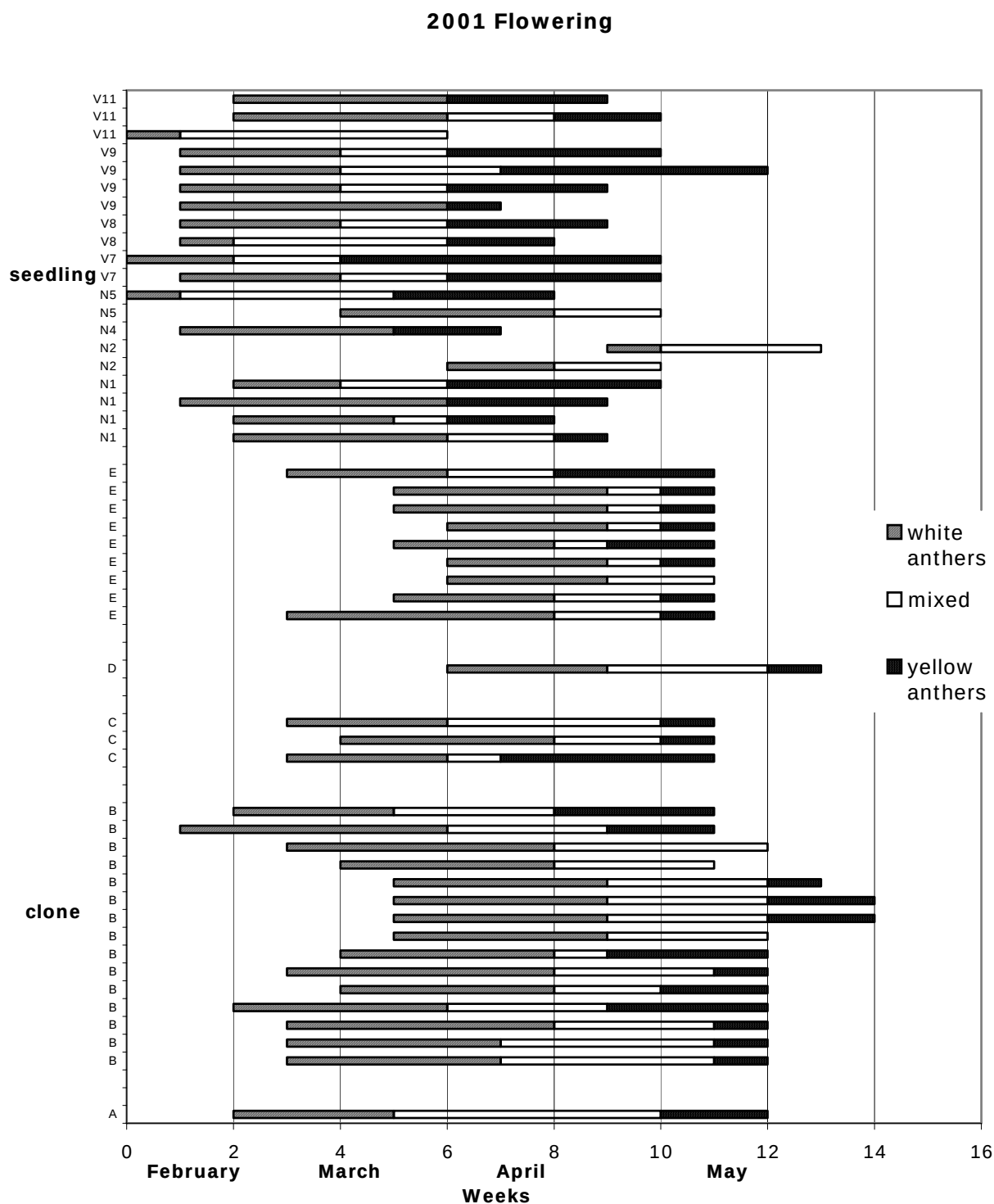


Figure 4.2 The flowering period for each tree which produced flowers in 2001, in the seedling and clonal orchards.

