Chapter 2 Background to the study

2.1 The need for the study

The literature revealed that *E. radiata* subsp. *radiata* can produce a high quality, high value oil, offering potential to be commercially produced on-farm. However, only limited data is available on tree growth, essential oil traits, improvement potential and economics for commercial production. It appears that in order for the potential of *E. radiata* subsp. *radiata* oil production to be realised, much further research and development work is required. This study addresses some of the information gaps identified in the literature review, and forms a basis for further research and expansion of the industry.

The objectives of the 1996-2001 'Eucalyptus' component of the Essential Oils Research and Development Plan compiled by the Australian federal governments Rural Industries Research and Development Corporation (RIRDC, 2001) were: (1) to lower the cost of production; (2) to add value to eucalyptus oils; and (3) to identify and develop new markets. That plan implemented a strategy involving the following components: select and test improved genetic lines; devise and evaluate agronomic and silvicultural techniques that increase yield and lower production costs; apply world best practice to harvesting and distillation; identify and test alternative extraction techniques; develop new cineole-based medicinal products; develop new industrial solvents based on a knowledge of the solvent and flammability properties of eucalyptus oil mixtures; reconnoitre world markets; provide technical support for promotion of new products; and provide representation at international trade shows (RIRDC, 2001).

The objectives of the RIRDC Research and Development Plan for Essential Oils and Plant Extracts 2002-2006 are: (1) to improve understanding by potential researchers and producers

of markets for essential oils and plant extracts; (2) to improve existing products and encourage the development of new crops and new products (including uses for existing products); (3) to support the development of sustainable and profitable production systems; (4) to facilitate regulatory approvals for essential oils and plant extracts; (5) to promote cost effective post harvest and extraction technology to improve yield and quality; and (6) to encourage the development of essential oils and plant extract industries.

In order to develop grower uptake, and increase the efficiency and profitability of *E. radiata* subsp. *radiata* essential oil production, further research and development activities must be carried out along the lines of those proposed in the RIRDC R&D Plans. Addressing the information gaps identified in Chapter 1 of this study is consistent with the desired direction of the industry, and forms the basis of this research project.

2.2 Aims

The aims of this study are: (1) to assess the potential gain in *E. radiata* subsp. *radiata* tree growth and essential oil traits achieved through selection and breeding; and (2) to determine the economic viability of a farm-scale eucalyptus oil enterprise through utilising selected *E. radiata* subsp. *radiata* stock, and varying a number of key production parameters.

2.3 Objectives

The objectives of this study are:

- to determine variation in tree growth and essential oil traits of a number of families of *E. radiata* subsp. *radiata* in a provenance/progeny field trial at Brogo near Bega in southern NSW;

- to estimate heritability of tree growth and essential oil traits of families in the provenance/progeny trial;
- to determine genetic and phenotypic correlations within and between tree growth and essential oil traits of provenances and families in the field trial;
- to determine potential gains in tree growth and essential oil traits achievable through the selection and breeding of superior trees in the trial;
- to compare, through economic modelling, the financial performance of various farm-scale *E. radiata* subsp. *radiata* oil production enterprises using unimproved and improved germplasm, and the potential gain in oil production through selection and breeding;
- to compare, through economic modelling, the financial performance of various farm-scale *E. radiata* subsp. *radiata* oil production enterprises achieved by adjusting key production cost and product price parameters; and
- to outline a practical breeding strategy to capture the potential gains.

It is intended that the results of this study be applicable to the current and future commercial eucalyptus oil production industry, and as such, all data are based on realistic information and estimates obtained from review of the literature, personal communication, field trials, statistical analyses and personal experience.

Chapter 3 Genetic parameters - variability, heritability, correlations and gain in growth and essential oil traits of *E. radiata* subsp. *radiata*

3.1 Introduction

Tree improvement is generally carried out in order to establish new populations with superior key economic traits such as, in the context of this study, leaf oil concentration and chemical composition. In order to develop improved stock, a detailed process of assessing variation and the determination of key genetic variables must be carried out. This chapter describes the determination of key factors that would underpin a tree improvement program for *E. radiata* subsp. *radiata* using the provenance/progeny trial at Brogo, NSW, as a realistic example.

The aim of this component of the study is to determine potential gain in oil production per unit area of land as a result of using seed produced from mating of selected superior trees amongst families in an *E. radiata* subsp. *radiata* provenance/progeny trial at Brogo.

The objectives are:

(1) to calculate variation in survival rate, number of stems per tree, basal area, leaf oil concentration and 1,8-cineole content in the provenances and families in the provenance/progeny trial via field sampling and laboratory and statistical analysis;

(2) to estimate phenotypic and genetic correlation(s) between the key tree growth and essential oil traits based on the data collected in Objective 1;

(3) to estimate heritability of the key tree growth and essential oil traits; and

(4) to determine the potential gains in oil production, particularly through increased leaf oil concentration, as a result of mating of selected superior trees.

3.2 Materials and methods

3.2.1 Field trial site

The trial block of *Eucalyptus radiata* subsp. *radiata* studied in this research project is located on private property at Brogo (near Bega) in southern New South Wales (36° 35' S, 149° 45' E, Figure 3.1). Elevation is 150 m above sea level. The trial is privately owned and was established for three purposes: commercial eucalyptus oil production, research, and ultimately the development of a seed orchard through selection of outstanding individuals with coppicing of the remaining trees. Development of the seed orchard will involve completely removing the seven poorest yielding families from seed production, and thinning the surviving trees in the best family plots to one tree (selection rate amongst surviving trees of one in ten for the 25 best families).

The trial site is, at the time of sampling, depicted in Figure 3.2. It has a northerly aspect and is of moderate to gentle slope, with soils of granitic parent material on the upper slope and sandy-silts on the lower slope running into 'Double Creek'. Prior to establishment of the trial, vegetation on the site included improved pasture with some black wattle (*Acacia mearnsii*), smooth-barked apple (*Angophora costata*), ribbon gum (*E. viminalis*) and river peppermint (*E. elata*) regrowth. The regrowth was pushed over, windrowed and burnt. The pasture was sprayed twice with glyphosphate (2 kg active ha^{-1}). Rows were mound ploughed along the contour. Pre-plant knockdown and residual herbicides (glyphosphate at 2 kg active ha^{-1} and Simazine ® at 5 kg active ha^{-1}) were applied along the mounded rows. The trial was planted in August 1993 and fertilised (100 g NPK $tree^{-1}$) in September 1993 (J. Doran, pers. comm. 1996).

