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Potential of Clones to Boost Yields in Tea Tree Plantations

Prastyono

B.Sc. (Forestry)
Bogor Agricultural University

A thesis submitted in fulfilment of the requirements for the degree of Master of Science

School of Environmental Science and Management
Southern Cross University

December 2008

Candidate declaration

I certify that the work presented in this thesis is, to the best of my knowledge and belief, original, except as acknowledged in the text, and that the material has not been submitted, either in whole or in part, for a degree at this or any other university.

I acknowledge that I have read and understood the University's rules, requirements, procedures and policy relating to my higher degree research award and to my thesis. **I certify that I have complied** with the rules, requirements, procedures and policy of the University (as they may be from time to time).

.....

Prastyono

22 December 2008

Abstract

This thesis reports on an investigation into variation in commercial oil traits of sets of *Melaleuca alternifolia* (tea tree) clones and improved seedling controls at up to three plant stockings (33,333, 22,222 and 16,667 plants/ha). The two trials examined in this work were planted in 2004 and 2006 at Bungawalbin by the Rural Industry Research and Development Corporation (RIRDC)/Australian Tea Tree Industry Association (ATTIA) tea tree breeding programme managed by the New South Wales Department of Primary Industries (NSW DPI). The main aim of these trials was to determine if there is a financial advantage to growers in replanting their existing unimproved tea tree plantations with clones rather than using improved seedlings from seed orchards established by the breeding programme. This is addressed by the development of a financial model comparing the financial aspects of planting clones vs. seedlings at two stockings (33,333 and 16,667 plants/ha)

The 2006 clonal spacing trial (CST) was assessed prior to the first harvest when trees were 12 months old. In the 2004 CST they were assessed prior to the third harvest when coppice shoots were 18 months. All surviving trees were assessed for growth traits while a sampling scheme was used to estimate dry weights of key tree components associated with off-paddock oil yield and oil characteristics of the clones and seedlings in the trials. Determinations of dry weights of key tree components, extraction of leaf oil using solvent techniques, and gas chromatographic analysis to determine quantity and quality of oils were undertaken at the Essential Oil Unit of NSW DPI.

This study showed that plant stocking can have a significant effect on the growth and oil traits of tea tree. Trees at lower stocking (wider spacing) typically have a higher leafiness score as they are given more space and there is less competition for light. But they also had lower oil concentrations than those at higher stockings (narrow spacing). Dry weights of key tree components and oil yields of tea tree plantations on a per hectare basis were found to be greatest at the highest stocking of 33,333 plants/ha, which is typical of the stocking used in most commercial plantations of *M. alternifolia* for oil production.

Clones in the 2006 CST showed superiority in commercial oil traits over seedlings grown from improved seed from the breeding programme. Oil concentration of clones in this trial averaged 91.6 mg/g ODW and 86.69 mg/g ODW compared to seedlings that averaged 63.6 mg/g ODW and 55.77 mg/g ODW from the stocking of 33,333 plants/ha and 16,667 plants/ha respectively. Conversely, the three clones under trial in the 2004 CST were inferior in commercial oil traits to the improved seedling controls (averaged 75.68 mg/g ODW and 75.59 mg/g ODW cf. 81.32 mg/g ODW and 76.47 mg/g ODW), due to extraneous factors, particularly the fact that J-rooted clones had poorer growth. Consistency in 1,8-cineole content was a feature of each clone compared to greater variability amongst seedling stock. This is an advantage for marketing as the current market requires the oils' 1,8-cineole content to be 3% or lower due to the misconception that this constituent is an irritant to skin and mucous membranes.

The variation in growth and oil traits of clones in the 2006 CST indicates that further gains in oil yields and oil quality can be achieved by deploying only the very best clones. The trial data suggested that average oil yields of 522.6 kg/ha and 356 kg/ha might be obtained from plantations established using three best clones –clone 5 (C64), clone 6 (C66) and clone 9 (C70)– at stockings of 33,333 plants/ha and 16,667 plants/ha respectively. These yields are substantially greater than the mature oil yields recorded for CSO1 (ATTIA 2B) seedlings in breeding programme yield trials (357 kg/ha at a stocking of 30,000 plants/ha). A further advantage of clones over seedlings is that clones give mature oil yields from first harvest whilst it is not until year three that seedlings give a higher, mature oil yield.

