Abstract

Previous studies have indicated that *Eucalyptus radiata* Sieb. ex DC subsp. *radiata* has the potential to produce a high quality medicinal oil commanding a premium price over other species in a niche market. This study has two aims: (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 effect on 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.

The work is based on a 38-month-old provenance/progeny trial of 32 open-pollinated families from four south-eastern NSW provenances, at Brogo in southern New South Wales. Tree growth and essential oil traits were assessed. Heritability and genetic correlation of traits were determined, and estimations of gain in the key traits of leaf oil concentration, tree basal area and 1,8-cineole content through selection were calculated. A breeding strategy was proposed.

The number of stems per tree, tree basal area and 1,8-cineole content varied significantly between provenances, while families within provenance varied significantly in survival, leaf oil concentration and 1,8-cineole content. Growth characteristics demonstrated low heritability, while leaf oil concentration and 1,8-cineole content were highly heritable ($h^2 = 0.35$ and 0.42, respectively). There were strong positive genetic correlations between: the number of stems per tree and tree basal area ($r_G = 0.59$); leaf oil concentration and 1,8-cineole content ($r_G = 0.52$); and leaf oil concentration and tree basal area ($r_G = 0.76$). An estimated absolute gain in leaf oil concentration of 0.97% (w/w DW) from the trial mean of 8.3% to 9.3% was calculated, representing an 11.7% relative increase from the trial mean through selection. Relative gains in tree basal area and 1,8-cineole content were calculated as 2.4% and 5.4%, respectively.

The 'baseline' oil production venture was marginal, with a NPV of $68 and an IRR of 5.02 percent over a 20-year timeframe. The financial performance of the venture improved when the calculated gain of 11.7% in leaf oil concentration was achieved (NPV = $6,861 and IRR = 6.67%). Reducing harvesting and distillation costs by 50% through increased efficiencies such as mechanical harvesting resulted in a NPV of $119,255 and an IRR of 17.5 percent. An increase in farm-gate price received for the oil of 50% resulted in a NPV of $221,772 and an IRR of 37.4 percent. Conversely, increasing production costs or reducing the sale price by just a small amount resulted in the venture becoming non-viable.

Rudimentary farm-scale production of bulk medicinal eucalyptus oil is likely to be a marginal business, being highly sensitive to minor fluctuations in production costs and product sale price. Use of selected stock to achieve a high yield per unit area of land, efficient production techniques and realising a premium price for the product are essential to successfully competing in the current market and returning a profit.

Chapter 1 Introduction and overview of commercial eucalyptus oil production

1.1 A brief history of eucalyptus oil production in Australia

Eucalyptus oil production can claim the distinction of being the first truly Australian primary and secondary production industry (Abbott, 1989). It was also one of the first exports from Australia following European settlement (Brooker, 1989). Denis Considen, assistant to the Surgeon-General of the First Fleet, was responsible for the collection and dispatch of one quarter of a gallon of steam-distilled eucalyptus oil to England in 1789 (Boland *et al.,* 1991). This oil was distilled from a natural stand of Sydney peppermint *(Eucalyptus piperita).*

Narrow-leaved peppermint (then *E. amygdalina*) was later discovered as being a higher oil-yielding species and became the basis of a small eucalyptus oil industry in the 1850s (Brooker, 1989). Boland *et al.* (1991) reported that Joseph Bosisto, a Victorian pharmacist, began the industry in 1852 at Dandenong Creek (Victoria), distilling *E. amygdalina* in a crudely constructed still. Sales were restricted to a local market until overseas interest grew and Bosisto began exporting to England in 1865 (Abbott, 1989).

In the late 1860s, Bosisto received financial support from Melbourne entrepreneurs, Alfred Felton and Frederick Grimwade, and the first large commercial eucalyptus oil operation was established in the Mallee-Wimmera settlement in North-Western Victoria. Davis (1995) indicates that the main species distilled was blue mallee *(E. polybractea).* The mallee oil was exported to England, Germany, Canada, South Africa, India, China, and New Zealand (Boland *et al.*, 1991). Felton and Grimwade were responsible for establishing one of the main eucalyptus oil producers in Australia today, Felton Grimwade and Bickford Pty Ltd.

E. globulus, E. oleosa and *E. cneorifolia* were distilled for commercial purposes in the early 1880s (Abbott, 1989) and by the mid 1870s, *E. globulus* oil, mainly from Victoria and Tasmania, had become renowned worldwide as a medicinal product and was being exported to England and Europe (Boland *et al.*, 1991).

The industry was well established by 1900, and Australia remained the largest supplier in the eucalyptus oil trade for the next 50 years (Small, 1981). The industry experienced its first boom around World War I. The oil was used as a medicine and antiseptic (Boland *et al.*, 1991). In the 1920s, prices began to fall and the boom declined. During the 1930s, a number of overseas producers established *E. globulus* plantations and began producing oil at a cheaper cost than could be achieved in Australia (Boland *et al.,* 1991). The depression years of 1930-1935 also contributed to low sales.

In 1946-1947, the industry reached its peak in Australia when production totalled approximately 1000 t of which 70% was exported (Small, 1981). Cineole-rich *E. australiana* (now *E. radiata* subsp. *radiata*) and *E. polybractea*, and phellandrene-rich *E. dives* were the main species harvested at that time. After this peak in production, the industry declined. The major reason for the decline has been the ability of other countries to produce oil much cheaper than Australia.

1.2 Current eucalyptus oil industry

1.2.1 Australian production

The Australian eucalyptus oil industry is currently structured to supply local and export markets through refiners and distributors (Small, 1981). There are approximately 25 producers of crude eucalyptus oil in Australia. Most are small-scale operators working on a part-time basis as an additional source of income. Australia's market share has fallen and production is now less than 5% of world requirements (Abbott, 1989). *E. polybractea* is the major commercial species currently harvested in Australia, followed by *E. radiata* and *E. dives* (broad-leaved peppermint).

There are currently three major commercial producers: G. R. Davis Pty Ltd, Felton Grimwade and Bickford Pty Ltd and the Banalasta Oil Plantation Pty Ltd. G. R. Davis Pty Ltd has major land holdings near West Wyalong in New South Wales and a rectification factory in Sydney. Felton Grimwade and Bickford Pty Ltd has crown leases near Inglewood in Victoria and a factory in Melbourne where the crude oil is used to produce a range of health and personal care products. Both producers utilise natural stands of *E. polybractea*, while G. R. Davis Pty Ltd also has an area of *E. polybractea* plantation. The Banalasta Oil Plantation was formed as a prospectus investment-driven project in 1996 and has since established approximately 250 ha of irrigated *E. radiata*[1] subsp. *radiata* plantation at Bendemeer, between Armidale and Tamworth in New South Wales (Kar *et al.*, 2000).

A large mallee eucalypt planting effort is currently under way in Western Australia led by the Oil Mallee Association of WA, the Oil Mallee Company and the Department of Conservation and Land Management (CaLM). The aim is to develop a eucalyptus oil industry integrated with a land degradation (salinity) remediation program in the low-rainfall wheatbelt area of the state. The oil mallee species currently under investigation include oil mallee (*E. kochii* subsp. *kochii* and subsp. *plenissima*), red-flowered mallee (*E. erythronema*), sand mallee (*E. eremophila*), oil mallee (*E. horistes*), york gum (*E. loxophleba*), swamp mallee

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[1] *Eucalyptus radiata* Sieber ex DC subsp. *radiata*. This is the taxonomic description used for the species studied in this thesis.

spathulata) and square-fruited mallee (*E. calycogona*). The project is being implemented on a large scale, with the trees being established as wide-spaced plantings integrated into existing farming operations in a system called alley cropping.

Investigation has identified a range of potential markets for the produce of the Oil Mallee project including industrial solvents (Holt, 1998), biomass for electricity generation and transport fuels (Bartle, 2001), as well as activated carbon (CSIRO Forestry and Forest Products, 1999). Feasibility studies have determined that it is critical that integrated markets are developed for the products including those for the wood as well as the leaf biomass components (Bartle, 2001).

Bartle (2001) reported that between 1994 and 1996, approximately 6 million seedlings were planted (2400 ha) in the wheatbelt area of Western Australia, and that between 1996 and 2000 another 11 million seedlings (4400 ha) were planted. Grower numbers have reached 900 and expenditure on the planning, establishment, feasibility studies, research and development has been approximately $21 million. More than 20 million seedlings in total have now been planted as part of the project (J. Bartle, *pers. comm.*, 2001).

Both Abbott (1989) and Davis (1995) reported that although Australian eucalyptus oil production declined steadily through the 1950s, 1960s, and 1970s, mechanisation in the 1980s enabled Australia to regain some of its lost markets. Coppen (2002) estimated current Australian production of cineole oil to be around 100 t/year or less, while McKelvie *et al.* (1994) cited in Coppen (2002) estimated it to be around 80 t/year. Australian production is also supplemented by imports of lower quality oils, particularly from China, which are rectified and then blended with locally produced oils (Abbott, 1989; Davis, 1995; Coppen, 2002). Much of the subsequent re-exports are in the form of the final, formulated product, rather than the crude oil. Coppen (2002) reported that according to Chinese trade statistics, Australian imports of eucalyptus oil averaged 155 t/year for the years 1991 and 1993-2000 with a rising trend. Piperitone-rich *E. dives* oil is no longer imported into Australia from South Africa, as it used to be, for menthol production. Recent Australian exports (1997-2000) averaged 113 t/year. If the plans to produce the large quantities of cineole-rich oil in Western Australia through the Oil Mallee project come to fruition, oil production in Australia could eventually exceed that of China (Coppen, 2002).

1.2.2 International production and markets

J. Coppen (pers. comm., 2001) provided recent statistics on world production and markets for eucalyptus oil, and all figures presented in this section have been extracted from Coppen (2002) unless otherwise referenced. China remains the dominant producer and exporter of eucalyptus oil in the world. Current estimates of annual production ranged from 2000 t to 5000 t, of which it is estimated that between 1000 t and 3500 t are exported.

The USA is likely to be one of the largest consumer of eucalyptus oil worldwide, where in 1998 and 2000, imports reached just over 700 t/year (valued at US$3.2 million and US$2.9 million, respectively). China is the main source of US imports, but Brazil, Australia and South Africa have also been important suppliers. Current annual production in Portugal and Spain combined, from *E.* globulus plantations is probably less than 100 t/year. Brazilian production, as estimated by Couto (pers. comm., in Coppen, 2002) is currently around 900 t/year. Brazil has a large domestic market for eucalyptus oil but there is still a surplus for export. The USA, Mexico, Colombia, Spain and Sweden have been consistent export markets, while the UK, France and the Netherlands imported significant quantities of Brazilian oil in 2000.

Southern Africa remains a significant producing region. Davidson (pers. comm., 1998) in Coppen (2002) estimated current annual production of *E. smithii* oil to be 100 t, *E. radiata* oil to be 35 t and *E. dives* oil to be 200 t in South Africa. Most of the *E. radiata* and *E. dives* oils produced are exported but the *E. smithii* oil is mostly rectified locally for domestic consumption. South Africa also imports Chinese oil for compounding a variety of fragrance and flavour materials to meet local demand. Production in Swaziland and Zimbabwe has virtually ceased.

Coppen (2002) reviewed the outlook for worldwide eucalyptus oil production and claimed that given the early stage of development of Western Australian eucalyptus oil production, China will, for the foreseeable future, continue to be the dominant force in eucalyptus oil supplies. Events in China, therefore, will be of prime importance in determining prices, particularly for cineole-rich oils. However, it was suggested that while Chinese eucalyptus oil is currently a high-volume, low-priced oil, generally exported for use in blending and compounding by others, there are changes taking place. Notable amongst these is an expansion and consolidation in China of its own refining and marketing activities, often through joint ventures with foreign companies. Coppen (2002) stated that "increasing public awareness of 'green' issues and attention to product labelling will ensure that natural products such as eucalyptus oil will continue to find favour in the marketplace in the West, while in the East, the massive potential of increasing consumer demand in countries such as China, India and those of the former Soviet Union beckons". He concluded that future global production of eucalyptus oil would be driven by those in the industry who can adapt positively to the changes that are taking place.

Tree selection and breeding, clonal propagation, plantation research and development, advanced mechanised harvesting, efficient distillation methods, product development and effective marketing strategies are the key elements in the survival and expansion of the industry (Davis, n.d.). Such innovations are currently being introduced into Australian production.

1.3 Commercial species and their use

Botanists have described over 600 species of *Eucalyptus* and *Corymbia* in Australia. Most of these have been examined for their essential oil content (Barton and O'Reilly, 1986), but only 20 or so of all species examined have been exploited commercially.

1.3.1 Oil classification

Commercial eucalyptus oils are divided broadly into three classes depending on end use: medicinal, industrial, and perfumery or flavouring (Small, 1981; Barton and O'Reilly, 1986; Lassak, 1988; Abbott, 1989; Barton, 1989; Boland *et al*, 1991; Davis, n.d.; Doran, 1991; Milthorpe, n.d.). Classification of oil types depends upon their chemical composition. This allows the 'labelling' of species and chemotypes, but a number of oils span all three categories of end use (Doran, 1991). The principal constituent of medicinal oils is 1,8-cineole. Alpha-phellandrene and piperitone are the major constituents of the industrial oils, while citronellal, geranyl acetate, citral (neral and geranial), and E-methyl cinnamate are common components of the perfumery and flavouring oils. Table 1.1 lists the principal chemical composition and concentration, and the approximate average oil yield of a number of major commercial eucalyptus oil species as grouped by the three end use classes. Australia primarily produces medicinal and industrial oils (Small, 1981).