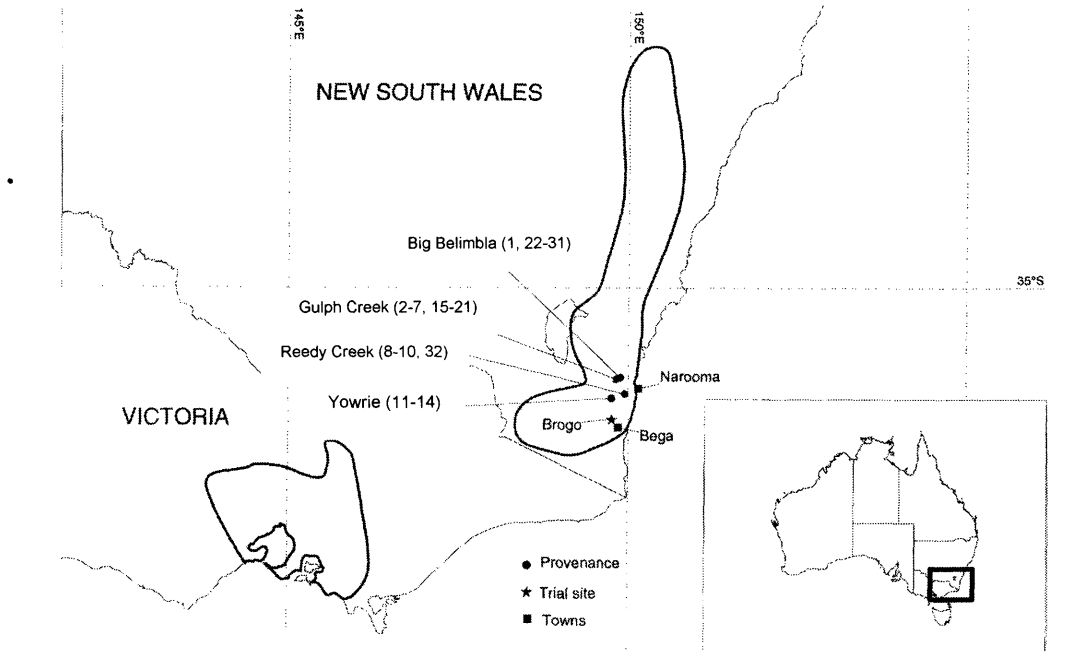

Figure 3.1 - Locations of *E. radiata* subsp. *radiata* (cineole variant) provenances (family numbers in brackets) and trial site at Brogo, NSW. The heavy black line indicates the approximate natural distribution of *E. radiata* subsp. *radiata* (all chemical forms) in mainland Australia (*source: Johnson and Hill, 1990*)

The location of the trial site, the four provenances and the approximate boundaries of the natural distribution of *E. radiata* subsp. *radiata* are shown in Figure 3.1. Table 3.1 gives origin details of families and provenances, while Table 3.2 gives climatic data for the four provenances and the Brogo trial site.

Figure 3.2 - The *E. radiata* subsp. *radiata* provenance/progeny trial site at Brogo, NSW (trees aged 38 months)

Table 3.1 - Origin of the 32 open-pollinated families of *Eucalyptus radiata* subsp. *radiata* in the trial at Brogo, NSW (*source: adapted from Doran* et al., *1998b*)

Provenance	No. of Families (Treatment no.)	Latitude	Longitude	Altitude (m asl)
Big Belimbla	11 (1, 22-31)	36°07' S	149°49' E	200
Gulph Creek	13 (2-7, 15-21)	36°05' S	149°53' E	80
Reedy Creek	4 (8-10, 32)	36°15' S	149°58' E	100
Yowrie	4 (11-14)	36°17' S	149°43' E	150

Table 3.2 - Climatic details for *E. radiata* subsp. *radiata* provenances and trial site at Brogo, NSW, as determined by BIOCLIM (*source: T. Jovanovic,* pers. comm., *2001*)

Provenance	Rainfall (mm)	MTHM (°C)	MTCM (°C)	MAT (°C)
Big Belimbla	977	25	2	14.1
Gulph Creek	980	25	3	14.7
Reedy Creek	967	25	4	14.9
Yowrie	961	25	2	14
Brogo Trial Site	940	26	2	14.3

NB - there are no months of the year with a mean rainfall less than 40 mm for any of the sites
MTHM: mean daily maximum temperature of the hottest month
MTCM: mean daily minimum temperature of the coldest month
MAT: mean annual temperature

The trees that provided the seed for the 32 families in the trial had already been selected from native stands for superior oil traits from 95 native trees tested for oil concentration and 1,8-cineole and α-phellandrene content (Section 1.3.3). In selecting the 32 mother trees, a balance was sought between the key traits of leaf oil concentration and 1,8-cineole content. All trees exhibiting α-phellandrene content of greater than 0.3% were eliminated (Section 1.3.3). The highest oil-yielding of the remaining trees were selected and those having a cineole content of greater than 70% were further selected and seed subsequently collected (Doran *et al.*, 1998b).

The trial uses a randomised complete block design comprising 32 treatments, being the open-pollinated families of *E. radiata* subsp. *radiata*. There are 14 trees per plot (2 rows of 7 trees) and 12 replicates, of which four were examined in this study. Spacing is 3.0 m between rows and 1.5 m between trees within rows (giving approximately 2200 stems hectare^{-1}). The trial is

buffered by one row of trees on the northern side, two rows on the eastern side and larger plantation areas to the south and west. Only four replicates (those on the lower slope) were studied for this research project (Figure 3.3).

3	28	4	13	20	24	4	13	5	16	3	29	27	32	24	7
5	6	2	27	21	7	9	25	32	22	14	23	12	20	1	19
7	18	19	10	32	11	26	27	9	21	31	6	9	13	3	8
9	26	20	30	2	29	17	14	30	19	28	2	15	16	29	14
8	22	1	23	15	10	22	16	10	7	4	24	22	11	17	23
15	21	31	14	5	18	23	8	13	17	8	25	10	21	18	31
11	32	16	29	30	3	19	31	11	15	18	27	30	6	5	26
25	24	17	12	28	12	1	6	12	20	26	1	28	2	25	4

Replicate 1 Replicate 2 Replicate 3 Replicate 4

Figure 3.3 - Randomised complete block design of the *E. radiata* subsp. *radiata* trial at Brogo, NSW, showing layout of replicates and families
(source: J. Doran, pers. comm., 1997)

3.2.2 Field sampling methodology

Fieldwork was carried out in October 1996 when the trees were 38 months old. All surviving trees were measured (Figure 3.4) for diameter at breast height (DBH). The DBH of each stem greater than one centimetre was measured for trees with multiple stems. Family means for individual tree basal areas (a tally of all stems on multiple-stemmed trees) were calculated from the diameter data incorporating survival.

Figure 3.4 – Measuring tree diameter of *E. radiata* subsp. *radiata* in the provenance/progeny trial at Brogo, NSW (tree age 38 months)

Leaf samples were collected in order to assess leaf oil concentration, oil chemical composition, and leaf moisture content. Four trees in each plot were chosen at random and a sample of approximately 20-30 g of mature leaf was cut from the upper crown of each. These were placed in labelled plastic bags and kept cool for processing. In a field laboratory (Figure 3.5) on the afternoon and evening of collection, exactly 6 g of leaf (petioles removed)

from each sample were weighed, cut into pieces and placed into labelled plastic screw top bottles along with 50 ml of high-grade ethanol for solvent extraction (Ammon *et al.*, 1985). A further 10 g of leaf from each sample was weighed and placed into paper bags for drying to determine moisture content. These were dried in drying ovens at 70°C for 12 hours on return from the field, as described in James (1991) and Doran and Matheson (1994).

Figure 3.5 – The field laboratory established near the trial site to process the leaf samples collected from the *E. radiata* subsp. *radiata* provenance/progeny trial at Brogo, NSW

3.2.3 Laboratory methodology to determine leaf oil concentration and 1,8-cineole content

The samples were kept under refrigeration for approximately 12 weeks to ensure that the ethanol extraction process was completed. Just prior to gas chromatograpy (GC) analysis, 120 mg (150 µl) of n-Tetradecane ($CH_3.(CH_2)_{12}.CH_3$) was added to each plastic bottle to act as an internal standard. A small sample (approximately 1.5 ml) of the ethanol extract solution was then taken from each bottle and placed into labelled vials and loaded into the carousels of the GC as described below.