Each period is subdivided to show when only white or yellow (dying) anthers or when a mix of both were present. The trees in the seedling orchard are labelled N1, V6 etc indicating the source (either NSW or Victoria respectively) and the identity of the family. The trees in the clonal orchard are identified by clone (A-E). Each tree occupies the same position in the figure as it does in Figure 4.1.



The very late flowering of one tree (N2) in the seedling orchard is noteworthy. White anthers were not present on this tree until the last week in April (week 9). All other trees had passed the white anther stage at this time and only two in the same orchard had trees with mixed white and yellow anthers (Figure 4.2). This tree might have had difficulty effecting mating with the other trees in the orchard.

Overall there were differences in flowering times in the different orchards in 2001 (Figure 4.2). The seedling orchard trees generally flowered earlier than the clonal material. Most seedling trees had commenced by week 1 (early March) and many had completed by week 9 (late April). The clonal trees only started flowering in weeks 2-5 (mid-late March) and did not complete until weeks 11 or 12 (mid May). This difference was also obvious from field observations in 2000 but was less obvious in 1999 (Figure 4.1).

4.3.3 Discussion

The results show a variation of flowering times in the species. Some trees are early flowering and others late flowering. There is a suggestion that the distinction may be consistent over different years.

It is not possible from this data to be sure about the trees' ability to interbreed. These macroscopic observations cannot detail the precise time of receptivity or the viability of the pollen. Studies in other eucalypt species have indicated viable pollen is shed very soon after the anthers appear whilst the flowers do not become receptive until several days later. In very broad terms the presence of the white anthers could indicate the presence of viable pollen and the presence of dead anthers the receptivity of the style.

Despite the limitations of the observations it is clear some trees may flower sufficiently out of phase as to make the likelihood of their mating remote. Also some combinations may not be possible. The flowering times do need to be borne in mind when managing orchards.

The possibility of trees being so out of phase as to be effectively isolated was reinforced by observations in 1998 in the clonal orchard. These showed trees in two clones to be extremely late flowering. In both the white anther stage was not recorded until mid July and the flowering continued into August. Both these clones were removed from the orchard before the 1999 flowering because of their poor oil quality but it is clear they would not have been useful contributors to the orchard anyway.

Flowering of trees so out of season as to make normal mating with their own species impossible has important implications. Such trees could self pollinate but would also be open to hybridization with other species flowering concurrently. The normal barriers to such hybridisation, firstly distance and secondly swamping of contaminating pollen by local heavy production, simply would be much less effective.

Indeed infrequent hybridisation of *Eucalyptus polybractea* could explain the unusually high levels of morphological variation present in the species. Certainly management of both seedling and clonal orchards will need to take flowering times into account. Flowering patterns may also need to be borne in mind in the management of conservation areas where contamination of the gene pool may be undesirable.

The data suggests a difference in flowering times between the clonal and the seedling orchards, with the seedling trees flowering before the clones. The difference became more pronounced as the trees aged. The two orchards are only about 1km apart so ambient climatic conditions seem unlikely to be the cause. The difference might be due to site fertility – the seedling orchard is on a less fertile site. This needs further study.

4.4 Bud retention

4.4.1 Procedure

Clusters of reproductive buds were tagged and the individuals in each cluster counted on six trees in the clonal orchard and three in the seedling orchard. The initial counts were in February prior to flowering and the counts were repeated at intervals up to October. The counting was very difficult during the flowering period as the flowers were very close together and interlocked. Counting without damaging was extremely difficult and frequently not attempted.

4.4.2 Results

The results of the count at different times are shown in Table 4.1. There were some minor losses before flowering but major losses were confined to the period after flowering (i.e. after May) and before fruiting.