Table 1.1 - Commercial eucalyptus oil species, grouped according to their end use classification (*source: adapted from Lassak, 1988; Boland et al., 1991*)

SPECIES	PRINCIPAL LEAF OIL CONSTITUENT AND % RANGE^1	AV. OIL YIELD (%) (on leaf FW unless specified)	MAJOR COUNTRY OF PRODUCTION ^{b}
MEDICINAL OILS			
E. calycogona	cineole, ?	2.6-2.9	(Australia)
E. camaldulensis	cineole, 10-90	0.3 - 2.8	(Nepal)
E. cneorifolia	cineole, 40-90	ca 2.0	
E. dives (cineole form) *	cineole, 60-75	3.0 - 6.0	(Australia)
E. Dumosa	cineole, 33-70	1.0 - 2.0	
E. eremophila	cineole, 33	1.75 (DW)	(Australia)
E. erythronema	cineole, 71	2.5 (DW)	(Australia)
E. goniocalyx	cineole, 60-80	1.5 - 2.5	
E. globulus #	cineole, 60-85	0.7 - 2.4	China, Portugal, Spain, India, Brazil, Chile, (Bolivia, Uruguay, Paraguay)
E. horistes	cineole, 73-83	2.1-4.7 (DW)	(Australia)
E. kochii subsp. *kochii*	cineole, 83-94	1.2 - 3.7	(Australia)
E. kochii subsp. *plenissima*	cineole, 83-94	2.2 -8.6 (DW)	(Australia)
E. leucoxylon	cineole, 65-75	0.8 - 2.5	
E. loxophleba	cineole, 67%	2.4	(Australia)
E. oleosa	cineole, 45-52	1.0 - 2.1	
E. polybractea *	cineole, 60-93	0.7 - 5.0	Australia
E. radiata ssp *radiata (cineole form)* *	cineole, 65-75	2.5 - 3.5	(South Africa, Australia)
E. sideroxylon	cineole, 60-75	0.5 - 2.5	
E. smithii #	cineole, 70-80	1.0 - 2.2	Sth Africa, Swaziland, (Zimbabwe)
E. spathulata	cineole, ?	1.43	(Australia)
E. tereticornis	cineole, 45	0.9 - 1.0	
E. viridis *	cineole, 35-40	1.0 - 1.5	(Australia)
E. exserta			China
INDUSTRIAL OILS			
E. dives (phellandrene form)	phellandrene, 60-80	1.5 - 5.0	
*E. dives (piperitone form)**	piperitone, 40-56	3.0 - 6.5	South Africa, (Australia)
E. elata (piperitone form)	piperitone, 40-55	2.5 - 5.0	
E. radiata ssp *radiata (phellandrene form)*	phellandrene, 35-40	3.0 - 4.5	
PERFUMERY AND FLAVOURING OILS			
E. citriodora (citronellal form) #	citronellal, 65-80	0.5 - 2.0	China, Brazil, India
E. macarthurii	geranyl acetate, 60-70 60-68 ++	0.2 - 1.0 0.1 - 0.4 ++	
E. olida	E-methyl cinnamate, 95	1.6 - 6.1	(Australia)
E. staigeriana	citral, 16-40	1.2 - 1.5	Brazil

[1] *% of total oil volume*

* *main Australian commercial species*

\# *main overseas commercial species*

++ *bark oil*

b *parenthesis indicate a minor producer*

FW is fresh weight and DW is dry weight. A yield based on fresh weight is approximately double that of a FW yield, as moisture content of mature eucalypt leaves is approximately 50%

1.3.2 Essential oil quality criteria

The quality of an essential oil is specified by minimum standards that are defined in Australian, British, United States, and other national and international pharmacopoeias and standards. The International Organisation for Standardisation (ISO) is a worldwide federation of national standards institutes. It issues standards that may be formally approved by member bodies of individual countries and go on to be adopted as national standards (Coppen and Hone, 1992). Table 1.2 lists the major current international and Australian standards applying to eucalyptus oil production. The specifications of the *British Pharmacopoeia* (BP) are often used as the international benchmark, but are only applicable to oil produced from some species. The United States is not a member of the ISO and their standards are set down by the Food Chemicals Codex 1981 (Coppen and Hone, 1992). The oils must meet a number of requirements as set out in the standards, such as the minimum percentage of the major chemical component, the relative density of the oil, its refractive index, optical rotation, and its solubility in ethanol (Coppen and Hone, 1992).

Table 1.2 - Major international standards (ISO) and Australian standards (AS) relating to eucalyptus oil production
(*source: adapted from Standards Australia (2002), Online Search Catalogue, http://www.standards.com.au/catalogue*)

STANDARD	YEAR	DESCRIPTION
AS 2113.1	1998	Oil of Australian *Eucalyptus* - 70 to 75 percent cineole
AS 2113.2	1998	Oil of Australian *Eucalyptus* - 80 to 85 percent cineole
AS 2116	1977	Oil of *Eucalyptus citriodora*, Australia
AS 2247.1	1999	*Eucalyptus* oil fractions – 1,8-cineole (Eucalyptol)
AS 2247.2	1999	*Eucalyptus* oil fractions – *Eucalyptus* oil terpenes (phellandrene fraction)
AS 2700S	1996	Colour standards for general purposes – *Eucalyptus*
ISO 3044	1997	Oil of *Eucalyptus citriodora* Hook
ISO 3065	1974	Oil of Australian *Eucalyptus*, 80 to 85% cineole content
ISO 4732	1983	Rectified oil of *Eucalyptus globulus* Labillardiere, Portugal
ISO 770	1980	Oil of *Eucalyptus globulus*

1.3.3 Medicinal oils

The medicinal properties of 1,8-cineole-rich eucalyptus oil were first known to the Aboriginals, but were initially exploited commercially in Australia by Bosisto (Coppen and Hone, 1992) as discussed earlier. Currently medicinal eucalyptus oil has many applications, having a multi-purpose 'cure all' reputation.

Barton (1989) listed some of the qualities of cineole, including its biodegradability, low toxicity, solvent ability for a range of other materials, ability to penetrate tissues rapidly, mild bactericidal qualities, limited insecticide and insect deterrent qualities, synergistic effects in medicinal behaviour when combined with other materials, and its odour which most people find acceptable and non-threatening. These qualities make such oils suitable for use as major ingredients in various inhalants, embrocations, soaps, gargles, sprays and lozenges (Small, 1981). Other uses include antiseptics, spot and stain removers, wool wash and deodorisers.

Barton (1989) reported that one of the significant features of 1,8-cineole is its ability to prevent the phase separation of blended liquid motor fuels containing ethanol, hydrocarbon, and small amounts of water. The addition of a small amount of eucalyptus oil as a co-solvent overcomes problems of corrosion, poor engine performance, and starting trouble (Ammon *et al.*, 1986). When this fuel replaces petrol, there is little change in octane rating or engine performance, and emission levels are generally lower.

Medicinal oils are generally rich in 1,8-cineole and free of α-and β-phellandrene (Barton and O'Reilly, 1986; Brooker *et al.*, 1988; Lassak, 1988; Abbott, 1989; Doran, 1991; Milthorpe, n.d.). To be classed as suitable for medicinal purposes under the British Pharmacopoeia (BP), an oil must have a minimum 1,8-cineole content of 70% and be virtually free of α- and β-phellandrene (Doran, 1991). A range of medicinal oils with various cineole contents is produced. The major grades are 70-75% cineole, 75-80% cineole, 80-85% cineole, 85-90% cineole, and 90-95% cineole. An oil of near 100% cineole is known as Eucalyptol (Barton, 1989) and is produced by a number of refining processes. Oils that have a low concentration of 1,8-cineole are often blended or rectified to adjust oil characteristics to meet pharmacopoeia and market requirements (Doran, 1991).

1.3.4 Industrial oils

Industrial oils contain α-phellandrene and piperitone as their main chemical constituents. These oils generally have two different use categories. Some are refined for the isolation of individual chemical compounds which are later used for specific applications, while others are used in raw form, usually as industrial deodorisers and scenting products.

Piperitone from various species (particularly *E. dives*) was used for the production of synthetic menthol and as the basis for a fungicide (Doran, 1991). Both these applications have now ceased (Coppen and Hone, 1992). Chemical components such as α- and β-pinene have been used in paint manufacture and as an oil-based paint thinner (Doran, 1991). Phellandrene-rich oils are often used for scenting cheap disinfectants and industrial soaps (Doran, 1991), and have been used as general solvents, as well as mineral flotation agents in mining operations.

1.3.5 Perfumery/flavouring oils

Perfumery and flavouring oils are dominated by the chemical compounds of citronellal, geranyl acetate, citral (neral and geranial), geraniol and E-methyl cinnamate. Very few species of eucalypt have ever been exploited to supply oils for these industries (Doran, 1991). Some uses include a component in soaps and perfumes, and as a lemon flavouring.

1.4 By-products from eucalyptus oil production

Apart from some minor chemical by-products and residues, the distillation process results in the generation of a large amount of spent leaf matter. Felton Grimwade and Bickford Pty Ltd sells spent leaves after the oil has been extracted as a mulch and ground cover (Abbott, 1989). It is claimed that the mulch has a natural appearance and fragrance, is weed and insect free, suppresses weed growth, allows good drainage, aeration, and water penetration, and after 2-3 years it breaks down into an organic humus. Allan Coates (pers. comm.,1994), from Uralla NSW, sold spent leaf material from *E. radiata* mixed with cow manure as a potting mix, often generating a greater income from it than from the sale of oil. G. R. Davis Pty Ltd spread the spent leaves over recently harvested areas of *E. polybractea* with a dramatic beneficial effect on regrowth, presumably from improved moisture retention (J. Doran, pers. comm., 2001). *E. polybractea* leaf residues go to horse stables as bedding in some areas of Victoria (J. Doran, pers. comm., 2001). The spent leaf is also often used to fire the stills.

1.5 Oil gland morphology

Oil glands are small globular cavities filled with oil composed of odorous terpenes such as 1,8-cineole, phellandrene and piperitone, and are distributed more or less abundantly throughout the leaf parenchyma of most eucalypt species (Doran, 1991). Oil glands appear to originate from single cells within the epidermis or in the mesophyll layers of the developing leaf blade (Doran, 1991). They are also associated with the epidermis of the midrib, veins and margins of the leaf, and undergo a complex sequence of intracellular divisions to form new cells that play an essential role in the formation and development of the gland.

Carr and Carr (1970) carried out extensive work on the anatomy of *Eucalyptus* leaves, with a number of findings. Superficial oil glands (close to the leaf surface) originate from single epidermal cells of the developing leaf by a series of periclinal and anticlinal divisions, while internal oil glands, which may account for some or all of the oil glands present in secondary seedling and adult leaves, originate from mesophyll cells. Oil glands may also be formed from epidermal cells from the midribs or other veins as well as those of the margins of the leaf. The oil cavity of the gland is formed schizogenously (i.e. from an intercellular space) following localised thickening of the surrounding cell walls.

The exact place of the formation of essential oil is unsure (Doran, 1991), but Haagen-Smit (1949) suggested that it may be produced in the region of photosynthetic activity in the epithelial and membranous cells of the oil gland, and then passed through the cell wall to the inside of the gland. Timing of the formation and decline of the oil glands and essential oils are important processes in determining the oil concentration and the oil retention capacity of the various species. This becomes critical with regard to time of harvesting and other processes for optimum production in commercial operations.

It appears that there is little correlation between size and density of oil glands and oil concentration. Brooker (pers. comm. in Doran, 1991) stated that the correlation is poor, with several species that possess conspicuously large and numerous glands ranking poorly in terms of oil concentration.

1.6 Chemistry of eucalyptus oil

Eucalyptus oil is comprised of many volatile organic compounds, often involving a large number of separate chemical types, such as hydrocarbons, alcohols, aldehydes, ketones, acids and esters. They are terpenoid in nature, most commonly composed of monoterpenes and sesquiterpenes. Terpenes are biosynthesised from mevalonic acid formed from photosynthates, and are generally recognised as compounds containing two or more isoprene units (C_5H_8) joined together. Two isoprene units joined together form geranyl pyrophosphate which is a precursor to the monoterpenes (C_{10}). Three isoprene units joined head-to-tail form farnesyl pyrophosphate which produces the sesquiterpenes (C_{15}) (Erman, 1985).

The monoterpenes are divided into three major groups, acyclic (open chain), monocyclic (one ring), and bicyclic (two rings), while the sesquiterpenes are divided into four major groups, acyclic, monocyclic, bicyclic and tricyclic (Doran, 1991). These groups contain the specific chemical compounds that make up the major components of eucalyptus oil (Fig 1.1).

MONOTERPENES

acyclic	monocyclic	bicyclic
myrcene	*1,8-cineole*	*α-pinene*
geranial	*α-phellandrene*	*β-pinene*
nerol	*α-terpineol*	*camphene*
geranial	*piperitone*	*borneol*
neral	*d-limonene*	*sabinene*
linalool	*p-cymene*	*3-carene*
citronellal	*α-terpinene*	*trans-pinocarveol*
citronellol	*γ-terpinene*	

SESQUITERPENES

acyclic	monocyclic	bicyclic	tricyclic
nerolidol	*humulene*	*β-caryophyllene*	*aromadendrene*
farnesol		*α-eudesmol*	*globulol*
		β-eudesmol	*spathulenol*
		γ-eudesmol	
		bicyclogermacrene	

Figure 1.1 - A selection of terpenes commonly found in the leaf oil of eucalypts, grouped according to their type and chemical structure (*source: Boland et al., 1991*)

1.7 Sources of variation in tree growth and essential oil traits in *Eucalyptus* species

Baker and Smith (1920) carried out the first work regarding the chemistry and yield of essential oils in Australia. Much of this early work was indicative only, mainly due to the large area and number of species examined, and the fact that in most cases only one or two bulk samples of leaf were distilled for the analysis of any one species (Bryant, 1950). Since then, much work has been carried out in studying variation (McKern and Smith-White, 1948; Bryant, 1950; Fluck, 1963; Simmons and Parsons, 1987; Brooker *et al.*, 1988; Murtagh *et al.*, 1990; Slee, 1992; Doran and Bell, 1994; Milthorpe *et al.*, 1994; Grant, 1997; Doran *et al.*, 1998b).