A Varian 3400 Gas Chromatography instrument with flame ionisation detector was used to analyse the relative components of each sample. Samples were loaded using an 8200 autosampler from a 48-vial carousel. The column was an Alltech Heliflex AT-35 of 30 m length and 0.25 mm diameter, with a 0.25 µm film thickness. Helium was used as the carrier gas and was set at a flow rate of 1.16 ml min^{-1}. Split injection was set at approximately 40:1. The column temperature was initially set at 80°C and held for 30 seconds, then programmed to rise to 100°C at 5°C min^{-1}, and then rise to 210°C at 10°C min^{-1}. The final temperature of 210°C was held for 5 minutes. Run time was approximately 21 minutes for each sample.

Varian Star software was utilised to process the results from the GC analysis. Typically 30-40 components were detected within the oil as indicated in the chromatogram in Appendix A. The 1,8-cineole content (%) of the oil was determined from the GC data output and calculation in an Excel Spreadsheet (i.e. the peak area for 1,8-cineole divided by the total peak area of the 30-40 compounds detected and expressed as a percentage).

Leaf oil concentration was estimated as a percentage of dry leaf weight (w/w% DW) using the following equation as described in Butcher (1994):

$$\mathbf{O_s = (R_{oil} \times A_s \times M_i \times 100) / (A_i \times M_s)} \quad \text{... (Equation 3.1)}$$

where O_s = oil concentration of sample (s); R_{oil} = response factor of the oil (in this case taken to be 1.0); A_s = total peak area for sample (s); Mi = mass of the internal standard; A_i = peak area of the internal standard; and M_s = dry weight of the leaf in the 50ml ethanol sample.

3.2.4 Statistical methodology to determine variation in tree growth and essential oil traits

The raw field and laboratory data were initially entered into an Excel spreadsheet and sorted into a format compatible with the DATAPLUS package (Williams *et al.*, 2000). DATAPLUS addresses the layout of the trial in data entry requirements, screens for outlying values and creates data files suitable for statistical analyses packages such as SAS and GENSTAT (Williams and Matheson, 1994).

Following data pre-processing by DATAPLUS, and removal of outlying values (data transformation was not required), plot means and variances for each trait were calculated. Subsequently, analyses of variance were carried out using GENSTAT, based on the following linear model:

$$\mathbf{Y_{ijk} = u + R_i + P_j + F_{k(j)} + e_{ijk}} \quad \text{... (Equation 3.2)}$$

where Y_{ijk} is the plot mean of the k^{th} family within j^{th} provenance group in the i^{th} replicate; u represents the overall mean; R_i represents the effect of the i^{th} replicate; P_j represents the effect of the j^{th} provenance group; $F_{k(j)}$ represents the effect of the k^{th} family which is nested within the j^{th} provenance group; and e_{ijk} represents the residual error with a mean of zero.

The traits examined were survival rate (%), the number of stems per tree, tree basal area (cm^2), plot basal area (cm^2), leaf oil concentration (w/w% DW) and 1,8-cineole content (%).

3.2.5 Statistical methodology to estimate heritability of tree growth and essential oil traits

Genetic and phenotypic variance components and standard errors were estimated through further GENSTAT REML (Reduced Maximum Likelihood) analyses and calculation in order to determine heritability, genetic and phenotypic correlation, and potential genetic gain.

Where open-pollinated families can be assumed to be truly half-sib families, the coefficient of relationship is 1/4. This cannot be assumed for eucalypts because of selfing and the likely high level of neighbourhood inbreeding (Eldridge *et al.*, 1994). For the purpose of estimating heritabilities in this study an outcrossing rate of 70% was assumed, corresponding to an average coefficient of relationship among open-pollinated progeny of r = 1/2.5 (Cotterill and Dean, 1990). Williams and Matheson (1994), also recommend the use of a coefficient of relationship of 1/2.5 (0.4) be adopted in such a study to account for the family structure and an expected degree of inbreeding.

Heritability (h^2) for each trait was estimated through the application of Equation 3.3 (Falconer, 1989):

$$\mathbf{h^2 = \sigma^2_a / \sigma^2_p} \qquad \text{... (Equation 3.3)}$$

σ_a^2 is the additive standard deviation:

$$\sigma^2_a = 1/r \times \sigma^2_f$$

$$= 2.5 \times \sigma^2_f \quad \text{... (Equation 3.3.1)}$$

where r represents the coefficient of relationship (i.e. 1/2.5) and σ^2_f represents family variance component.

σ^2_p is the phenotypic standard deviation:

$$\sigma^2_p = \sigma^2_t + \sigma^2_m + \sigma^2_f \quad \text{... (Equation 3.3.2)}$$

where σ^2_t represents the family x block variance component; σ^2_m represents the within plot variance component; and σ^2_f represents the family variance component.

The standard error of heritability was estimated according to Becker (1984) as follows:

$$\textbf{SE } h^2 = 4 \times (\text{s. e. } \sigma^2_a / \sigma^2_p) \quad \text{... (Equation 3.4)}$$

3.2.6 Statistical methodology to determine phenotypic and genetic correlation(s)

Phenotypic correlation (r_P) between two traits is calculated as the covariance divided by the product of the standard deviations of two traits (Cotterill and Dean, 1990) as follows:

$$r_P = \sigma^2_P(x.y) / \sigma_P(x)\, \sigma_P(y) \quad \text{... (Equation 3.5)}$$

where σ^2_P (x.y) represents the covariance of the measured values for traits x and y; $\sigma_P(x)$ represents the standard deviation of the measured value for trait x; and $\sigma_P(y)$ represents the standard deviation of the measured value for trait y. Phenotypic correlations between key tree

growth and essential oil traits in this study were calculated using the linear regression function in an Excel spreadsheet.

After Williams and Matheson (1994), genetic correlation (r_G) between two traits x and y is defined as:

$$\mathbf{r_G = \sigma^2_{xy} / \sqrt{(\sigma^2_x \sigma^2_y)}} \quad \text{... (Equation 3.6)}$$

where σ^2_{xy} represents the covariance component for traits x and y; σ^2_x represents the variance component for trait x; and σ^2_y represents the variance component for trait y.

Standard error of genetic correlation was calculated as follows (Becker, 1984):

$$\mathbf{SE\ (r_G) = [(1 - r^2_G) / \sqrt{2}] \times \sqrt{[(SE\ h^2_1 \times SE\ h^2_2) / (h^2_1 \times h^2_2)]}} \quad \text{... (Equation 3.7)}$$

where r^2_G represents the square of the genetic correlation; h^2_1 represents heritability of trait 1 and h^2_2 represents heritability of trait 2. The variance and covariance components necessary for estimation of genetic correlations between growth and essential oil traits in the trial were calculated using the REML procedure in GENSTAT.

3.2.7 Statistical methodology to determine potential gain

Genetic gains in individual traits expected from single trait selection were estimated from the following equation (Cotterill and Dean, 1990):

$$\mathbf{\Delta G = i \times \sigma_p \times h^2} \quad \text{... (Equation 3.8)}$$

where ΔG is the absolute gain (change) in the trait of interest expected as a consequence of selection for that trait; i is the standardised selection differential; σ_p is the phenotypic standard deviation for the trait; and h^2 is the individual heritability for the particular trait. In this case it was assumed that selection is at a rate of one in every ten, giving a standardised selection differential of 1.54 (Becker, 1984).