**Table 4.1 Results of the counts of the reproductive buds at various dates
February 2001 – October 2001 (d/m/y)**

Tree Identity	23/2/01	13/3/01	9/5/01	16/10/01
Clonal 1-3	46	46	35+ ⁽ⁱ⁾	Lost
Clonal 1-7	41	32+ ⁽ⁱ⁾	30+ ⁽ⁱ⁾	37
Clonal 2-7	56	50	flowering	15
Clonal 3-10	61	59	46+ ⁽ⁱ⁾	30
Clonal 4-9	98	91	flowering	60
Clonal 5-3	74	flowering	70	63
Seedling 2-9	54	52	37	Lost
Seedling 5-3	34	34	21	Lost
Seedling 6-13	32	32	30	12

+ ⁽ⁱ⁾ indicates the count was incomplete as the crowding of flowers made counting impossible

4.4.3 Discussion

The loss of developing flowers and fruits was only important in the period after pollination. This is useful information for seed orchard management and prediction of seed yields. It is rather different than for *E regnans* where losses can take place throughout that species' much longer period (4y) of flower and fruit development (Griffin 1980).

Chapter 5. Conclusions

5.1 Breeding strategies

The project compared the gains achieved using three different yet complementary breeding strategies. The three – use of seed trees, development of a clonal orchard and development of a seedling orchard are commonly used in tree breeding programmes throughout the world. The seedling orchard resulted from the conversion of a completed provenance trial and this too is a common procedure especially where a new species is being introduced to a region.

All three breeding strategies produced gains in characteristics that were selected for – namely oil quality (% cineole) and the proportion of oil in the leaves (measured as percentage of either the fresh or dry weight of leaf.). However it was not possible to select for tree vigour or total production so there has been no gain in production. Phenotypic selection for vigour is always difficult and especially so in natural forests or with individual specimen trees, as here. The most reliable procedure when selecting for vigour is to use the results of progeny tests. Such tests also need to be used to assess overall oil production. This study has shown how progeny tests can be used to assist breeding programs.

The procedure advocated as a result of this project is to use large scale tests and commercial harvesting to get direct results. This compares with the usual procedures where forest trees are compared in small scale tests (possibly even with single tree plots) and the results extrapolated. The difficulty in using small scale tests with blue mallee (or other species) is that measurement of both biomass and oil production per tree is so cumbersome, time consuming and expensive as to be unrealistic for even quite small numbers of trees. In blue mallee this is compounded by the extreme variation exhibited by the species in almost every characteristic examined. So there are problems for extrapolation and prediction.

Therefore the procedures used in this project have potential. When progeny testing, the important operation is to plant each treatment as lines right through the experiment. This allows the harvester to pass right through and collect the material in a container dedicated to that treatment. The oil content can then be determined for each line (or combination of lines if desired) and for a certain number of trees. If the material does not fill the container then spent leaf can be used to ensure a full load.

The procedure is simple and has direct application because it uses standard commercial procedures. There is a cost in that organization of the planting, harvesting and distillation means these operations are slowed compared to commercial runs. However this is minimal compared with much experimental work.

5.2 Flowering

The flowering studies have shown there is a spread of flowering times and this is probably genetically controlled in that some trees are early flowering and some late. It may be that some particular trees are precluded from a mating with others because of the difference in flowering times. This needs to be borne in mind in management of seed orchards.

Some trees flowered at a distinctly different time from the other trees in the species. Any flowering of trees outside of the normal time has very important implications. Not only will any such trees be unable to mate with their own species but they would be liable to hybridisation or to self pollination and fertilization. The occurrence of such trees has

important direct practical implications for seed orchard and conservation management and more generally for understanding of the evolution and ecology of the species.

E. polybractea exhibits several very unusual features especially the extreme variation in almost every characteristic studied. One explanation of this variation could be out of phase flowering with hybridisation followed by backcrossing and genetic mixing. Indeed it is possible there may be an incipient hybrid swarm developing with the other mallee species around. The scientific importance of this has been mentioned above. But it is also of extreme practical importance for breeding programs, seed collection and species introductions. Any one seed collection in the wild may not be repeatable subsequently! If so the establishment of reliable seed orchards appears a most sensible procedure.