The four major factors that can cause qualitative and quantitative variation in oil composition between and within species are genetics, type and age of leaf, environment, and techniques of extraction and analysis (Doran, 1991). Volatile oils are under genetic control, but are also influenced by non-genetic factors such as leaf age and seasonal effects (Simmons and Parsons, 1987). It is necessary to consider these factors when comparing leaf oil concentration and chemical composition of different species or individuals of the same species.

1.7.1 Variation in tree growth and essential oil traits between and within species due to genetic factors

Variation within species is generally quantitative in nature. In breeding programs for oil production in eucalypts, the main traits for selection are quantitative traits for which the variation is continuous, such as growth rate and leaf oil concentration. Thus the genetics of breeding eucalypts for oil falls predominantly in the realm of quantitative genetics. The number of different chemical forms present within some species of *Eucalyptus* is an example of extreme quantitative genetic variation (Doran, 2002).

The genetic components of continuous variation are additive, dominance and epistatic variation. Gene effects are said to be 'additive' when many genes each of small effect all influence the same trait; the more genes there are for that trait in an individual, the bigger the effect (Eldridge *et al.*, 1994). Additive genetic variance can be estimated from analysis of open-pollinated progeny in suitable field trials using the 'general combining ability' (GCA) of many individuals whose progeny are under test, that is, the average performance of each individual parent in crosses with several other trees (Eldridge *et al.*, 1994).

Bryant (1950) and McKern *et al.* (1953) reported marked variation both in leaf oil concentration and in oil chemistry not only between species but also within species. Recent studies of the oils of various *Eucalyptus* species using quantitative genetic methods have indicated moderate to strong genetic control of oil composition and concentration, with substantial levels of genetic variation (Doran, 2002).

Doran *et al.* (1998b) studied the variation in first-harvest oil production in *E. radiata* (cineole form). Tree growth and essential oil traits of 32 open-pollinated families of *E. radiata* from four different provenances in a provenance/progeny trial were assessed at first harvest (23 months). Percentage survival and number of stems varied significantly between provenances, while families within provenances varied significantly in survival, basal area per tree and leaf biomass per tree. There was also substantial evidence of variation both within and between provenances in oil concentration. The data from this trial did not allow estimation of genetic parameters for oil concentration due to the limited extent of data collected rendering it unsuitable for statistical analysis (Doran *et al.*, 1998b). Studies of other number of different chemical forms present within some species of *Eucalyptus* is an example of extreme quantitative genetic variation (Doran, 2002).

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Defining genetic variation is one of the first tasks in tree breeding (Eldridge *et al.*, 1994). Well-designed and replicated provenance and progeny trials are an essential part of the study into variation and genetic heritability in oil traits, and in selecting superior oil producing trees. Estimating genetic parameters such as heritability and genetic correlations for tree growth and essential oil traits is discussed in detail in Sections 1.9 to 1.11.

1.7.2 Variation in tree growth and essential oil traits between and within species due to the type and age of leaf

Typically, eucalypts develop up to five distinct morphological types of leaf during their lifetime, each type corresponding to different ontogenetic stages of development. The types are: (1) the cotyledons, (2) the seedling leaves (for about 5-10 leaf pairs above the cotyledon), (3) the juvenile leaves (often persisting for a number of years), (4) the intermediate leaves, and (5) the adult leaves (Penfold and Willis, 1961).

Leaf age is a separate classification to leaf type, and is generally categorised as: (1) young leaf (mean age of about one month or less - the growing tips or flush growth of the tree), (2) mature leaf (mean age of about 6 months), and (3) aged leaf (about 12-18 months old) (Penfold and Willis, 1961).

Any of the three age groups can, therefore, exist in any one of the three ontogenetic stages - juvenile, intermediate, and adult (Doran, 1991). Juvenile leaf on coppice growth may often be aged and indeed such leaf usually stays on the tree much longer than the adult-type leaves on trees in virgin stands (Bryant, 1950; Doran, 1991).

The differences in oil composition between young and mature leaves are often marked, but there appears to be a high level of variability in the direction and extent of these changes, not only between species but between individuals in the same population (Doran, 1991). Bryant (1950) also stated that juvenile and adult foliage differ in oil concentration and oil constituents. Bryant (1950) described trials carried out by Smith-White, comparing oil concentration in relation to leaf type between *E. dives* and *E. radiata*. It was found that there is generally a higher concentration of oil in young leaf than older leaves in *E. radiata* and *E. dives*. However, similar trials carried out on *E. smithii* indicated no difference in that species.

Coppen (1992) studied *E. smithii* in Swaziland and reported that both oil concentration and cineole content were highly dependent on the type of leaf sampled (juvenile *vs* adult) and the sampling position within the crown. However, Brooker *et al.* (1988) in their study of *E. kochii* and *E. plenissima* and Doran (1992) in his study of *E. camaldulensis* reported that there is no change in oil concentration in leaves from different positions in the crown. These findings raise important points with regard to sampling methods for the determination of oil concentration and composition.

1.7.3 Variation in tree growth and essential oil traits between and within species due to environmental factors

A number of environmental factors can affect the quantity and chemical characteristics of essential oils (Bryant, 1950). Studies into the effect of climate, topography, soil type, and other factors on tree survival and growth are extensive. Much less work has been done on the effects upon essential oil production. The main areas of work have been studies in seasonal and diurnal variation. There is general agreement that the yield and concentration of constituents of essential oils changes during the course of the year or the growing season (Fluck, 1963). Conflicting results have been found for diurnal variation (Fluck, 1963).

Williams and Home (1988) in their studies of *Melaleuca alternifolia* determined that there is significant fluctuation in seasonal leaf oil concentration. There was a notable annual cycle of oil accumulation with the level of oil increasing from September-October to November-January, and declining from May to August. Fluctuations in the composition of the major components were minor and not related to the seasonal change in leaf oil concentration. Murtagh (1999) also studied seasonal and diurnal variation in *M. alternifolia.* He reported that oil concentration varied according to season, being highest in summer, and that the greatest variation between seasons occurred in the cooler locations. Additional short-term variation occurred within the seasonal trend where oil concentration increased with increasing temperature and humidity. This was more marked in plants with a high oil concentration (Murtagh, 1999). Brooker *et al.* (1988) demonstrated that eucalypts also tend to have varying leaf oil concentrations over a season. Leaf oil concentrations in *E. kochii* and *E. plenissima* were generally greater around January-February and were at a minimum during August. The results of Berry (1947) indicated that leaf oil concentration in *E. cneorifolia* peaks in December, fluctuates through autumn, and reaches its lowest concentration in August. Milthorpe *et al.* (1994) also observed cyclic patterns in leaf oil concentration in *E. polybractea* with an April peak and August low.

1.8 Heritability of tree growth and essential oil traits between and within species

Heritability is a measure of how strongly a trait is influenced by genetics and conversely, how much by the environment (Hanson, 1963). It is the statistical expression for the relative contributions of genotype and environment to phenotype. Heritability can be calculated on a family, within family, individual tree or clonal basis (Cotterill and Dean, 1990). Individual (or narrow-sense) heritability is relevant to estimating gains from selecting individual trees, and is the type of heritability considered in this study. It is calculated as the ratio of additive genetic variance to the total phenotypic variance. Phenotypic variance is the sum of additive variance, non-additive variance and environmental variance (Falconer, 1989).

Individual heritability (h^2) expresses the proportion of the total variance of measured values among trees that is due to additive effects (or the genes that the trees are carrying) (Cotterill and Dean, 1990). Additive genetic variance is associated with average gene effects that parents pass on to their progeny resulting from sexual reproduction. When two superior trees are mated, their progeny are not identical. The average performance of the progeny is the additive genetic effect, and individual deviations from the family (progeny) mean can be due to non-additive genetic causes (Cotterill and Dean, 1990). Individual heritability of $h^2 = 0.20$, for example, indicates that for the particular population, 20% of the total phenotypic variance among trees is due to additive genetic effects. Where heritability is high, gain from selection will be high (Eldridge *et al.*, 1994). Heritability of $h^2 = 0.1 - 0.2$ is considered low, while 0.3 - 0.5 is high (Eldridge *et al.*, 1994). Cotterill and Dean (1990) defined low heritability as $h^2 < 0.1$, intermediate heritability as $0.1 \leq h^2 \leq 0.3$ and high heritability as $h^2 > 0.3$.

There is a substantial amount of data published on the heritability of growth traits relevant to wood production for a number of eucalypt species, but there appears to be only limited

There is a substantial amount of data published on the heritability of growth traits relevant to wood production for a number of eucalypt species, but there appears to be only limited published data on the heritability of essential oil traits in eucalypts. Published estimates of narrow sense heritability for a number of growth traits for six commercial timber species are shown in Table 1.8 (Eldridge *et al.*, 1994).

Brooker (1989) found that high oil-yielding parent trees produce, on average, high yielding progeny, that is oil production is strongly heritable. Barton *et al.* (1991) examined the heritability of 1,8-cineole content in *E. kochii*, indicating that (at that time) there appeared to be no other published estimates of heritability of 1,8-cineole content for any species of *Eucalyptus*. Doran and Matheson (1994) investigated *E. camaldulensis* and found that 1,8-cineole content and total monoterpene content (as an indication of oil concentration) were highly heritable traits. Grant (1997) reported high heritability of oil concentration and 1,8-cineole content in *E. polybractea*. Doran (2002) commented that much work remains to be done before reliable estimates of the heritability of oil traits are available for the major eucalyptus oil-producing species. He noted, however, that current *Eucalyptus* oil trait heritabilities are in line with those reported in Australian tea tree (*Melaleuca alternifolia*) (in a closely related genus). For that species, Butcher *et al.* (1996) reported a high heritability of oil concentration and 1,8-cineole content, and Doran *et al.* (1997) reported a high heritability for oil concentration. There appear to be no published data on the heritability of either growth or essential oil traits of *E. radiata* subsp. *radiata*.

Table 1.3 - Published estimates of narrow sense heritability (h^2) from control-pollinated (C) and open-pollinated (O) progeny tests of six eucalypt species (*source: Eldridge et al., 1994*)

Trait	Species	Pollination	Heritability (h^2)	Age (yrs)	Families	Source*
Volume per tree	*E. globulus*	O	0.19	6	45	1
	E. grandis	C	0.10	1	51	2
		C	0.53	8	71	3
		O	0.31	5	529	4
	E. regnans	C	0.18	4	21	5
		O	0.45	4	13	5
Height	*E. globulus*	O	0.12	6	45	1
		O	0.29	4	36	11
	E. grandis	C	0.11	1	51	2
		C	0.45	8	18	10
		O	0.39	5	529	4
	E. nitens	O	0.07	2	45	6
	E. regnans	O	0.13	4	16	7
		O	0.23	6	14	7
		C	0.18	4	21	5
		O	0.43	4	13	5
	E. teriticornis	O	0.16	4	15	8
Diameter	*E. globulus*	O	0.24	6	45	1
	E. grandis	C	0.30	8	18	10
		O	0.39	5	529	4
	E. obliqua	O	0.57	13	216	9
	E. regnans	O	0.10	10	64	7
		C	0.19	4	21	5
		O	0.46	4	13	5
	E. teriticornis	O	0.11	4	15	8
Sectional area	*E. globulus*	O	0.13	18	36	11
Crown form	*E. globulus*	O	0.29	6	45	1
	E. grandis	C	0.33	8	71	3
	E. regnans	O	0.15	7	64	7
Stem form	*E. globulus*	O	0.22	6	45	1
	E. grandis	C	0.46	8	71	3
	E. nitens	O	0.07	3	38	6
Wood density	*E. grandis*	C	0.45	8	18	10
Fibre length	*E. grandis*	C	0.54	8	18	10

* 1 - Volker *et al.* (1990), 2 - Van Wyk (1976), 3 - Van Wyk (1990), 4 - Reddy and Rockwood (1989), 5 - Griffin and Cotterill (1988), 6 - Purnell (1986), 7 - Eldridge (1972), 8 - Kedharnath and Vakshasya (1978), 9 - Matheson *et al.* (1986), 10 - Malan (1988), 11 - Borralho *et al.* (1992)

1.9 Genetic and phenotypic correlation of tree growth and essential oil traits

Put simply, phenotype is what a particular tree looks like (resulting from genetic and environment interaction) and genotype is the composition of genes (or hereditary constitution) of that tree. Phenotypic correlation is the correlation between measured values for two traits such as height and straightness for each of several trees in a population (Cotterill and Dean, 1990; Eldridge *et al.*, 1994). In the case of essential oil production, traits of interest might include the number of stems and leaf biomass, or tree basal area and oil yield. Total oil production from a harvesting operation is the product of leaf biomass and oil yield (or concentration). Leaf biomass of an individual tree is typically highly correlated with stem diameter and height (Doran *et al.*, in press). Doran *et al.* (1998b) confirms a strong association between tree diameter and leaf biomass in *E. radiata* subsp. *radiata*. Phenotypic correlation is calculated as the covariance of the measured values divided by the product of the standard deviations of the measured values for each trait (Cotterill and Dean, 1990).