3.3 Results

3.3.1 Variation in tree growth and essential oil traits

There was notable variation in tree growth and essential oil characteristics between provenances and between families within provenances in the Brogo *E. radiata* subsp. *radiata* provenance/progeny trial at age 38 months. Provenance and family means are presented in Table 3.3 and Table 3.4, respectively. Figures 3.6 to 3.10 present variation in the specific traits ranked by family within provenance groups.

Table 3.3 - Mean growth and essential oil traits at 38 months of the four provenances in the *E. radiata* subsp. *radiata* provenance/progeny trial near Brogo, NSW

Provenance	Survival (%)	No. Stems per Tree	Tree Basal Area (cm^2)	Oil Conc. (w/w% DW)	1,8-Cineole Content (%)
Big Belimbla	75	2.51	45.8	8.54	62.96
Gulph Creek	64	2.45	41.0	8.22	60.95
Reedy Creek	71	2.62	45.4	7.96	61.95
Yowrie	71	2.01	35.6	8.12	62.07
Std error	44.7	0.04	15.7	0.14	1.03

Table 3.4 – Family means for growth and essential oil traits at 38 months by family in the *E. radiata* subsp. *radiata* provenance/progeny trial near Brogo, NSW

Family	Provenance*	Survival (%)	No. of Stems per Tree	Tree Basal Area (cm^2)	Oil Conc. (w/w% DW)	1,8-cineole Content (%)
1	BB	80	2.48	52.2	8.32	61.9
2	G	59	2.90	41.5	7.85	62.9
3	G	61	2.54	38.7	7.16	59.2
4	G	61	2.56	36.6	7.08	55.2
5	G	70	2.43	37.4	8.56	61.0
6	G	86	2.27	40.8	9.02	58.6
7	G	64	2.93	43.3	6.54	55.5
8	R	64	2.31	46.6	7.95	58.0
9	R	70	2.35	42.1	7.77	66.1
10	R	75	2.71	49.7	8.16	61.0
11	Y	71	2.20	41.4	8.36	61.6
12	Y	86	1.65	39.5	8.40	61.8
13	Y	79	2.23	34.5	7.52	63.7
14	Y	48	1.97	27.0	8.19	61.1
15	G	71	2.09	42.2	9.15	63.5
16	G	43	2.62	38.3	8.60	62.7
17	G	70	2.09	36.5	8.70	63.8
18	G	79	2.03	44.8	9.38	63.2
19	G	77	2.71	58.1	8.65	62.3
20	G	86	2.56	42.5	8.46	62.1
21	G	13	1.82	24.6	7.25	63.8
22	BB	84	2.78	40.9	8.71	64.2
23	BB	71	2.46	45.1	8.56	61.4
24	BB	63	2.00	38.7	7.78	64.8
25	BB	66	2.52	47.8	8.24	62.2
26	BB	52	2.77	36.8	7.18	61.3
27	BB	82	2.63	52.7	7.60	66.2
28	BB	75	2.70	44.2	8.87	63.9
29	BB	82	2.75	52.3	9.37	63.3
30	BB	88	2.68	48.2	10.70	63.1
31	BB	79	1.90	44.5	8.58	60.3
32	R	75	3.12	43.2	7.96	62.7
Mean		69.48	2.43	42.3	8.29	62.0
Std error		44.7	0.04	15.7	0.14	1.03

* BB = Big Belimbla; G = Gulph Creek; R = Reedy Creek; Y = Yowrie

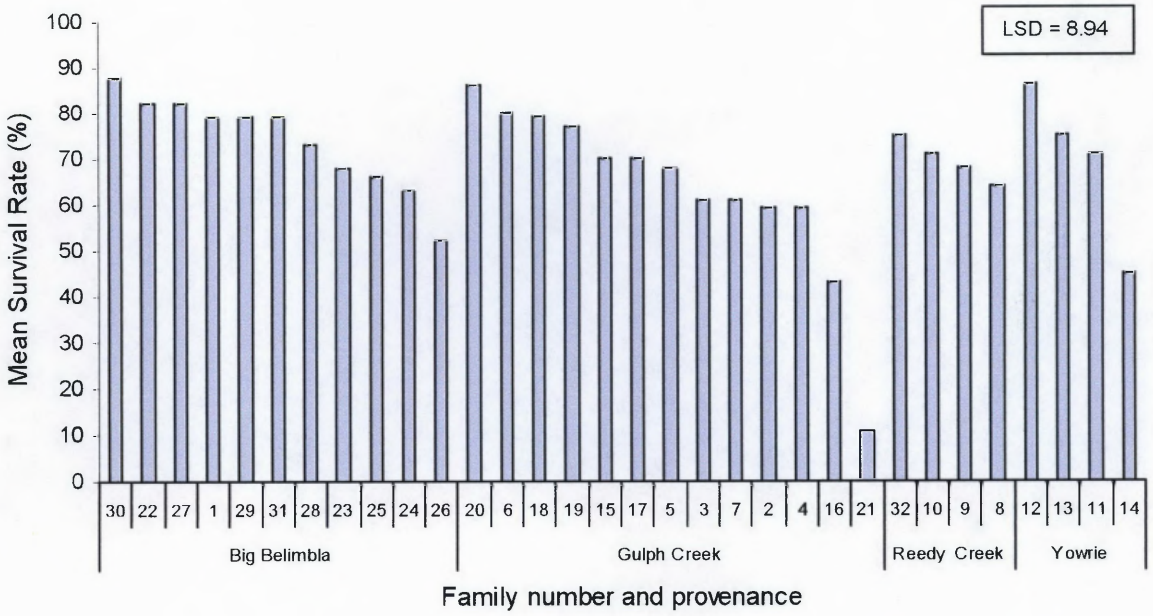

Figure 3.6 – Ranked family means for survival in the *E. radiata* subsp. *radiata* trial at Brogo, NSW, at age 38 months

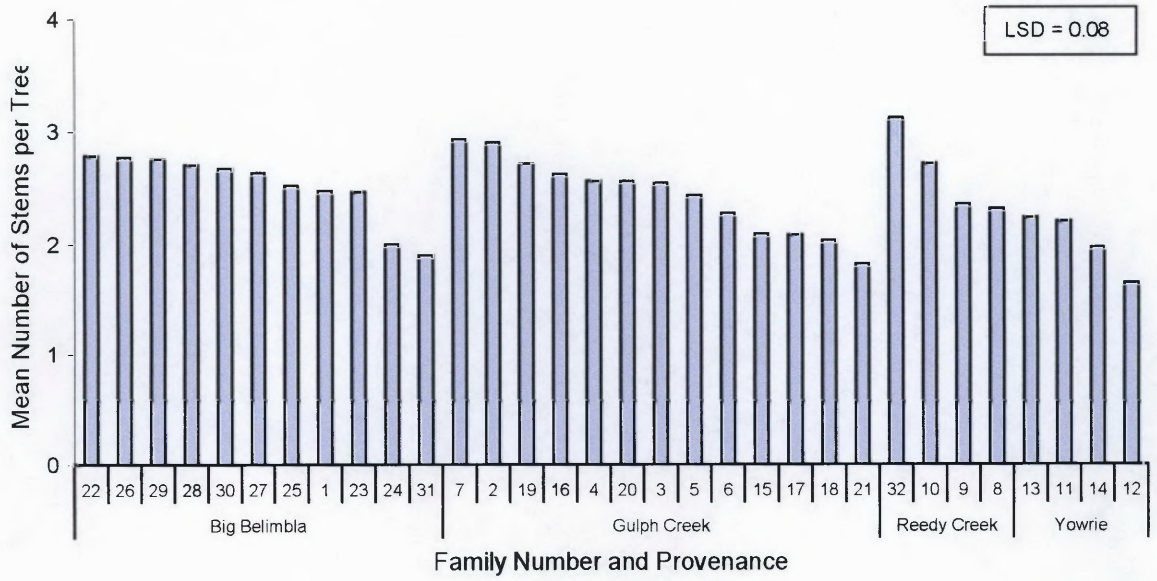

Figure 3.7 – Ranked family means for the number of stems per tree in the *E. radiata* subsp. *radiata* trial at Brogo, NSW, at age 38 months