A possible difference in flowering times as a result of site differences also needs study. This could militate against converting trials to seedling orchards. Provenance and progeny trials are necessarily established on typical plantation sites. These may not be the best for seed production so this common practice needs to be carefully considered.

However in this study, the gain achieved in the seedling orchard that resulted from converting a provenance trial does support the procedure. It is especially valuable with species introductions where there may not be a local source of seed.

Chapter 6. Recommendations

This project builds on previous research which investigated blue mallee propagation, compared oil production of progeny from trees sourced from New South Wales and Victoria (James 1991, Grant 1997) and initiated a breeding program (Slee 1996). The 1991 provenance (seed tree) trial was subsequently thinned and converted to a seedling orchard; and used as the 'seedling seed orchard' treatment in the project reported here. The genetic base for that treatment was thus progeny from 7 parent trees. The recommendations below encompass the need to broaden the genetic base for future breeding and selection, as well as to implement developments based on the results reported above. Future research may be able to use material from the range-wide provenance-progeny trial established in 1997 at Tallimbalong (see Slee 1999).

1. A procedure should be developed to select for gain in blue mallee oil yield (overall yield and leaf quantity). This should complement the improvements in oil quality (cineole percentage and oil content per leaf) gained in this study.
2. Prior to further breeding for oil quality and overall oil yield, larger-scale plantings should be established using a broader genetic base.
3. Tests of oil quality and yield should be large scale and based on commercial harvesting methods. The key recommendation for progeny trials is to plant each treatment as lines right through the experiment, so that the harvester can pass right through and collect the material in a container dedicated to that treatment, from which the oil content can be determined for each line. While organisation of the planting and distillation will be slower, the method will allow use of commercial procedures and reduce problems with extrapolation from conventional tree breeding experimental plots with few trees. The benefit of this approach is that it will (a) compensate for the substantial variation in tree vigour and total production in *E. polybractea* material collected from natural populations, (b) avoid cumbersome and expensive measurement of biomass and oil production per individual tree.
4. For seed production, larger plantings using a broader genetic base are recommended, to ensure adequate cross-pollination and seed set despite asynchronous flowering between individuals.
5. Research should explore the effect of site fertility on flowering time, and its impact on choosing sites for seed orchards.

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Appendix. Germination Summary

Sowing

The following seedlots were sown into tubes at Tallimbalong in late December 2000. They were set to germinate under mist sprays and high humidity conditions

- Unselected material from CSIRO Seed Section collection batch 19649 (75 trees)
- Local seed tree (Buddigower)
- Ex- the converted provenance trial (seedling orchard)
- Ex – GR Davis clonal orchard.

Material other than that from CSIRO was collected and extracted by local staff.

Germination

Detailed observations of germination were effected for 100 tubes of each batch except the clonal orchard in which 400 tubes were monitored. The results are summarized in Table 1.

Appendix Table 1- Summarized details of germination rates for the four batches at West Wyalong in early 2001.

Numbers of germinants in each 7 day period are shown as is the date at which the last germinant appeared and the percentage of tubes with no germinants.

	Jan	Jan	Jan	Jan	Jan	Feb	Feb	Feb	Feb	Mar	Mar	Mar
Date – Batch	1-7	8-13	14-20	21-27	28-3	4-10	11-17	18-24	25-3	4-10	11-17	17 and later
Unselect	3	11	3			47	44	19				
Seed Tree		1				54	80	30	1	2	2	1
Clonal O					1	47	59	33	11	5	4	12
Seedl O.	9	50				5	56	36				3

Batch	Date of last germinant	Total plantable seedlings	Tubes with nil germ.
Unselect	24/2	127	49%
Seed Tree	10/4	171	15%
Clonal O	24/4	172	66%
Seedl O.	16/5	159	25%