Genetic correlation is an expression of correlated response to selection, that is, the effect on one trait if the other trait is selected for improvement. 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). Cotterill and Dean (1990) described genetic correlation as representing the correlation between breeding values for different traits and it is thought to be caused mainly by genes influencing more than one trait. Falconer (1989) referred to this feature as pleiotropy. Genetic correlation is much more important than phenotypic correlation to the breeder as it is the correlation of breeding values (Falconer, 1989). Breeding value is the value of an individual judged by the mean value of its progeny (Eldridge *et al.*, 1994). Genetic correlations are based on estimates of genetic covariance between traits from a progeny test (Eldridge *et al.*, 1994), and determined as the correlation of the breeding values of two traits expressing the extent to which these traits are influenced by the same genes (Falconer, 1989). The estimate of genetic correlation is calculated as the ratio of the additive genetic covariance of the measured traits over the square root of the additive genetic variance for each trait (Cotterill and Dean, 1990).

Williams and Matheson (1994) commented that genetic correlation is mainly used for four different purposes:

(1) to help predict response to selection carried out in young trees (rather than actually waiting until harvest to estimate heritabilities for the traits under improvement);
(2) to predict response in a trait which is hard to measure from one which is easy to measure;
(3) to predict response to selection at one site when selecting at another; and
(4) to maximise gain in specific traits selected at the same time, through use of selection indices constructed using genetic correlations and heritabilities.

Genetic correlations influence the way in which breeding programs are structured. When there are strongly negative genetic correlations between two traits, it is difficult to breed for them both in one breeding population and it may be necessary to divide the population. Strong positive genetic correlations, however, are useful in reducing the number of traits for selection, as selection for one of the traits will be effective in improving the other trait (Eldridge *et al.*, 1994).

There is a substantial literature regarding phenotypic and genetic correlation between growth traits in trees grown for wood production (Shelbourne *et al.*, 1972; Wilcox *et al.*, 1975;

Cotterill and Zed, 1980; Shelbourne and Low, 1980; Burdon and Namkoong, 1983; Dean *et al.*, 1983; Cotterill and Dean, 1990; Volker *et al.*, 1990; Eldridge *et* a*l.* 1994). Table 1.9 and Table 1.10 present published data on phenotypic and genetic correlations of a number of growth traits for *Pinus radiata* in Australia (Cotterill and Dean, 1990) and *E. globulus* (Volker *et al.*, 1990). However, there is only limited published data on phenotypic and genetic correlations relevant to essential oil traits such as leaf oil concentration and chemical composition in eucalypts.

Table 1.4 - Estimates of genetic correlations (above the diagonal) and phenotypic correlations (below the diagonal) for *Pinus radiata* in Australia (*source: Cotterill and Dean, 1990*)

Trait	Height	Diameter	Stem Straightness	Branch Quality	Wood Density	Source*
Height		0.72	0.29	0.01		1
		0.87	0.74	0.23	-0.31	2
Diameter	0.71		0.48	-0.28		1
	0.56		0.35	-0.24	-0.45	2
Stem Straightness	0.25	0.19		0.11		1
	0.12	0.01		0.59	-0.03	2
Branch Quality	-0.27	-0.49	-0.01			1
	-0.16	-0.12	0.4		0.01	2
Wood Density	0.1	0	-0.18	0.05		2

* 1 - Cotterill and Zed (1980); 2 – Dean *et al.* (1983)

Table 1.5 - Estimates of genetic correlations (above the diagonal) and phenotypic correlations (below the diagonal) for 45 open-pollinated families of *E. globulus* in a progeny test at age 6 years (*source: Volker et al., 1990*)

Trait	Height	Diameter	Volume	Stem form	Branch size
Height		0.55	0.70	0.43	-0.38
Diameter	0.66		0.98	0.07	-0.45
Volume	0.75	0.95		0.13	-0.28
Stem form	0.09	-0.03	0.0		0.76
Branch size	-0.04	-0.38	-0.33	0.21	

Grant (1997) estimated genetic correlations between oil traits and leaf biomass production in *E. polybractea*. He found a strong genetic correlation between oil concentration (w/w DW%) and 1,8-cineole content (%) and negative genetic correlations between both oil concentration and dry leaf weight, and 1,8-cineole content and dry leaf weight. Doran and Matheson (1994) reported small genetic correlations between growth traits and 1,8-cineole content, and moderately negative genetic correlations between growth traits and total yield of monoterpenes (as a measure of leaf oil concentration) in *E. camaldulensis*. Butcher *et al.* (1996) reported a negative genetic correlation between leaf oil concentration and plant dry weight in *Melaleuca alternifolia.* Later, more extensive studies of this species found neither negative nor positive genetic correlations between these traits (Doran *et al.*, 1997). Chen (1997) studied genetic variation in growth and oil traits (including leaf biomass, hedge height and crown width, yield of various oil components and leaf oil concentration) in *Backhousia citriodora* (lemon myrtle) and found a strong positive genetic correlation between most oil and growth traits. There appears to be no published data on phenotypic or genetic correlations of growth and oil traits of *E. radiata* subsp. *radiata.*

1.10 Gain in growth and essential oil traits through selection and breeding

Gain is a measure of the response to selection. The improvement of a desired trait such as total oil production from a plantation as a result of selecting high-yielding individuals is the ultimate goal of the selection and breeding process. Genetic parameters have fundamental roles in practical tree breeding which centre around predicting genetic gains from selection. Breeders need to know what influence selection on one trait can be expected to have on both the trait itself and on other correlated traits (Cotterill and Dean, 1990). Genetic parameters are needed to predict the influence that increasing or decreasing intensities of selection are likely to have on genetic gains for a range of traits (Cotterill and Dean, 1990).

The simplest expression of genetic gain through selection is the product of the intensity of selection, the phenotypic standard deviation of the population and an estimate of the narrow sense heritability (Eldridge *et al.,* 1994). The intensity of selection is expressed in units of standard deviation and is obtained from standard tables such as in Becker (1984) and Cotterill and Dean (1990). It is a function of the number of individuals selected for superior traits from a population of a specific size, such as one in ten. The estimate for genetic gain expressed above only applies to mass selection (i.e. selection among individual trees) without reference to pedigree (i.e. selection within and between families). Gain is estimated in this study using this method.

There are a number of different approaches to predicting gain. Shelbourne (1991) predicted genetic gains from between- and within-family selection under a number of different breeding population options and plant production population options. The breeding populations included open-pollinated, controlled-pollinated (full sib) and cloned open-pollinated options. The production populations included four different open-pollinated seedling orchards and six clonal orchards, as well as two schemes of selecting clones for clonal forestry. This was a comprehensive study, and the methods of estimating gain under the different options involved detailed breeding plan development and estimate calculation. Shelbourne (1991) noted that in terms of achieving genetic gain for a minimal investment, phenotypic selection or mass recurrent selection is a good option, but there is a complete lack of pedigree control which could eventually lead such populations to accelerated relatedness and inbreeding.

There are few data published on gain in tree growth and essential oil traits specifically for eucalyptus oil production. Grant (1997) reported significant potential gains in both 1,8-cineole and leaf oil concentration in *E. polybractea* from the first generation as a result of selection and breeding. Similarly, Barton *et al.* (1991) predicted a significant gain in 1,8-cineole production (i.e. leaf oil concentration) from *E. kochii.* Doran and Matheson (1994) also found that significant gains in 1,8-cineole and total monoterpene concentration could be achieved in selecting and breeding of *E. camaldulensis*. There are no published data on the potential gains in key oil production traits for *E. radiata* subsp. *radiata.*

1.11 Breeding strategies for improving efficiencies in *Eucalyptus* oil production

In the past, tree breeders often chose their strategies for breeding and plant production solely on intuition (Shelbourne, 1991). Quantitative estimates of gain from different procedures and also cost estimates are necessary in planning a modern breeding program (Shelbourne *et al.*, 1989). Information on heritabilities of production traits in forestry, and the genetic and phenotypic correlations between these traits, is fundamental to efficient tree breeding (Cotterill and Dean, 1990).

Tree improvement programs aim to develop superior plantations in one or several key economic traits (Doran *et al.*, 1998b). The main activity in improvement of eucalypt breeding populations is recurrent selection for general combining ability, seeking to increase the frequency of favourable genes in populations to improve traits of economic value (Eldridge *et al.*, 1994). Breeding programs usually begin with selections from a starting or base population. Selections are made and crossed, and their progeny tested in a progeny test. Selections are made in the progeny test and the process continues. Progress depends on several matters (Williams and Matheson, 1994):

(1) how variable the trees are in the population from which parents were selected (phenotypic standard deviation, σ_p).

(2) how intensively selection is carried out (selection intensity, i); this is the difference between the mean of the selected individuals and the overall mean, divided by phenotypic standard deviation (σ_p); and

(3) what proportion of the phenotypic variance is due to genetic variation (i.e. heritability, h^2).

Parents chosen to cross are based on what they look like (their phenotype), hoping that this reflects the genes they carry (their genotype). The closer these two are, the higher the heritability (Williams and Matheson, 1994).

There is now ample evidence that faster growth and greater oil yields of eucalypts in plantations can be obtained through selection of provenances and genetic improvement (Eldridge *et al.*, 1994). As such, the primary tree breeding objective for the eucalyptus oil producer is to maximise the yield of high quality oil per unit area, thereby increasing productivity and economic return.

Eldridge *et al.* (1994) described the key components to tree breeding as the breeding strategy and the breeding plan. The strategy is the overall concept on how to go about breeding. It is a conceptual plan of genetic improvement of a population including ideas on possible selection, mating and mass propagation methodologies of superior individuals. The breeding plan includes a set of objectives and a flow diagram of what is to be done each month for several years ahead. As an ongoing and recurrent process, a breeding strategy accumulates benefits over successive generations through a cycle of testing, selection and mating. Eldridge *et al.* (1994) described that every effective breeding strategy involves the maintenance of a hierarchy of three major types of population which can continue to meet the demand for genetically improved planting stock for the production population. These populations are: the base population; the breeding population; and the propagation population. The base population is the genetic resource population consisting of a large number of trees of a particular species in a native forest or a plantation in which selection can be carried out. The breeding population is the selected trees and their progeny (as many as 300-400 families of 100 trees in each) in a series of progeny trials and clonal archives in which the breeding cycle of selection and mating will be repeated over many generations. This is the tree breeder's main area of work. The propagation population is the intensively selected trees (commonly fewer than 100 trees) established in seed orchards or cuttings in clonal hedges where combinations of genes selected in the breeding populations are mass-produced as genetically improved planting stock. The production population is the commercial plantation established using the improved germplasm. Figure 1.5 illustrates that a breeding strategy accumulates benefits over successive generations through a cycle of testing, selection and mating (Doran *et al.,* 1998a).

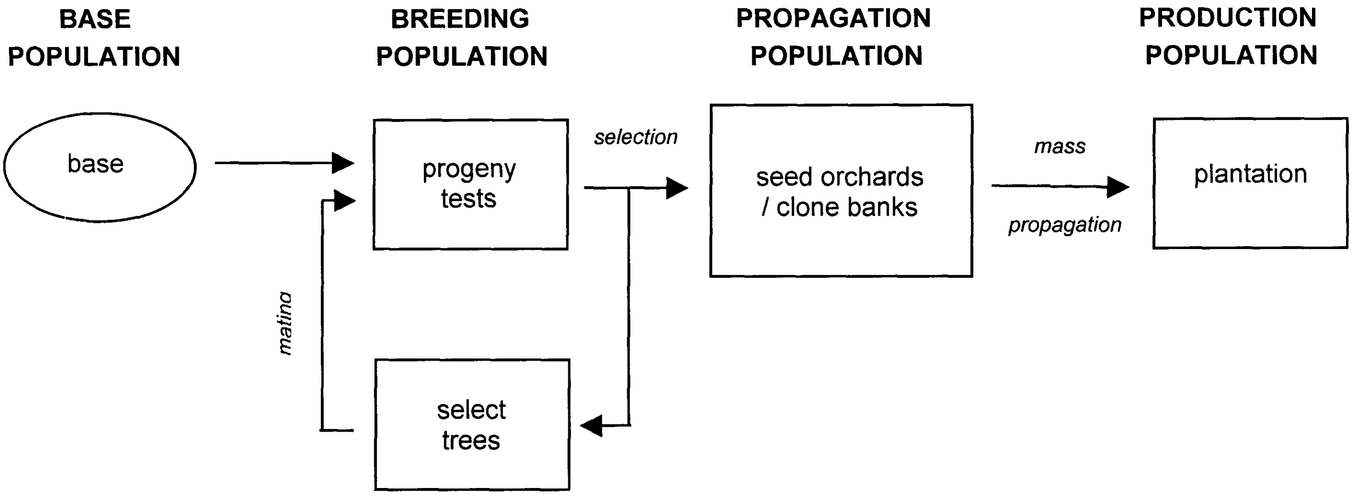

Figure 1.2 - Simplified diagram of the activity cycle for breeding and the hierarchy of four populations (*source Doran et al., 1998a*)

Doran *et al.* (1998a) designed a simple and flexible breeding strategy for *Melaleuca cajuputi* subsp. *cajuputi* in Java, Indonesia (Figure 1.6). The strategy is relatively simple and cheap to apply (once the complexities and skills required in establishment and assessment of tests and data analysis have been addressed), because it is based on open pollination. The breeding plan resulting from this strategy would involve a detailed month-by-month planning program based on the key components of the strategy.