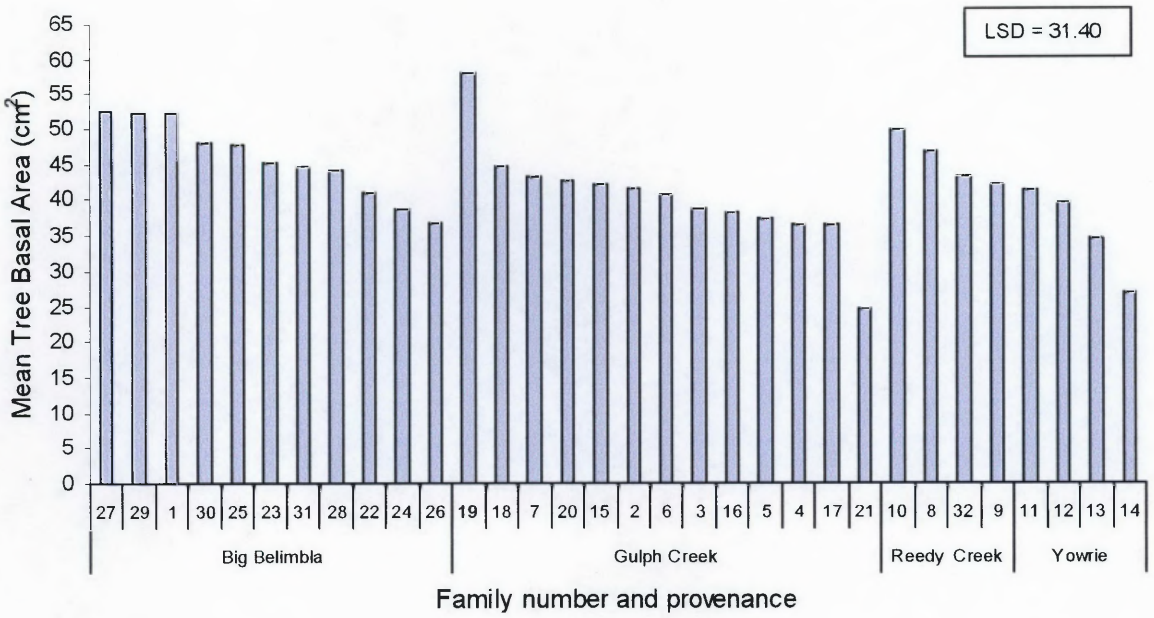

Figure 3.8 – Ranked family means for tree basal area (cm^2) in the *E. radiata* subsp. *radiata* trial at Brogo, NSW, at age 38 months

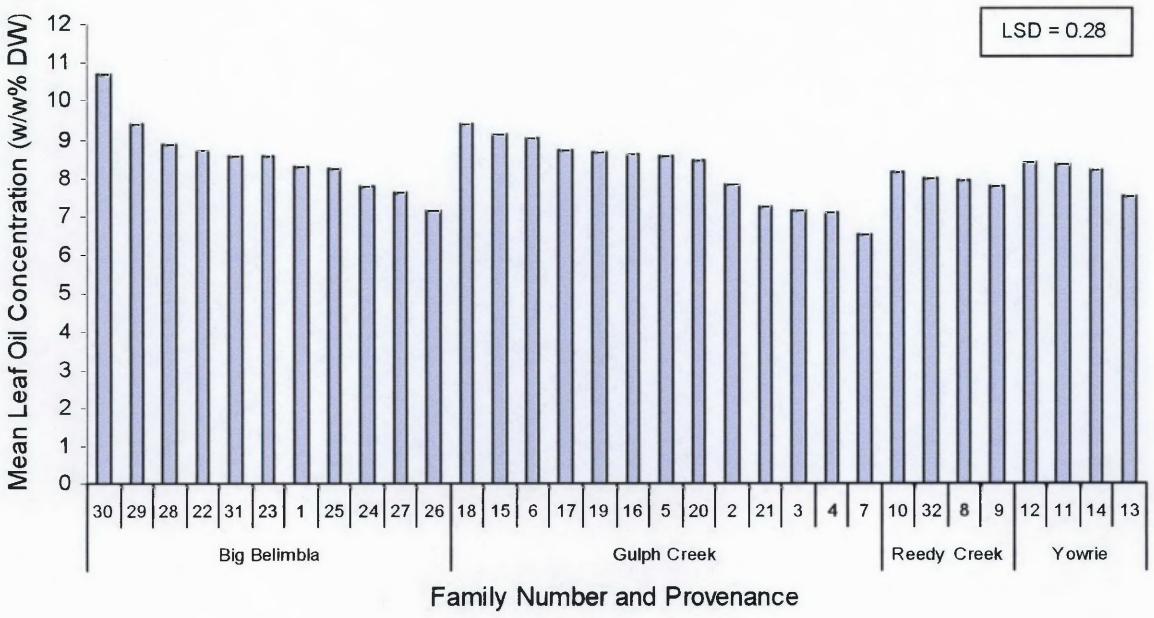

Figure 3.9 – Ranked family means for oil concentration (w/w% DW) in the *E. radiata* subsp. *radiata* trial at Brogo, NSW, at age 38 months

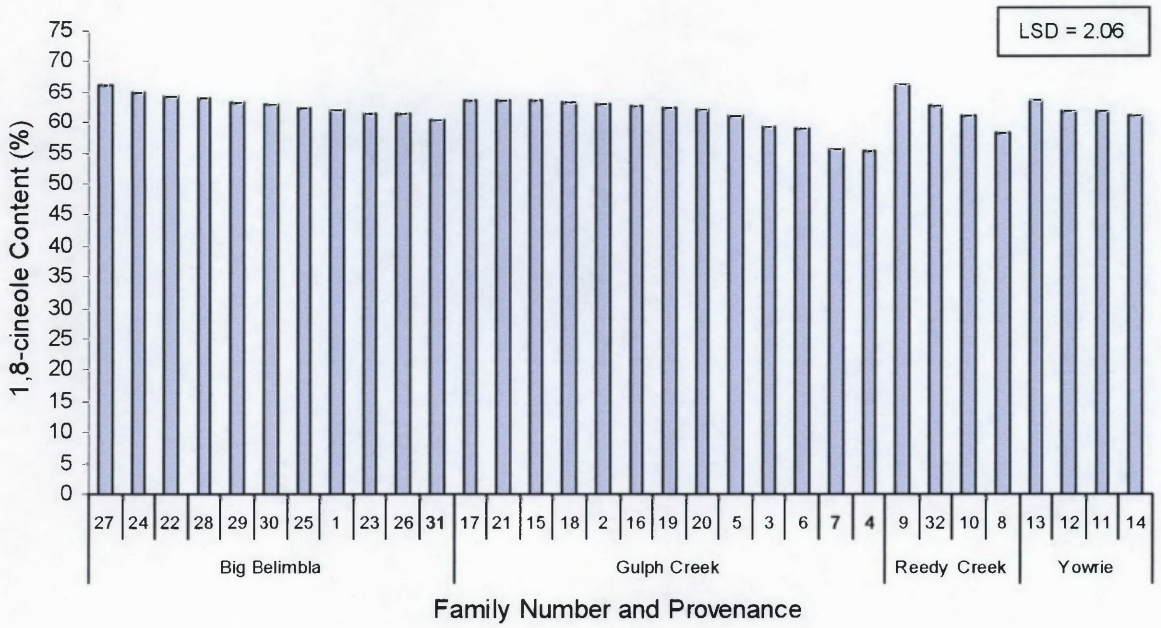

Figure 3.10 – Ranked family means for 1,8-cineole content (% of total oil) in the *E. radiata* subsp. *radiata* trial at Brogo, NSW, at age 38 months