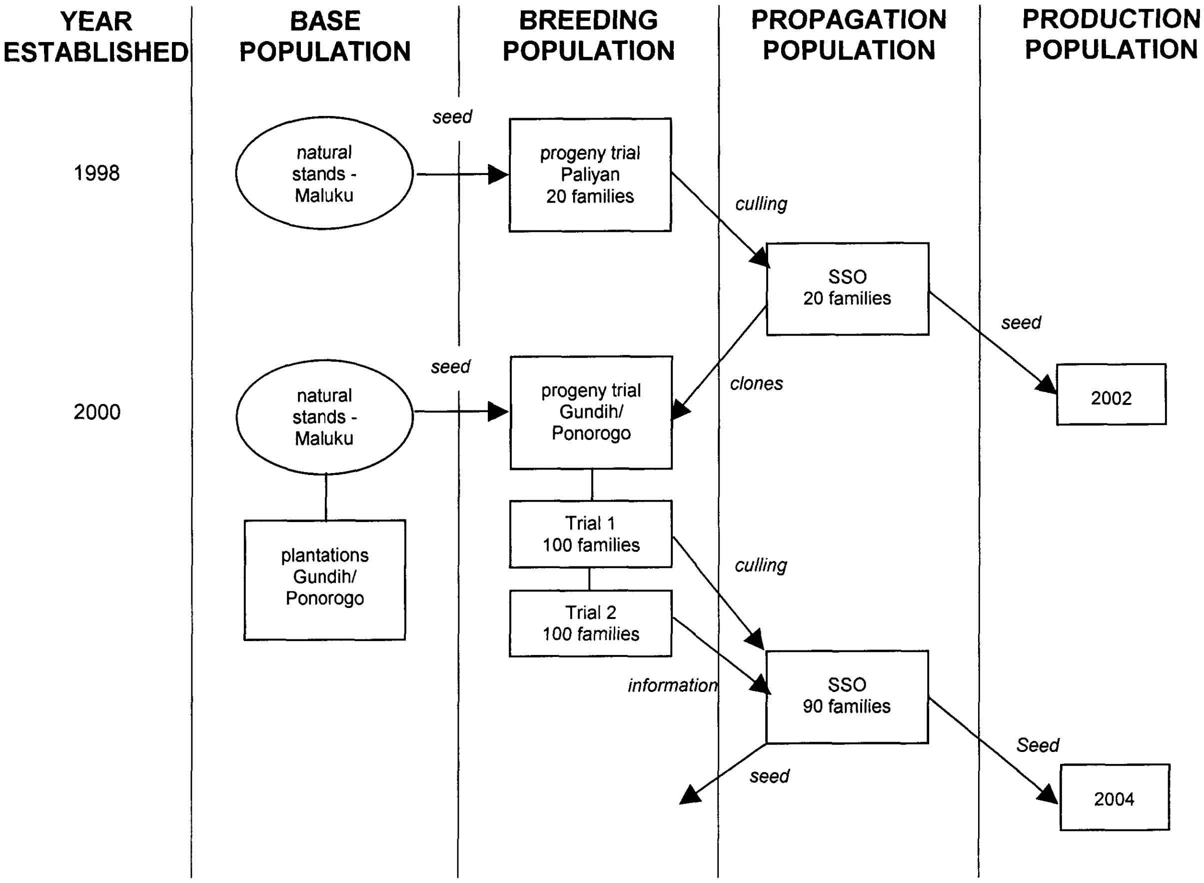

Figure 1.3 - Diagrammatic representation of proposed breeding strategy for improving oil yields in *Melaleuca cajuputi* subsp. *cajuputi* in Indonesia. SSO is seedling seed orchard (*source: Doran et. al., 1998a*)

1.12 *Eucalyptus radiata* as an essential oil producing species

1.12.1 General tree description, distribution and native growth habits of *E. radiata*

Eucalyptus radiata subsp. *radiata* is one of the more important sources of cineole-rich eucalyptus oil (Boland *et al.*, 1991). In its native habitat, *E. radiata* varies in size from a bushy tree 10-15 m tall, to a medium-sized to tall tree of moderately good form, 20-30 m tall and up to 1 m in diameter at breast height (Boland *et al.,* 1984).

The species is endemic to the Northern Tablelands and south east of New South Wales and eastern Victoria (Figure 1.4) (Boland *et al.*, 1984). Brooker and Kleinig (1999) have recorded it in limited areas of Tasmania. There are two chemical forms of the species: 1,8-cineole and α-phellandrene. The phellandrene form of *E. radiata* has been harvested primarily around Braidwood, Captains Flat and Mongarlowe, NSW while the cineole form has been worked near the Tumut, Batlow and Tumbarumba area, and around Bega, Nerrigundah, Yowrie, and Quaama in the south-east of NSW (Boland *et al.*, 1991). Harvest of the 1,8-cineole form has also be carried out from plantations near Armidale on the Northern Tablelands of New South Wales and near Bega in south-eastern NSW (pers. obs.). Table 1.6 outlines some of the main geophysical features determining the distribution of *E. radiata.*

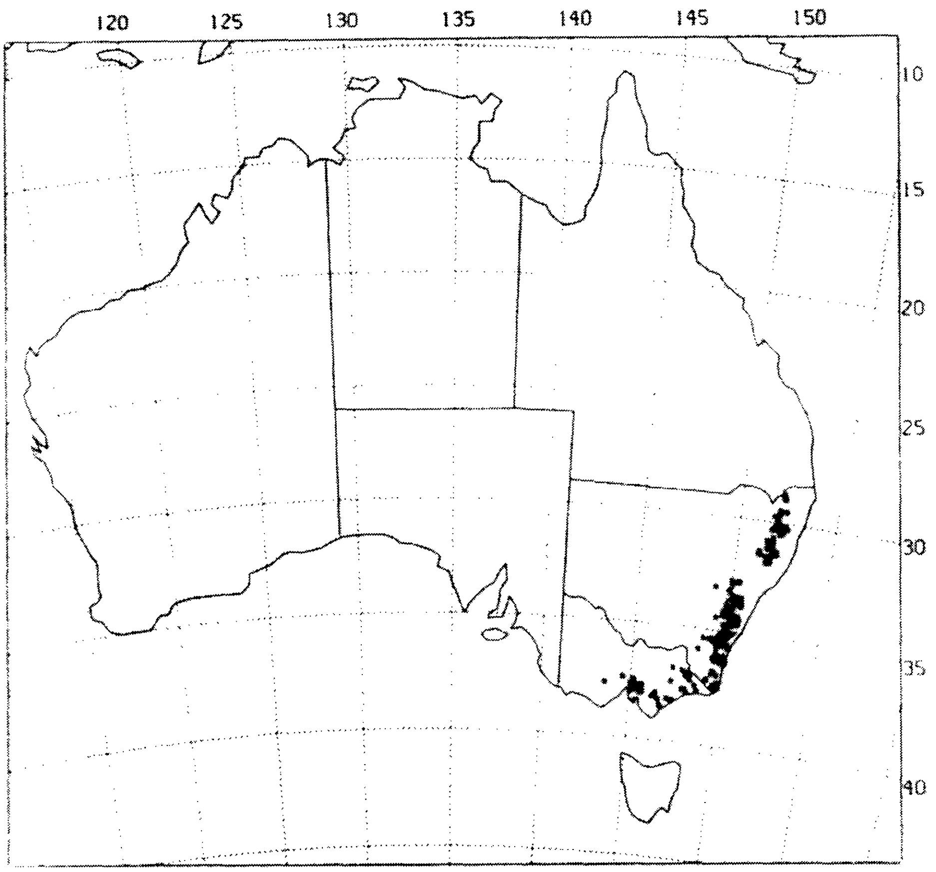

Figure 1.4 – Natural distribution of *E. radiata* in Australia (*source: Boland et al., 1984*)

Table 1.6 - Geophysical features commonly associated with the mainland occurrence of *E. radiata* (*source: Johnson and Hill, 1990*)

Geophysical Variable	**Attributes Pertinent To *E. radiata***
latitudinal range	28 - 39 °S
altitudinal range	50 - 1200 m asl
frost frequency	few per yr at low altitude, up to 70 per yr at higher altitude
mean annual rainfall	650 - 1100 mm
mean max temp	24 – 27 °C
mean min temp	-1 – 5 °C
soil types	wide range: sand, skeletal, volcanic loam

1.12.2 Botanical description of *E. radiata*

Boland *et al.* (1984) provided the following botanical description of the species:

Bark. Persistent on the trunk and larger branches, peppermint type, finely sub-fibrous with shallow longitudinal fissures, grey or brownish; upper branches smooth, greyish [Fig 1.5.1].

Leaves. Seedling - opposite, sessile, broad-lanceolate to lanceolate, 5.5-10 x 1.4-2.8 cm, green, discolorous [Fig 1.5.3]. Juvenile - opposite, sessile, broad-lanceolate to narrow-lanceolate, 9-18 x 1.5-3.5 cm, green, discolorous. Stems at seedling and juvenile stages have many raised oil glands [Fig 1.5.4]. Intermediate - alternate, petiolate, lanceolate to narrow-lanceolate, 13-18 x 1.5-2.5 cm, green, concolorous [Fig 1.5.8]. Adult - alternate, petiolate, narrow-lanceolate to almost linear, 7-15 x 0.7-1.5 cm, green, concolorous, thin [Fig 1.5.5].

Inflorescences. Simple, axillary, 11- to more than 20-flowered; peduncles more or less terete, 0.2 to 0.8 cm long; pedicels 0.1 to 0.5 cm long, buds clavate, 0.3-0.5 x 0.2-0.3 cm [Fig 1.5.6]; opercula low conical or hemispherical-apiculate. Flowers October to January.

Fruits. Pedicellate, more or less hemispherical to globular-truncate, 0.3-0.7 x 0.4-0.7 cm; disc relatively broad, more or less level; valves 3-5, generally enclosed or to near rim level, sometimes slightly exserted [Fig 1.5.9].

Distinctive features. A medium sized tree with fine, peppermint-type bark to large limbs; juvenile leaves opposite and sessile for many pairs; adult leaves narrow-lanceolate to linear with strong peppermint smell when crushed; small many-flowered inflorescences (11 to more than 20 buds per umbel).

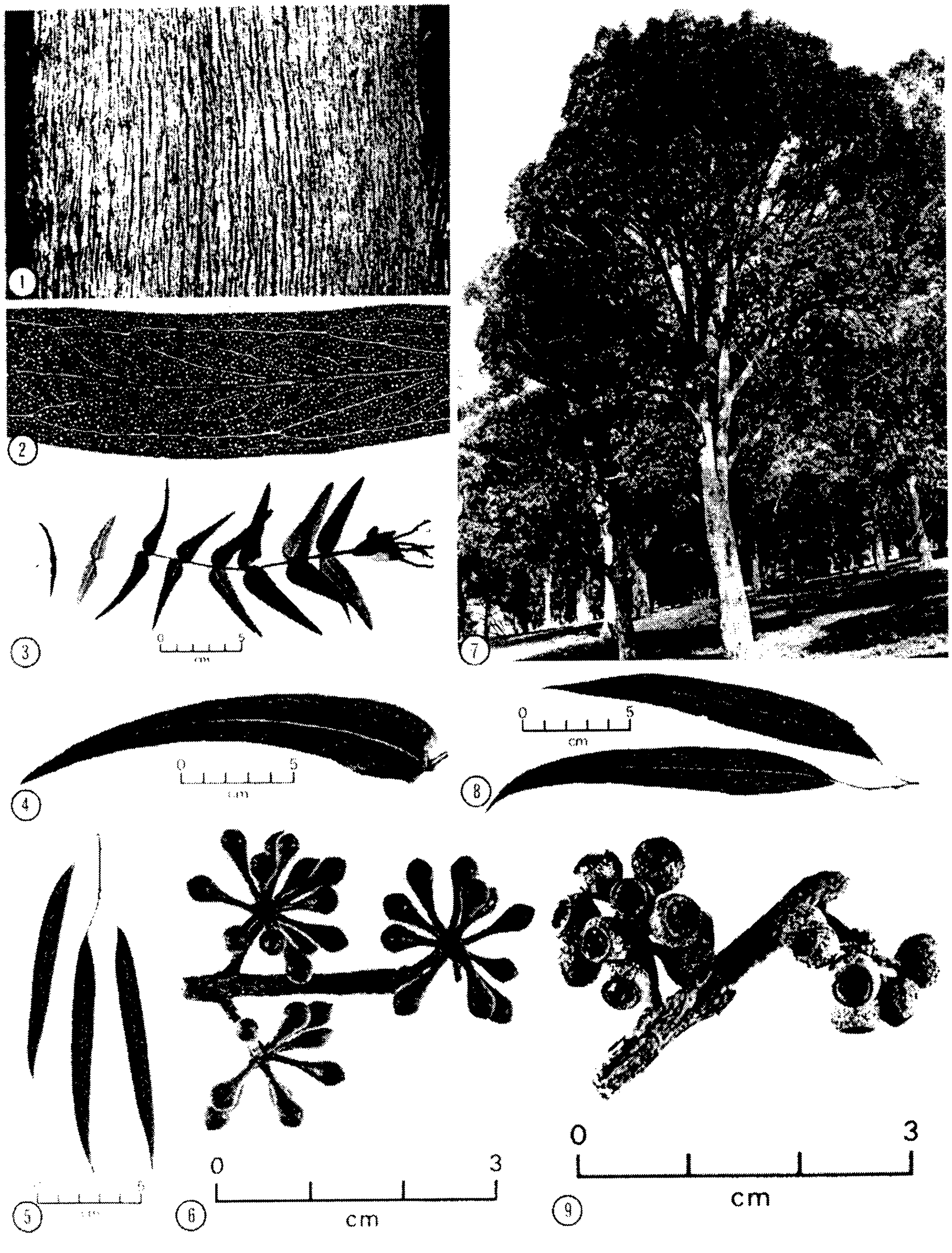

Figure 1.5 - Botanical features of *E. radiata* subsp. *radiata* (*source: Boland et. al., 1984*)

1.12.3 Taxonomy and chemical forms of *E. radiata*

Lassak (1988) reported that the unsettled and incomplete state of eucalypt taxonomy was one of the greatest problems that the early eucalyptus oil industry faced, resulting in its inability to produce and market oils of consistent quality. Narrow-leaved peppermint is a notable example of a species that has been subject to a multitude of nomenclatural descriptions in the past. Table 1.7 summarises Johnstone (1984)'s commentary on the turbulent nomenclatural history of the species.

Table 1.7 - Summary of Johnstone (1984)'s commentary on the nomenclatural history of narrow-leaved peppermint

Author	Description
Sieber (1828)	Designated narrow-leaved peppermint *Eucalyptus radiata*
Bentham (1866)	Amalgamated a number of taxa of peppermints (incl. *E. radiata*) into a single species designated *E. amygdalina* Labill., including several varieties, one being *E. amygdalina* Labill. var. *radiata* (Sieb.) Bentham (given to be synonymous with *E. radiata* Sieb.).
Wools (1880)	Described Benthams *E. amygdalina* Labill. var. *radiata* (Sieb) Benth. as "whitegum ..." indicating that it was not *E. radiata* (Sieb.) *sensu stricto* but more likely *E. elata* Dehnh. Considered his description of *E. radiata* should be distinct from *E. amygdalina* of the Mittagong Ranges, indicating his *E. amygdalina* is the narrow-leaved peppermint described as *E. radiata* by Sieber. *E radiata* Sieb. (in the sense of Wools) is incorrectly applied and actually refers to *E. elata* Dehnh.
von Mueller (1880)	Broadly combined many taxa as *E. amygdalina*. This was a very broad definition even claiming *E dives* as an aberrant form of *E. amygdalina.*
Maiden (1889)	Adopted von Mueller's broad form of *E. amygdalina*.
Baker and Smith (1902)	Followed Wools' 1880 nomenclature of *E. amygdalina* as the narrow-leaved peppermint and *E. radiata* as the river whitegum. Differentiated the Tasmanian and mainland forms of *E. amygdalina*, determined on the basis of essential oil composition, calling the mainland form *E. australiana* Baker and Smith. Therefore Baker and Smith's *E. radiata* refers to *E. elata* Dehnh. and their *E. australiana* refers to *E. radiata* Sieb. in its correct sense.

Maiden (1917)	Gave the name *E. numerosa* to the river whitegum and confirmed *E. radiata* as the correct name for the mainland form of *E. amygdalina*. This made Baker and Smith's use of *E. radiata* synonymous with *E. numerosa* Maiden, and their *E. australiana* a synonym of the older name *E. radiata* Sieb. Argued against the proposal of Baker and Smith in 1915 to give species status to *E. australiana* on economic grounds.
Baker and Smith (1920)	Included a new variety of *E. australiana*, viz *E. australiana* var. *latifolia* Baker and Smith (a southern form of the species), and a new peppermint species *E. phellandra* Baker and Smith (a mainland peppermint separated from *E. amygdalina*). This raised the point that they had previously assigned "mainland *E. amygdalina*" to *E. australiana* and were taxonomically splitting off more mainland *E. amygdalina*. They regarded the morphological similarity between *E. amygdalina*, *E. australiana* and *E. phellandra* as the reason these taxa were considered as one species. *E. phellandra*, like *E. australiana*, was separated from *E. amygdalina* on its essential oil components. The economic importance of the different oil compositions necessitated their separation.
Penfold and Morrison (1932, 1935, 1937, 1940)	Followed Baker and Smith (1920).
Blakely (1927)	Added another species of narrow-leaved peppermint – *E. robertsonii* Blakely (a rough barked form of the river whitegum (*E. numerosa* Maiden, syn. *E. elata* Dehnh.)), separated from *E. radiata* Sieb. (*sensu* Maiden) on morphological grounds. Blakely described the synonymy of *E. robertsonii* as including *E. amygdalina* of many authors, but not of Labill., *E numerosa* Maiden (partim), *E. australiana* Baker and Smith (partim) and *E. phellandra* Baker and Smith (partim).
Blakely (1934)	Designated three mainland narrow-leaved peppermints: *E. lindleyana* DC. (syn. *E. numerosa* Maiden, *E. elata* Dehnh.), *E. robertsonii* Blakely and *E. radiata* Sieb. His *E. radiata* Sieb. had three varieties; var. *australiana* (Baker and Smith) Blakely, var. *subexerta* Blakely, var. *subplatyphylla* Blakely and McKie.
Penfold and Willis (1961)	Followed Blakely.
Johnson and Marryatt (1965)	Revised Blakely's classification absorbing *E. radiata* var. *subexerta* Blakely into the species removing the varietal epithet.
Pryor and Johnson (1971)	Proposed the establishment of two subspecies of *E. radiata*; subsp. *radiata* and subsp. *robertsonii*. They also suggested that *E. radiata* var. *australiana*, var. *subexerta* and var. *subplatyphylla* were chemical, local, and local variants, respectively, and absorbed them into the new subsp. *radiata.* Baker and Smith's *E. phellandra* is also fully absorbed into this subspecies.
Johnson and Blaxell (1973)	Carried out the necessary nomenclatural changes proposed by Pryor and Johnson (1971). Now the narrow-leaved peppermints of the mountain ranges of mainland Australia belonged to one species (*E. radiata* Sieb. Ex DC.) which has two subsp.: subsp. *radiata,* and subsp. *robertsonii* (Blakely) Johnson and Blaxell.
Chippendale (1976)	Based on that of Pryor and Johnson 1971 - no changes made to the peppermints.

Johnstone (1984) identified three distinct populations of *E. radiata* in three major geographical regions: southern Victoria, northern Victoria and southern and central New South Wales, and the New England Tablelands. Based on the existence of significant isolation between the three major forms, Johnstone (1984) considered them to be three distinct species proposing the names *E. polymorpha*, *E. radiata* and *E. secendens* (southern Victoria; northern Victoria and southern and central New South Wales; and the New England Tablelands, respectively) comprising the superspecies *E. radiata.* Johnstone (1984)'s *E. radiata sensu stricto* corresponds to Sieber's original description and excludes the populations from the New England Tablelands and many populations from southern Victoria, and also includes *E. robertsonii.*

Varying nomenclatural descriptions for narrow-leaved peppermint are still evident. Chippendale (1988) described two subspecies of *E. radiata* Sieb. ex DC; subsp. *radiata* and subsp. *robertsonii.* Johnson and Hill (1990) described *E. radiata* as having two subspecies; subsp. *radiata* and subsp. *sejuncta. E. radiata* subsp. *radiata* in this sense is synonymous with *E. phellandra* Baker and Smith, *E. australiana* Baker and Smith, *E. radiata* Sieb. ex DC. var *australiana* (Baker and Smith), *E. amygdalina* Labill. var. *australiana* Baker and Smith and *E. radiata* Sieber. ex DC. var. *subexerta. E. radiata* subsp. *sejuncta* in this sense replaces *E. radiata* Sieb. ex DC var. *subplatyphylla* and occurs on the New England Tablelands of New South Wales (Figure 1.6). Johnson and Hill (1990) described *E. robertsonii* as a distinct species synonymous with *E. radiata* Sieb. ex DC subsp. *robertsonii* (Blakely) Johnson and Blaxell, and occurring as *E. robertsonii* subsp. *robertsonii* and *E. robertsonii* subsp. *hemisphaerica.* It is Johnson and Hill (1990)'s description of *E. radiata* Sieb. ex DC subsp. *radiata* that will be referred to in this thesis.

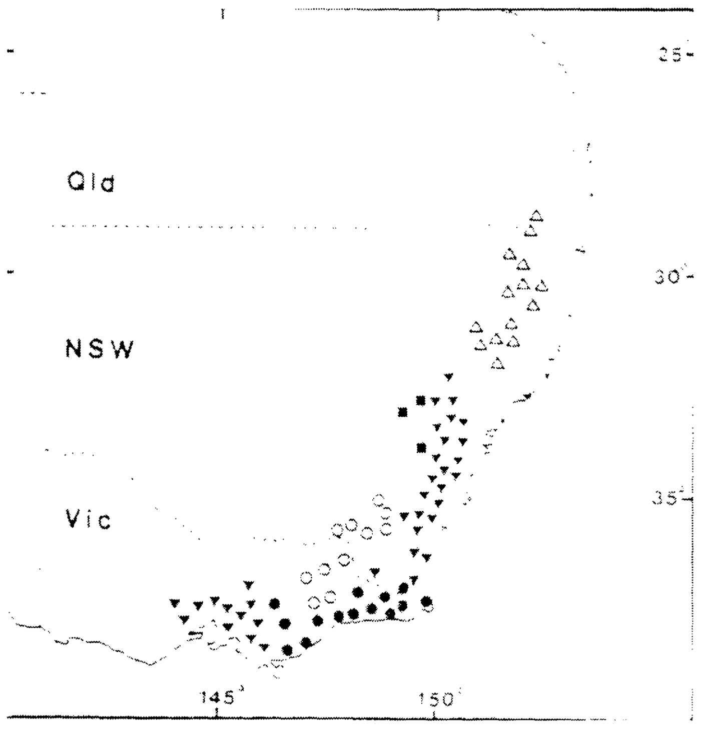

Figure 1.6 - Distribution of *E. radiata* subsp. *radiata* (closed triangle) and subsp. *sejuncta* (open triangle), *E. robertsonii* subsp. *robertsonii* (open circle), and subsp. *hemisphaerica* (square) after Johnson and Hill (1990) p97.

Chemical forms are plants in naturally occurring populations which cannot be separated on morphological evidence, but which are readily distinguished by marked differences in the chemical composition of their essential oils (Penfold and Willis, 1953). Chemical forms are generally well defined and readily distinguishable from each other by quantitative differences in major oil components. The eucalypts demonstrate extreme variations in chemical form both between and within species.

Penfold and Morrison (1927) first reported such forms in *E. dives* and called the variants ‘physiological forms’. To distinguish four separate and distinct oil forms they called them ‘Type’, ‘Variety A’, ‘Variety B’, and ‘Variety C’ in order of discovery. The different biochemical variants are generally now referred to as variants, forms, chemoforms, chemovars or chemotypes with the major oil component highlighted, for example, *E. dives* (cineole variant) (Lassak, 1988).

Penfold and Morrison (1935, 1937 and 1940) recorded three chemical forms of *E. australiana* Baker and Smith (syn. *E. radiata* Sieb.) in New South Wales and one in Victoria and northern NSW. These were named 'Type', Var. A, Var. B and Var. C respectively, differing in the amounts of cineole and phellandrene and the presence or absence of piperitone. The major chemical components reported by Penfold and Morrison in the four physiological varieties of *E. australiana* Baker and Smith (syn. *E. radiata* Sieb.) are presented in Table 1.8.

Table 1.8 - Chemical composition of Penfold and Morrison's four physiological varieties of *E. australiana* Baker and Smith (syn. *E. radiata* Sieb. ex DC) (*source: Penfold and Morrison, 1935, 1937,1940*)

Variant	Principal Chemical Constituents
'Type'	1,8-cineole (65-72%); α-pinene; α-terpineol; geraniol; citral
Var. 'A'	α- and γ-terpinene; β-phellandrene; terpinen-4-ol
Var. 'B'	α-phellandrene (35-40%); 1,8-cineole (20-50%); terpineol; citral
Var. 'C'	piperitone (30-50%); piperitol; α-phellandrene; ρ- cymene

Johnstone (1984) identified six chemoforms of *E. radiata*, defined primarily by the amounts of cineole and piperitone in the oil, and suggested that this feature of the chemoforms together with the close relationships between the taxa indicate that the chemical variants may be common to the three different species (*E. polymorpha, E. radiata and E.* secendens) that he had proposed. Table 1.9 presents the range in abundance in four key compounds in the oils of the six identified chemical forms in a population of *E. radiata.*

Table 1.9 - The range in abundance in four key compounds in the oils of six chemical forms in a population of *E. radiata*
(*source: Johnstone, 1984*)

Chemoform name	1,8-cineole (%)	α-phellandrene (%)	piperitone (%)	terpinene-4-ol (%)
R:Ai	2 – 12	4 – 27	21 – 55	2 – 26
R:Aii	7 – 27	3 – 33	0.8 – 10.5	2 – 37
R:Aiii	4 – 27	3 – 23	0 – 19	12 – 36
R:Aiv	9 – 23	1 – 7	1 – 6	14 – 28
R:Bi	30 – 60	3 – 20	0 – 6	2 – 23
R:Bii	58 – 67	1 – 20	1 – 3.5	1 – 6

Boland *et al.* (1991) discussed the chemistry of two major chemotypes of *E. radiata.* The first, predominantly monoterpenoid in character with major compounds comprising 1,8-cineole and α-pinene, is referred to as the 'cineole form'. The second type has a more even distribution of monoterpenes, with the major compounds comprising α-phellandrene, 1,8-cineole, ρ-cymene, mentha-2-en-1-ol, piperitone, piperitols, and terpinen-4-ol, and is referred to as the 'α-phellandrene form'.