Table 3.5 presents the analyses of variance of tree growth and essential oil traits between provenances and between families within provenances. There was no significant difference in the survival rate between the four provenances. There was, however, a significant difference in the number of stems per tree ($P < 0.01$) and tree basal area ($P < 0.01$) between the four provenances. The Reedy Creek provenance had the greatest number of stems per tree (2.62) while the Yowrie provenance had the least number of stems per tree (2.01). The Big Belimbla provenance had the largest basal area per tree (45.77 cm^2), while the Yowrie provenance had the smallest basal area per tree (35.6 cm^2). There was no significant difference in leaf oil concentration between the four provenances (ranges 7.96% - 8.54%). However, there was a significant difference in 1,8-cineole content ($P < 0.01$) between the four provenances, with Big Belimbla having the highest (63.0%) and Gulph Creek the lowest (61.0%).

The trial mean survival rate was 69 ± 45%. Survival rates between families within the provenance groups were significantly different ($P < 0.001$). Survival ranged from 52% to 88% in the Big Belimbla provenance; from 13% to 86% in the Gulph Creek provenance; from 64% to 75% in the Reedy Creek provenance; and from 48% to 86% in the Yowrie provenance (Figure 3.6). There was no significant difference in the number of stems per tree or tree basal area between families within the provenance groups. Differences in leaf oil concentration and 1,8-cineole content between families within the provenance groups were both highly significant ($P < 0.001$). The trial mean leaf oil concentration was 8.29 ± 0.14% (w/w DW). Leaf oil concentration ranged from 7.17% to 10.7% in the Big Belimbla provenance; from 6.54% to 9.38% in the Gulph Creek provenance; from 7.77% to 8.16% in the Reedy Creek provenance; and from 7.52% - 8.40% in the Yowrie provenance (Figure 3.9). The trial mean for 1,8-cineole content was 61.9 ± 1.03%. 1,8-Cineole content ranged from 60.3% to 66.2% in the Big Belimbla provenance; from 55.2% to 63.8% in the Gulph Creek provenance; from 58% to 62.7% in the Reedy Creek provenance; and from 61.1% to 63.7% in the Yowrie provenance (Figure 3.10).

It is worth noting that only examining 4 of the 12 replicates in the trial could have contributed to the large standard of error in some of the traits measured. It would be useful to repeat the assessments in all 12 replicates or carry out further trials to confirm these findings.

Table 3.5 - Analyses of variance for survival (%), number of stems per tree, tree basal area (cm^2), leaf oil concentration (w/w% DW), and 1,8-cineole content (%) of provenances and families within provenances for the *E. radiata* subsp. *radiata* trial at Brogo, NSW

Trait	Source of Variation	Degrees of Freedom	Mean Squares	Variance	F Probability	Sig.
Survival (%)	Provenance	3	512.24	1.78	0.156	ns
	family within prov.	28	739.64	2.57	0.0004	***
	Residual	93	287.60			
No. of Stems per Tree	Provenance	3	1.23	4.16	0.008	**
	family within prov.	28	0.400	1.34	0.149	ns
	Residual	91	0.30			
Tree Basal Area (cm^2)	Provenance	3	494.04	4.67	0.004	**
	family within prov.	28	139.34	1.32	0.166	ns
	Residual	91	105.90			
Leaf Oil Concentration (w/w% DW)	Provenance	3	1.71	1.77	0.159	ns
	family within prov.	28	2.71	2.81	0.0001	***
	Residual	91	0.97			
1,8-cineole Content (%)	Provenance	3	31.73	4.58	0.005	**
	family within prov.	28	24.70	3.56	0.000002	***
	Residual	91	6.93			

*** $p \leq 0.01$; *** $p \leq 0.001$; ns = not significant at $p \leq 0.05$*

3.3.2 Heritability estimates for tree growth and essential oil traits

Individual heritabilities and standard errors for the number of stems per tree, tree basal area, leaf oil concentration and 1,8-cineole content are presented in Table 3.6. The number of stems per tree and tree basal area demonstrated low heritability of 0.05 (SE = 0.09) and 0.03 (SE = 0.07), respectively, while leaf oil concentration and 1,8-cineole content were highly heritable traits, $h^2 = 0.35$ (SE = 0.24) and $h^2 = 0.42$ (SE = 0.25), respectively.

Table 3.6 – Heritabilities and their standard errors for four traits in the *E. radiata* subsp. *radiata* provenance/progeny trial at Brogo, NSW, at age 38 months

Trait	Heritability (h^2)	Standard Error of Heritability
No. of Stems per Tree	0.05	0.09
Tree Basal Area (cm^2)	0.03	0.07
Leaf Oil Concentration (w/w% DW)	0.35	0.24
1,8-cineole Content (%)	0.42	0.25

3.3.3 Phenotypic and genetic correlations of tree growth and essential oil traits

Table 3.7 presents the genetic correlations (above the diagonal) and phenotypic correlations (below the diagonal) between the four traits (number of stems per tree, tree basal area, leaf oil concentration and 1,8-cineole content). There was a strong positive genetic correlation between the number of stems per tree and tree basal area (r_G = 0.59 (SE = 0.95); between leaf oil concentration and 1,8-cineole content (r_G = 0.52, SE = 0.33); and between leaf oil concentration and tree basal area (r_G = 0.76, SE = 0.38). There was a moderate positive genetic correlation between tree basal area and 1,8-cineole content. Strong negative genetic correlations were determined between the number of stems per tree and leaf oil concentration (r_G = -0.56, SE = 0.54), and between the number of stems per tree and 1,8-cineole content (r_G = -0.42, SE = 0.61). Phenotypic correlations between the traits were negligible (Table 3.7), except for a positive correlation between the number of stems per tree and tree basal area (r_P = 0.43).

Table 3.7 - Estimates of genetic correlations (above the diagonal) and phenotypic correlations (below the diagonal) for the *E. radiata* subsp. *radiata* provenance/progeny trial at Brogo, NSW, at age 38 months (standard errors are shown in parentheses).

Trait	No. of Stems per Tree	Tree Basal Area (cm^2)	Leaf Oil Concentration (w/w% DW)	1,8-cineole Content (%)
No. of Stems per Tree		0.585	-0.564	-0.415
		(0.953)	*(0.536)*	*(0.606)*
Tree Basal Area (cm^2)	0.430		0.758	0.360
			(0.381)	*(0.725)*
Leaf Oil Concentration (w/w% DW)	-0.030	0.060		0.519 *(0.330)*
1,8-cineole Content (%)	0.030	0.020	0.090	

3.3.4 Potential gain in tree growth and essential oil traits

Table 3.8 presents the gain or improvement in the number of stems per tree, tree basal area, leaf oil concentration and 1,8-cineole content that could be expected as a result of mating between selected superior trees in the provenance/progeny trial. It is important to note that the calculation of gain has been based on the assumption that selection is carried out on a single trait only. A selection intensity of one in ten (i = 1.54, after Becker, 1984) has been used.