There is vague correspondence between some of Johnstone's chemoforms, Penfold and Morrison's "physiological varieties" and the chemotypes of Boland *et al* (1991). Johnstone (1984) reported that his chemoform R:Ai has at least 20% piperitone and occurs predominantly in Victoria, and therefore could correspond to Penfold and Morrison (1940)'s 'Var. C' and the α-phellandrene form of Boland *et al* (1991). Penfold and Morrison's 'Type' appears to correspond to Johnstone (1984)'s chemoform R:Bii having high cineole. This corresponds with the cineole form of Boland *et al* (1991).

In a study of 50 *E. radiata* trees in a single population containing three distinct chemical forms, Whiffin and Bouchier (1992) concluded that the forms appear to be the result of the actions of the enzymes which control terpenoid biosynthesis modifying a basic monoterpene pool. Each form may occur in separate distinct populations but they often mingle on the one site with individual trees (or chemotypes) locking crowns with other chemical forms, suggesting that oil traits are under strong genetic control (Doran, 1991). Johnstone (1984) also suggested the mosaic of chemical forms within populations indicate that the determination of the forms is under simple genetic control. The distribution and frequency of the chemical forms suggested that the genes responsible for the forms are not universal for the taxa, but are restricted in geography. The geographic trends in both morphological and chemical characteristics show that gene flow is restricted between populations (Johnstone, 1984).

1.12.4 Leaf oil concentration of *E. radiata* subsp. *radiata*

Leaf oil concentration as defined here is the concentration of oil in the leaves of an oil-bearing species. It is generally measured as the mass of oil extracted from a known mass of foliage and is expressed as a percentage of either leaf fresh weight (w/w% FW) or leaf dry weight (w/w% DW). Leaf oil concentration can be highly variable within a species. Oil yield describes the amount of oil produced per unit of land area, and is usually measured as kilograms of oil produced per hectare of plantation (kg oil ha^{-1}).

Table 1.10 lists some typical leaf oil concentration figures quoted by various authors for *E. radiata*. The oil concentration ranges presented by Lassak (1988) and Boland *et al.* (1991) are average leaf oil concentrations from unselected trees. Doran *et al.* (1998b) presented figures from a provenance/progeny trial of selected stock where superior trees are being produced for oil production. Kar *et al.* (2000) cited figures from the Banalasta Oil Plantation from trees also grown from selected seed stock collected from native stands in south eastern New South Wales.

Table 1.10 - Typical *E. radiata* leaf oil concentration figures as determined by various authors

Leaf oil concentration (w/w% DW)		
cineole form	**phellandrene form**	**Author**
3.4-4.0	4.9	Boland *et al.*, 1991
5.0-7.0	3.0-4.5	Lassak, 1988
5.1-10.3		Doran *et al.*, 1998b
6.0		Donald, 1980
6-7		Kar *et al.*, 2000

1.13 Technical aspects involved in *E. radiata* subsp. *radiata* plantation establishment and essential oil production

Plantation establishment for essential oil production requires careful planning and management, and presents an opportunity to introduce significant efficiencies in commercial production.

1.13.1 Plantation site selection

Eucalyptus radiata subsp. *radiata* grows on a wide range of soils from sand to loams. It will not generally tolerate extreme heat, but may withstand a cold climate with some frost. The species grows naturally in an altitudinal range of 50 to 1200 m above sea level, and in a rainfall range of 650 to 1100 mm per annum (Table 1.6). Matching a suitable provenance of *E. radiata* subsp. *radiata* from the wild (or seed orchard) to the plantation site is critical to its survival and productivity.

1.13.2 Ground preparation

Standard forestry ground preparation techniques have been employed in the establishment of *E. radiata* subsp. *radiata* plantations for eucalyptus oil production. The initial step is to assess the site and compile a plantation establishment plan (PEP) taking into account the natural features of the site. Features such as drainage lines, soil types, potential erosion hazards, areas of significant vegetation, fauna habitat, heritage features, roading and firebreak requirements, establishment techniques and ongoing management requirements should be considered (pers. obs.).

Once the PEP is completed, the establishment work generally consists of: clearing (subject to legislative requirements and a possible development consent process); pre-cultivation broadcast knockdown herbicide application; deep ripping and mounding; second cultivation; pre-plant knockdown and residual herbicide application; planting; fertilising; and post-plant herbicide application (pers. obs.). Mounding of planting rows is common practice in timber plantation establishment, however consideration must be made of the possible impacts of this, such as access to trees or machinery damage in mechanical harvesting of trees in eucalyptus oil production. It may be necessary to create smaller mounds or not mound at all.

1.13.3 Tree spacing

The major difference to plantation establishment for timber production in plantation establishment for essential oil production is the spacing of the rows and the trees within the rows. Timber production generally has a spacing of 3 to 4 m between the rows and around 2 to 3 m between the trees within the rows (giving an initial density of approximately 800 to 1200 trees per ha), while planting densities for oil production vary significantly.

One of the major goals for achieving a high plantation oil yield is maximising oil-yielding leaf biomass production per ha. This is primarily achieved through optimising tree spacing between the trees for this purpose (as well as maximising coppice regrowth following harvest). The trial block studied in Doran *et al.* (1998b) was established with a density of approximately 2,222 trees/ha (3 m between rows and 1.5 m between trees within rows) (Doran, pers comm., 1996). Turvey Agricultural Consulting (Anon., 1994) recommended a density of 4,000 to 10,000 trees/ha. Allan Coates (pers. comm., 1994) planted *E. radiata* near Armidale on the Northern Tablelands at a density of 4,444 trees/ha (a spacing of 1.5 x 1.5 m) for oil production with adequate results. A number of plantation densities have been trialled for *E. polybractea* at the G. R. Davis Pty Ltd plantations, but current commercial plantings are spaced at 3 m x 0.5 m, giving a stocking rate of 6666 trees/ha (R. Davis, pers. comm., 2002). Consideration must be given to between-row spacing to allow for plantation maintenance and harvesting operations to be carried out without damaging machinery or trees.

The economic analysis carried out as part of this study assumes a tree spacing of 3 m between rows and 1.5 m between the trees within the rows based on the *E. radiata* subsp. *radiata* trial block of Doran (1998b)'s study. This stocking rate is not necessarily the optimum for oil production. Some well-designed spacing trials are still required to determine the most productive rate.

1.13.4 Irrigation requirement

A number of reports claim that irrigation will increase plantation productivity and overall oil yield. Irrigation offers scope for carrying an increased density of trees per hectare, as well as increased growth and production reliability in eucalyptus oil production (Anon., 1994). Irrigation may ensure a more reliable pattern of production and maximise productivity per hectare (Colton and Murtagh, 1990). Milthorpe *et al.* (1994) found that irrigated *E. polybractea* produced a higher oil yield than non-irrigated plantations. Yields ranged from 3.1 to 7.0 t ha^{-1} of leaf biomass and 117 to 230 kg ha^{-1} of oil on non-irrigated sites to 4.8 to 10.3 t ha^{-1} of leaf biomass and 176 to 343 kg ha^{-1} of oil on irrigated plots.

The establishment and maintenance of irrigation infrastructure is expensive and the cost of irrigation must be weighed up against the increased financial benefits that may be gained. The trees in the Banalasta Oil Plantation are drip-irrigated with water pumped from a large on-farm water storage. However, economic data on this production method were unavailable.

1.13.5 Fertiliser requirements

Determining fertiliser requirements for a eucalypt plantation is dependent on a number of factors such as previous land use, current nutrient status and availability, and tree species being planted (Florence, 1996). Common practice involves the application of a 'starter' dose, often 50 to 100 g of a nitrogen phosphorous and potassium (NPK) blend, nitrogen and phosphorous diammonium phosphate (DAP), or ammonium phosphate sulphate compounds buried 10 to 15 cm from the tree roots 4 to 8 weeks after planting (CSIRO Forestry and Forest Products, nd; Florence, 1996; Northern NSW Forestry Services, nd; State Forests NSW, 1998; pers. obs.). Seedlings generally respond with a flush of new growth and an increase in height and stem diameter a few weeks after fertilising.

Davidson (1996) emphasised the necessity of adequate nutrition for fast-growing trees in plantations, noting that many trials have demonstrated the importance of NPK fertilisers, with evidence of increased growth rates from the application of up to about 120 kg N ha^{-1}, 50 kg P ha^{-1} and 100 kg K ha^{-1} near the trees at the time of planting. Milthorpe *et al.* (1994) found that the only response to fertiliser application in an *E. polybractea* trial for essential oil production occurred in conjunction with some irrigated phosphorous plots. However, that result was inconsistent across the trial.

Doran (pers. comm., 1996) applied 100 g of NPK per tree 4 weeks after planting in the provenance/progeny trial block of *E. radiata* subsp. *radiata* at Brogo studied in this research project. The Banalasta Oil Plantation incorporated sulphate of potash, chicken manure and Growmag (32% Ca and 8% MgO) into the mounded rows prior to planting *E. radiata* subsp. *radiata* in northern NSW (Kar *et al.,* 2000).

Soil tests should be carried out prior to plantation establishment to determine the nutritional status (including trace elements) of the soil and advice should be sought to determine an efficient fertiliser type and application rate (Florence, 1996).

1.13.6 Leaf harvesting

The initial harvesting operation for *Eucalyptus radiata* subsp. *radiata* oil production is generally carried out 2 years after planting, and then every 2 years after that (A. Coates, pers. comm., 1994; J. Doran, pers. comm., 1996). There are anecdotal reports that annual harvesting is too frequent in this species and may lead to rapid tree decline (G. Davis, pers. comm., 2000). In subsequent harvests, and under optimum conditions, the trees are approximately 3 to 4 m tall and have taken on a shrubby form brought about by producing a number of stems and a large amount of foliage from the coppice regrowth. Traditionally, in native forests where the trees are older and larger, they have been cut off just above ground level at harvest, but Kar *et al.* (n.d.) reported that production of coppiced leaf biomass from 2-year-old trees was greatest following a high cut (1 m) as opposed to a low cut (20 cm). Harvesting at 1 m above ground level is routinely applied in *E. radiata* subsp. *radiata* plantations in South Africa that are first cut when basal diameters reach a minimum of 4.5 cm (J. Owen, pers. comm., 2001).

To optimise efficiency, large-scale commercial forests of *E. polybractea* are mechanically harvested using tractors and forage harvesters. The trees are generally cut off close to ground level, promoting coppice growth as described above. The plant material is conveyed directly into large mobile distillation vats, ready for transport to the distillery. Small scale *E. radiata* subsp. *radiata* plantations for essential oil production are harvested by hand using chainsaws, cane knives and manual loading of leaf material into the still. Although this is time consuming and costly, the initial costs involved in mechanical harvesting may be too great for a small-scale operation.

The time of harvest is important in maximising oil yield. This will vary between different localities, and also between subsequent years. Kar *et al.* (2000) reported that oil yield of *E. radiata* subsp. *radiata* grown at the Banalasta Oil Plantation was highest in summer and lowest in winter. Donald (1991) and D. Donald (pers. comm., 1989) reported that harvesting of *E. radiata* is carried out in the summer months in the southwest Cape of South Africa because the yield drops from approximately 22 L per 500 kg charge of wet leaf, twig and small branches in summer to as low as 10 L during the winter. Donald (1991) attributed this to the rapid growth of the trees over the winter in a winter rainfall area where temperature is not limiting, and to most of the photosynthate going into leaf growth rather than oil production.

J. Doran (pers. comm., 2001) found that a small percentage of the *E. radiata* subsp. *radiata* trees in the provenance/progeny trial studied by Doran *et al.* (1998b) dies following each commercial harvest. This implies that total plantation oil yield will gradually decline over a number of years, and Doran suggested that replanting may have to be considered after ten harvests, or approximately 20 years.

1.13.7 Oil distillation

Distillation involves extracting the oil from the plant material by steam. The five major components of a distillation still are the distillation vat, a water supply, the condenser, a receiver/separator and a heat source (Boland *et al.*, 1991). Wood-fired bush stills, often consisting of 400-gallon steel water tanks for vats with pipes submerged in cold dams or streams as condensers, still exist and are common in developing countries. However, the larger commercial operations in Australia such as Felton Grimwade and Bickford Pty Ltd and G. R. Davis Pty Ltd use large fixed boilers with mobile vats, stainless steel pipework, and multi-tubular condenser units.

Steam generated through large wood or gas fired boilers is pumped through the leaf charge. In contact with the steam, the compounds in the oil are volatilised and carried out through an exit pipe at the top or side of the distillation vat. The steam and oil vapour is then passed through a condenser where the oil and water return to the liquid phase and separate, forming two distinct layers when settled, with the oil floating on top of the water. The solubility of the major commercial eucalyptus oils in water at ambient temperature is negligible (Coppen and Hone, 1992). The oil is drained off the water into containers. Some oils such as *E. globulus* require secondary distillation or rectification to bring them to a suitable medicinal standard. *E. radiata* subsp. *radiata*, however, does not generally require this.

Distillation time depends upon a number of factors including species, the amount of heat applied and its uniformity, external temperature and the design of the still. Generally distillation should be stopped when the ratio of oil to water leaving the condenser is 1:50 (Boland *et al.*, 1991). For cineole-rich oils, extended distillation will result in a poorer quality oil, while for piperitone-rich oils, distillation should be continued for as long as possible (Boland *et al.*, 1991). Penfold (1945) reported that *E. australiana* was generally distilled for between two and three hours in bush stills. *E. radiata* subsp. *radiata* currently produced on the south coast of NSW is distilled for approximately two to three hours (J. Doran, pers. comm., 2001).