The gains in Table 3.8 are expressed as an absolute change from the mean as well as a percentage gain from the mean. The results indicate that an absolute increase in leaf oil concentration of 0.97% (w/w DW) from 8.29% to 9.26% could be achieved (representing a relative increase of 11.7% from the trial mean) following selection for that particular trait and mating among the best 10% of trees. If selection for 1,8-cineole content was carried out, an

absolute increase of 3.34% (or a relative increases of 5.4% from the trial mean) could be achieved. If selection for either, the number of stems per tree or tree basal area was carried out these traits could be expected to improve by a relative 3.3% and 2.4%, respectively. As the calculations of gain have been based on single trait selection, gain will only be realised in the one specific trait that has been selected for.

Table 3.8 - Predicted individual heritability and genetic gain over trial means for the four traits in the provenance/progeny trial of *E. radiata* subsp. *radiata* at Brogo, NSW, at age 38 months

Trait	Trial Mean	Selection Intensity (*i*) (1 in 10 selected)	Heritability (h^2)	Phenotypic Variance (σ^2_p)	Gain (absolute)	Gain (%)
No. of Stems per Tree	2.44	1.54	0.046	1.30	0.08	3.3%
Tree Basal Area (cm^2)	42.54	1.54	0.027	602.27	1.01	2.4%
Leaf Oil Concentration (w/w% DW)	8.29	1.54	0.348	3.09	0.97	11.7%
1,8-cineole Content (%)	61.92	1.54	0.418	27.04	3.34	5.4%

3.4 Discussion

3.4.1 Variation in tree growth and essential oil traits

As outlined in Chapter 1, qualitative and quantitative variation in essential oil can be attributed to four major factors: genetics, type and age of leaf, environment, and techniques of extraction and analysis. The results of this study confirmed that there is considerable variation between the four provenances and between the families within the provenances of *E. radiata* subsp. *radiata* in tree growth and essential oil traits in the provenance/progeny trial at Brogo. Doran *et al.* (1998b) found significant variation in survival and the number of stems per tree between provenances of *E. radiata* subsp. *radiata*, and in survival, tree basal area and leaf biomass per tree between families within provenance in this same trial at 23 months of age. Substantial variation in leaf oil concentration was also evident both within and between provenances of *E. radiata* subsp. *radiata* (Doran *et al.*, 1998b). Grant (1997) reported considerable variation within *E. polybractea* for a range of characteristics including leaf oil concentration and 1,8-cineole. This variation enables identification of superior provenances and families for oil production on this particular site, and suggests that further investigation into heritability of the desired traits, estimation of gain, and selection and breeding could be warranted.

The key traits of interest in maximising oil production are leaf biomass (of which Doran *et al.*, 1998b, determined as a surrogate for tree basal area) and leaf oil concentration. 1,8-cineole content can also be a key determinant of oil quality for certain markets. The significant difference in tree basal area between provenances, and their rankings, indicates that, on average, trees from Big Belimbla and Reedy Creek are likely to have a greater basal area than trees from the other provenances. These two provenances, therefore, would be the preferred

seed source on this site for maximising leaf biomass production. There was no significant difference in tree basal area between families within provenances.

There was no significant difference in oil concentration between provenances. There was, however, a highly significant difference in oil concentration between families within provenances. This indicates that by selecting and breeding from the best performing trees from the best families, a gain in oil concentration from the mean could be achieved, assuming oil concentration is a heritable trait. There were significant differences in 1,8-cineole content between provenances, and between families within provenances. Selecting and breeding from the best trees within the best ranked families and provenances could generate a gain in 1,8-cineole content, provided this trait is heritable.

It is important to note that the 1,8-cineole contents reported in this study (Table 3.3, Table 3.4 and Figure 3.7) were lower than expected (ranging from 56% to 66%, with a mean of 62%), considering that the mother trees of the Brogo trial families were originally selected from native stands on the basis of a 1,8-cineole content greater than 70%. Doran *et al.* (1998b) carried out a similar study based on the same provenance/progeny trial at Brogo (but at age 23 months), and reported a range of 64 to 76% 1,8-cineole, with a trial mean of 73%. The low 1,8-cineole values in this particular study (cf. Doran *et al.*, 1998b, and subsequent sampling by Doran) are most likely due to a technical programming error in the GC analysis throughout the process. The recorded values, although slightly lower than expected, remain relative to each other.

Pearson correlation analysis was carried out to compare the rankings of tree growth and essential oil traits determined by Doran *et al.* (1998b), where the trees in the Brogo provenance/progeny trial were 23 months old, and this study where the same trees were

38 months old. There was no significant difference in family rankings in any trait between the two sampling events. The stability of rankings at this early age bodes well for the reliability and effectiveness of early selection for growth and oil traits in *E. radiata* subsp. *radiata*. Rankings are presented in Appendix B.

3.4.2 Heritability estimates for tree growth and essential oil traits

Heritability of the number of stems per tree and tree basal area in *E. radiata* subsp. *radiata* were low (0.05 and 0.03, respectively) in this study. This indicates that only 5% and 3% of the total phenotypic variance in the number of stems per tree and tree basal area respectively, is due to additive genetic effects. Doran *et al.* (1998b) reported a moderate heritability (0.14) for basal diameter (a functional of basal area) in 16-month old *Melaleuca alternifolia* trials in northern New South Wales. Eldridge *et al.* (1994) presented a summary of individual heritability estimates in a number of eucalypt species determined by various authors. Diameter heritability estimates varied considerably, ranging from 0.11 in *E. tereticornis* (Kedharnath and Vakshasya, 1978), 0.10 to 0.46 in *E. regnans* (Eldridge, 1972 and Griffin and Cotterill, 1988, respectively), to 0.57 in *E. obliqua* (Matheson *et al.*, 1986). Cotterill and Dean (1990) also summarised individual heritability estimates in a number of traits in *Pinus radiata* from nine different authors. Diameter heritability estimates were generally low to moderate, ranging from 0.10 (Carson, 1986) to 0.25 (Wilcox *et al.*, 1975) with a mean of the authors combined estimates of 0.16.

Leaf oil concentration and 1,8-cineole content were found to be highly heritable (0.35 and 0.42, respectively) in *E. radiata* subsp. *radiata* in this study. Individuals selected for high leaf oil concentration or high 1,8-cineole content should therefore produce similarly high yielding offspring. Other recent studies of the oils of various species of *Eucalyptus* and *Melaleuca*

have also indicated moderate to strong genetic control of oil concentration and composition. Grant (1997) reported individual heritabilities of 0.61 for 1,8-cineole content (%) and 0.36 for leaf oil concentration in *E. polybractea* at 26 months in a progeny trial at West Wyalong. Doran and Matheson (1994) reported individual heritabilities for 1,8-cineole yield (w/100g, dry leaf) and total monoterpene yield in *E. camaldulensis* progeny trials at Mtao in Zimbabwe at 45 months of 0.53 and 0.54, respectively. They also reported individual heritabilities for 1,8-cineole yield and total monoterpene yield in *E. camaldulensis* progeny trials at Forest Hill in Zimbabwe of 0.61 and 0.42, respectively. Doran and Saunders (1993) reported individual heritabilities of 0.36 for 1,8-cineole concentration, and 0.27 for total oil yield in young provenance/progeny trials of *E. globulus.* Barton *et al.* (1991) reported a family heritability (h^2_f) for 1,8-cineole concentration (w/w fresh leaves) of 0.83 for 30-month old *E. kochii* progeny. Butcher *et al.* (1996) reported an individual heritability of 0.67 for oil yield in a *Melaleuca alternifolia* provenance/progeny trial at age 13 months in north-eastern New South Wales. Doran *et al.* (1997) also reported high individual heritability (0.51) for oil yield in 16-month old *M. alternifolia* trials in northern New South Wales.

In practice, total oil yield from a plantation is a function of leaf oil concentration and leaf biomass production. As such, a breeding program should consider improving the total leaf biomass produced per tree as well as improving oil concentration. Heritabilities on leaf biomass were not determined in this study, however, Grant (1997) determined that the estimated heritability of dry leaf biomass in *E. polybractea* was low (0.05). Barton *et al.* (1991) did not estimate heritability of foliage biomass, but indicated that they had no reason to believe that this trait could not be improved by selection.

3.4.3 Phenotypic and genetic correlations of tree growth and essential oil traits

The phenotypic correlations determined between the various tree growth and essential oil traits in this study were all close to zero, except for the correlation between tree basal area and the number of stems per tree. Falconer (1989) stated that phenotypic correlation is not as important to the tree breeder as genetic correlation, because it is the latter that is the correlation of breeding values. A knowledge of genetic correlations is necessary to predict correlated gains in one trait as a consequence of selection on another trait (Cotterill and Dean, 1990).

A strong positive genetic correlation between tree basal area and leaf oil concentration (r_G = 0.76, SE = 0.38) was determined in this study, indicating that selecting trees with a large basal (or diameter) area can be expected to result in an increase in leaf oil concentration. Doran and Matheson (1994), however, reported a negative genetic correlation between diameter (a function of basal area) and total monoterpene yield (an estimate of oil concentration) of r_G = -0.46 (SE = 0.33) for *E. camaldulensis*.

This study also identified a moderate positive genetic correlation between tree basal area and 1,8-cineole content (r_G = 0.36, SE = 0.73) in *E. radiata* subsp. *radiata* suggesting that selecting trees with a large basal area may result in an increase in 1,8-cineole content. There was also a strong positive genetic correlation between the number of stems per tree and tree basal area (r_G = 0.59, SE = 0.95).

The strong negative genetic correlation between the number of stems per tree and 1,8-cineole content, and also between the number of stems per tree and leaf oil concentration in this study indicates that making a selection based on the number of stems per tree would not result in an increase in oil concentration and 1,8-cineole content. A large number of stems is therefore an

undesirable selection parameter for attempting to generate a gain in oil production traits through a breeding program. The strong positive genetic correlation between leaf oil concentration and 1,8-cineole content indicates that selecting trees for high leaf oil concentration may also increase the 1,8-cineole content of the offspring. Grant (1997) also reported a moderate genetic correlation between 1,8-cineole content and leaf oil concentration ($r_G = 0.39$) in *E. polybractea*.

It can be inferred from the results of this study that selection of individual trees with the largest basal area will result in progeny likely to have high leaf oil concentration, high 1,8-cineole content and a greater number of stems per tree. Tree basal area (or diameter, as a derivative of basal area) is therefore seen as a suitable selection parameter in a breeding program aiming to increase oil quantity and quality, based on this trial.

It is important to be able to calculate the degree to which the genetic gain in one particular trait is affected by increasing or decreasing the number of traits under selection. If selection is applied to many traits at once, it is likely that none of them will be improved much, and there is danger that adverse genetic correlations could devalue one trait at the same time that another is being improved (Eldridge, *et al.,* 1994). There are three fundamental methods of selection including; tandem selection, which involves selecting for several traits one at a time over several generations; independent culling, which involves independent culling with respect to different traits carried out either simultaneously or at different times in one generation; and index selection, which integrates information from multiple sources for more than one trait into one index value. Independent culling and index selection allow a quicker response to selection than tandem selection, and minimise the effect of diminishing gains in one trait when selecting for two or three over a longer period of time (Cotterill and Dean, 1990).

It should be noted that the genetic correlations between all of the tree growth and essential oil traits determined in this study had large standard errors and must be interpreted with a degree of caution. It would be useful to repeat the assessments in all 12 replicates of the trial or carry out further trials to confirm these findings.

3.4.4 Potential gain in tree growth and essential oil traits

Relative gain (% from the mean) in the number of stems per tree or tree basal area as a result of mating amongst superior trees selected for those individual traits (one tree in ten selection intensity) from the provenance/progeny trial at Brogo are expected to be in the order of 3.3% and 2.4%, respectively. Relative gains (% from the mean) in leaf oil concentration or 1,8-cineole content from one generation of single trait selection are expected to be about 11.7% and 5.4% respectively. An 11.7% relative gain in leaf oil concentration would result in a population mean of 9.3% (w/w DW). This figure mimics that proposed by Doran *et al.* (1998b) as likely for the population mean after selection. The figure is also consistent with an oil concentration of 9.2% (w/w DW) obtained from *E. radiata* subsp. *radiata* plantations in South Africa established using selected seed (Donald, 1991).

These figures may not be high, but it is important to note that the provenances and families in this trial have already been selected from native stands for superior traits as described in Section 3.4.1. Based on experience in breeding *M. alternifolia* Doran *et al.* (1997) estimated that a 20% gain in leaf oil concentration may have already been achieved in the Brogo trial through selection of mother trees from native stands. Published estimates of gain in oil concentration from the selection of the best tree in 10 and their subsequent mating are generally in the order of 30%. Grant (1997) reported relative gains in oil concentration and 1,8 cineole content of 25% and 28% respectively for *E. polybractea* at 26 months in a

progeny trial at West Wyalong. Barton *et al.* (1991) determined that oil concentration in *E. kochii* was expected to increase from 2.0% (w/w FW) for native stands to 2.8% (a gain of 40%) following selection of the best parent in every ten on the basis of yield of 30 month old offspring. Doran and Matheson (1994) estimated gains in oil yield in *E. camaldulensis* in the first generation of selection of the best parent in every ten of 45-month-old offspring to be 25% for 1,8-cineole and 32% for total monoterpenes.

Maximising oil production from a plantation is achieved by maximising leaf oil concentration and leaf biomass production (a surrogate of tree basal area or diameter; Doran *et al.*, 1998b). This may not always be possible however, as moderate and unfavourable genetic and phenotypic correlations for oil concentration and dry leaf weight can occur as reported by Butcher *et al.* (1996) and Grant (1997). Based on the results of this study and the findings of Doran *et al.,* (1998b) however, selection should aim to achieve concurrent gains in tree basal area and leaf oil concentration in order maximise oil production from a breeding program,.

The implications of the gains achieved in essential oil traits in *E. radiata* subsp. *radiata* in the provenance/progeny trial at Brogo are examined in more detail in Chapters 4 and 5, where the potential boost in economic performance as a result of the gain is modelled, and a breeding strategy is discussed. It is important to note that the heritabilities, phenotypic and genetic correlations, and gains presented in this study are based solely on data from the Brogo trial. In general application, these estimates should be used with caution until confirmed by other similar studies on other sites.