1.13.8 Potential oil yield from commercial *E. radiata* subsp. *radiata* plantation

There is only limited practical information available on the potential oil yield that might be achieved from commercial *E. radiata* subsp. *radiata* plantations. In their research into growth and oil characteristics of the *E. radiata* subsp. *radiata* provenance/progeny trial at Brogo, Doran *et al.* (1998b) estimated a mean oil yield of approximately 131 kg ha^{-1} at age 23 months (first harvest) from selected stock. John Doran (pers. comm., 2001) has also carried out a subsequent commercial harvest of the trial block when the trees were aged 48 months and achieved a yield equating to approximately 340 kg ha^{-1}. It is expected that harvesting the plantation every 2 years will optimise production, and that 340 kg ha^{-1} could be sustained for a number of years if full tree stocking is retained (J. Doran, pers. comm., 2001). In South Africa, oil yield from an unselected seedlot averages 200 kg ha^{-1} (J. Owen, pers. comm., 2001)

Abbott (1989) stated that oil yield is critical in determining the profitability of a commercial eucalyptus oil venture. Planting trees with a leaf oil concentration twice that of otherwise identical trees will produce twice the amount of oil under the same harvesting and distillation regime, hence effectively halving the cost of production. If planting trees with only half the leaf oil concentration, the costs of production will effectively be doubled, because twice as many trees need to be established and twice as much harvesting and distillation must be carried out to produce the same amount of oil. Abbott (1989) believed that a viable plantation needs an oil yield of 5% or more on a fresh leaf basis (equating to approximately 10% on a dry weight basis). Tree selection and breeding for desirable traits such as high leaf oil concentration can help achieve theses targets.

1.14 Economic modelling of eucalypt plantations

There are a number of economic modelling approaches for estimating costs, cashflows and financial returns of various farm forestry timber plantation scenarios (O'Brien *et al.*, 1990; Eckersley *et al.*, 1993; Loane, 1993, 1994; Dunchue and Sinclair, 1994; Bulman, 1995; Bird and Jowett, 1996; Moore *et al.*, 1996; Townsend and Mahendrarajah, 1996; Greening Australia, 1997; Private Forests Tasmania, 1999; Thompson and Harris, 2000; Victorian Department of Agriculture and Rural Affairs and Victorian Department of Conservation and Environment, n.d.; Wilson, n.d.;). However only a limited number specifically address the economics of eucalyptus oil plantation ventures.

Allan Herbert is a Senior Economist in Agriculture Western Australia. In conjunction with the Western Australian Department of Conservation and Land Management and the Oil Mallee Association, he developed a model to evaluate the financial performance of oil mallees established in Western Australia as part of the Oil Mallee Project (Section 1.2.1). The model requires production and price information and determines break-even point, cashflow, net present value, internal rate of return and profitability compared to the existing agricultural enterprise. Using current production and price information, the model suggests that oil mallees should be financially attractive to farmers when the mallees are planted in paddocks with conventional production values of \$79 ha^{-1} or less (Agriculture Western Australia, 2000). This is based on a 2 year harvesting regime (first harvest at year 4) and a return of \$15 t^{-1} for biomass on-farm. The model also assumes the woody biomass is sold for use in an integrated wood processing plant that generates power, produces activated carbon and distils eucalyptus oil, as is currently being investigated in Western Australia. Figure 1.7 presents a typical graphical cashflow diagram generated by the model. In this particular case, a 15-ha mallee plantation (at a stocking rate of 2667 trees ha^{-1}) was established on a 100-ha land area currently producing an operating profit of approximately \$50 ha^{-1} under its existing agricultural regime. A 20-year rotation was modelled, with harvesting every 2 years. A discount rate of 7% was used. The present value of returns 'without' oil mallees was \$54,178, while the present value of returns 'with' oil mallees was \$78,186. The internal rate of return was 34%.

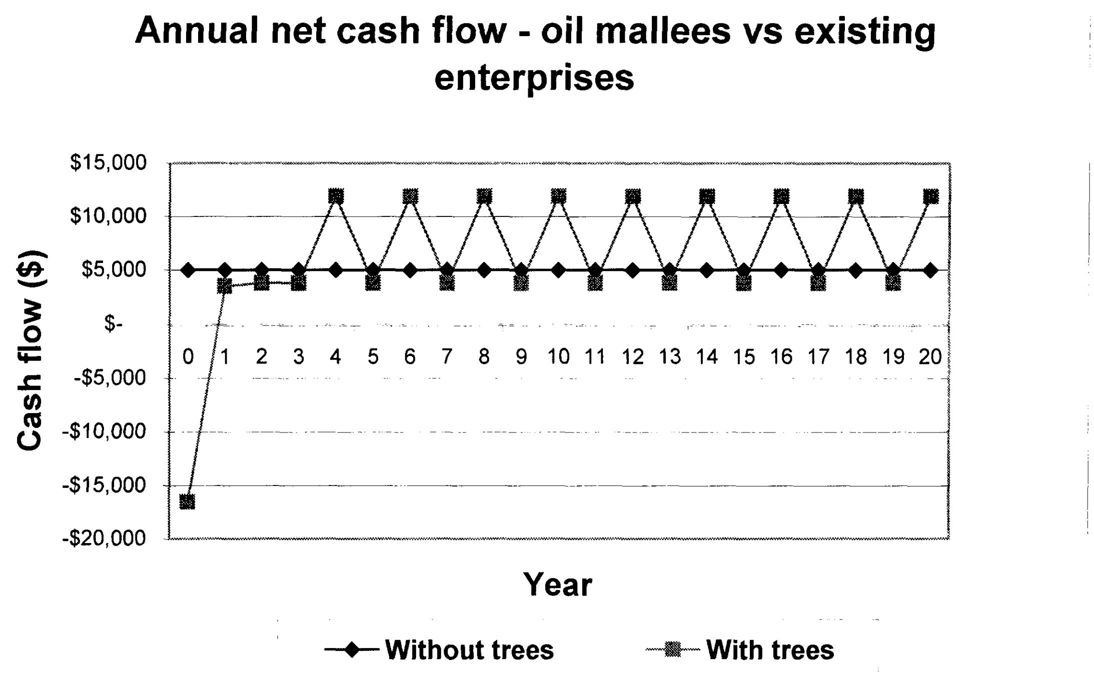

Figure 1.7 - Sample output of annual net cashflow of an oil mallee plantation vs the existing agricultural enterprise using the Agriculture Western Australia Oil Mallee model (*source: Agriculture Western Australia, 2001*)

Bartle *et al.* (1996) presented an economic analysis for a typical Western Australian wheatbelt farm incorporating a 10% alley distribution of oil mallee plantings. A 20-year timeframe was modelled using a discount rate of 6.5%. Planting was carried out over a 5-year period, and the first harvest was carried out in Year 2. Oil concentration was taken to be 4% w/w fresh weight or approximately 40 kg t^{-1} leaf, and fresh leaf yield was taken to be 5 t ha^{-1} yr^{-1}. Oil price was set at \$2 kg^{-1} to a local extraction plant. It was assumed that the only product extracted from the mallees was eucalyptus oil. Table 1.11 presents the results of the analysis in the form of break-even levels of performance for each oil mallee parameter, that is the level of performance that must be achieved for that single variable to maintain the net present value of overall farm production. It is evident that the venture is not viable at the assumed levels of performance. However, it shows the break-even points for oil content and harvesting and extraction costs were within reach, and Bartle *et al.* (1996) suggested that focus on a genetic improvement and modernising harvesting and distilling techniques could exceed the break-even points.

Table 1.11 - Break even production levels for an oil mallee venture on a typical Western Australian wheatbelt farm exploiting a eucalyptus oil market only (*source: Bartle et al., 1996*)

Parameter	Assumed Level Of Performance	Break-even
Leaf yield (FW)	5 t ha^{-1} yr^{-1}	11.5 t ha^{-1} yr^{-1}
Oil concentration	40 kg t^{-1} fresh leaf	53 kg t^{-1}
Harvesting/extraction cost	\$60 t^{-1} leaf	\$34 t^{-1} leaf
Establishment cost	\$1000 ha^{-1} (planted)	no break even
Oil price	\$2 kg^{-1}	\$2.65 kg^{-1}

Bartle *et al.* (1996) reworked the model to evaluate the financial impact of incorporating an integrated residues market (in the form of woody biomass for electricity generation) as well as the oil production. The results of the model presented in Table 1.12 illustrated that a dry biomass price of \$25 t^{-1}, in addition to the return from the eucalyptus oil, would improve farm net present value and make oil mallee a competitive crop at current levels of performance (Bartle *et al.*, 1996). The reworked scenario demonstrated the synergy in a 'dual purpose' oil mallee crop and highlighted the importance of integrated production and marketing.

Table 1.12 - Break even production levels for an oil mallee venture on a typical Western Australian wheatbelt farm exploiting a market for both eucalyptus oil as well as a woody biomass residue for electricity production (*source: Bartle et al., 1996*)

Parameter	Assumed Level Of Performance	Break-even
Leaf yield (FW)	5 t ha^{-1} yr^{-1}	4.5 t ha^{-1} yr^{-1}
Oil concentration	40 kg t^{-1} fresh leaf	38 kg t^{-1}
Harvesting/extraction cost	\$60 t^{-1} leaf	\$65 t^{-1}
Establishment cost	\$1,000 ha^{-1} (planted)	\$1,223
Oil price	\$2 kg^{-1}	\$2.65 kg^{-1}
Residue price (total dry biomass)	\$25 t^{-1}	\$20 t^{-1}

Paul Miller and Associates Horticultural Consultants (Anon., 1999) investigated the potential of production and marketing of *E. polybractea* oil in the north of Central Victoria. The work included the construction of a basic economic model to determine the viability of a 10-ha plantation. A requirement was that the venture had to return a profit after 10 years. To achieve that figure the study determined that the price received for the oil had to be \$14.21 kg^{-1}. The break-even production costs were \$7.55 kg^{-1}. It was ultimately determined that the operation was uneconomic. The plantation establishment costs used in the model were very high (\$7,500 ha^{-1}), and it is possible that reducing this component might have made the venture viable. There are many variations reported in the literature in plantation establishment costs depending on site conditions, species, planting density and ground preparation techniques. Greening Australia (n.d.) establishment costs ranged from \$3,080 to \$3,880 for a general commercial 'plantation' of 1100 seedlings ha^{-1} and up to \$8,400 to \$14,400 for an 'ecoforest' of up to 4000 seedlings/ha. Bartle *et al.* (1996) reported establishment costs of \$1,000 ha^{-1} for oil mallee plantings in Western Australia, while Agriculture Western Australia (2001) estimated establishment costs of an oil mallee planting of 2667 seedlings ha^{-1} to be \$1,300 ha^{-1}. Eckersley *et al.* (1993) reported establishment costs of approximately \$1,200 ha^{-1} for *E. globulus* for pulpwood production. Personal experience in commercial plantation establishment has led to an estimate of establishment and maintenance costs in the first 2 years for a eucalyptus oil plantation of 2200 seedlings ha^{-1} of approximately \$3,200 ha^{-1}.

There are only limited sources of published data on the economics of *E. radiata* subsp. *radiata* plantation establishment and essential oil production. Donald (1991) established an irrigated provenance/progeny trial of approximately 5 ha of *E. radiata* subsp. *radiata* at a spacing of 3 x 1.5 m near Stellenbosch in South Africa. An economic analysis of the operation was carried out, and it was determined that establishing such a plantation using selected seed gave a real rate of return of 13% on total investment, including establishment costs, construction of a 500-kg still, and all harvesting and transport costs, but excluding research costs (Donald, 1991). It must be noted, however, that labour costs were likely to be considerably less in South Africa at that time than in Australia at present.

Abbott (1989) reported financial figures associated with the commercial production of *E. polybractea* oil, estimating that the mechanical cutting and distillation of each 3 t load of leaf (producing up to 60 kg of oil) cost \$180. Abbott (1989) went on to say that the international market price for oil is highly variable, but *E. polybractea* averages around \$9 kg^{-1} for good quality oil and about \$5-6 for lower quality oil, and that returns could range from a loss of \$90 to a profit of \$180 from the 3 t distillation process. R. Davis from G. R. Davis Pty Ltd (pers. comm., 2002) indicated that small volumes of high quality oil such as *E. radiata* subsp. *radiata* could fetch a premium price in a niche market (of up to nearly twice as much) over readily available "bulk product" eucalyptus oils such as from *E. polybractea* or *E. globulus.*

Turvey Agricultural Consulting (Anon., 1994) developed a 70-ha plantation model to determine the economic viability of a number of alternative planting densities and oil yield possibilities for *E. radiata* on the Northern Tablelands of New South Wales. A summary of the results is presented in Table 1.13.

Table 1.13 - Net financial return (\$ per litre of oil) for three different oil yields (presumably fresh wt) and three different tree planting densities (*source: Anon., 1994*)

	Planting density (trees/ha)		
Oil yield (%)	**10000**	**4444**	**3333**
2.5	5.24	4.28	3.72
2.0	4.72	3.51	2.81
1.0	1.49	-0.93	-2.32

1.15 Conclusions

Australia is a net importer of eucalyptus oil. China dominates world production, producing large volumes of cheap "bulk product" oil. *Eucalyptus radiata* subsp. ra*diata* grows well in certain parts of Australia, and has the potential to attract a premium price in a niche market.

Review of the literature identified significant information gaps concerning *E. radiata* subsp. *radiata* essential oil production. Only limited tree growth, leaf oil concentration and plantation oil yield data were available, and there were no published data on heritability, genetic and phenotypic correlations or potential gain in oil production as a result of selection and breeding. No thorough economic analyses of farm-scale *E. radiata* subsp. *radiata* oil production ventures could be sourced.