Financial analyses to evaluate the viability of replanting 20-ha tea tree plantations using elite clones and improved seedlings over a 15-year time frame were carried out. Four plantation options were modelled i.e. (1) plantations established using ATTIA 2B seedlings planted at a stocking of 33,333 plants/ha and (2) 16,667 plants/ha, (3) plantations established using the three best selected clones planted at a stocking of 33,333 plants/ha and (4) 16,667 plants/ha. Capital costs e.g. purchase of land and machinery were not included in this analysis, as all plantation options involve replacement plantations.

The financial analysis showed that, at the current oil price of $45/kg, replacement plantations of either elite clones or improved seedlings are both highly profitable irrespective of the stocking employed. The NPV of the plantation per hectare at 7% discount rate was $109,584, $65,224, $164,921 and $105,638 for plantation options 1, 2, 3 and 4 respectively.

A clonal plantation at a stocking of 33,333 plants/ha was predicted to give the greatest profit at any of the oil prices tested, followed by plantations using improved seedlings at a stocking of 33,333 plants/ha, plantations using clones at a stocking of 16,667 plants/ha, and plantations using seedlings at a stocking of 16,667 plants/ha. The break-even prices for tea tree oil production, using the production parameters in this model were $11.3/kg, $15.5/kg, $10.4/kg and $12.5/kg for plantation options 1, 2, 3 and 4 respectively.

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Chapter 1 Introduction

1.1 Australian tea tree oil

1.1.1 Introduction

Australia has a flora rich in essential oils including many members from the family Myrtaceae such as the genera *Eucalyptus* and *Melaleuca.* The *Eucalyptus* oil industry in Australia was first initiated in the 1850s and these oils are commonly grouped into three categories based on their principal end use (i.e. medical, industrial and perfumery purposes) (Boland *et al.*, 1991). The genus *Melaleuca* contains hundreds of foliar essential oil bearing species (Brophy and Doran, 1996) but the oils of only a few species meet the Australian standard to qualify as Australian tea tree oil. Unlike the production of *Eucalyptus* oil in Australia which amounts currently to less than 5% of the world *Eucalyptus* oil production (Southwell and Lowe, 1999), Australia is a very significant player in production of Australian tea tree oil in the world market. Production in Australia of Australian tea tree oil represented 99% of the world trade during the 1980/90s (Davis, 2003; McCartney, 2003).

Although Australians use the name 'tea tree' to refer to many Australian native species from the genera of *Leptospermum*, *Melaleuca* and *Neofabricia* (family Myrtaceae) (Craven, 1999), it should be noted that the only source of Australian tea tree oil is in the genus *Melaleuca*, particularly from the species *Melaleuca alternifolia* (Maiden and Betche) Cheel. Other related species that can produce chemically similar oils but have seen little or no production are *M. linariifolia*, *M. dissitiflora* (Colton *et al.*, 2000; Southwell and Lowe, 1999; Williams and Lusunzi, 1994; Wrigley and Fagg, 1993) and *M. uncinata* (Brophy and Lassak, 1992), *M. hamata* and *M. halophila* (J. Brophy[1] pers. comm., 2008). Therefore the term 'tea tree oil' throughout this paper is referring to the oil derived from *M. alternifolia.* The oil type commonly referred to as Australian tea tree oil is rich in terpinen-4-ol and is

[1] School of Chemistry, University of New South Wales

traded under various standards like Australian Standard AS2782-1997: 5 August 1997 and International Standard Organisation 4730:1996 (Table 1.1) which stress that the oil should be comprised of 30 percent or more of terpinen-4-ol and 15 percent or less of 1,8-cineole (Colton *et al.*, 2000; Davis, 2003).

Table 1.1 Level of 15 components stipulated in the International Standard for oil of *Melaleuca*, terpinen-4-ol type (ISO 4730:1996)

Component	ISO 4730 range
α-pinene	1 - 6
sabinene	Trace – 3.5
α-terpinene	5 - 13
limonene	0.5 – 1.5
p-cymene	0.5 – 8
1,8-cineole	Trace – 15
γ-terpinene	10 – 28
terpinolene	1.5 – 5
terpinen-4-ol	30 – 48
α-terpineol	1.5 – 8
aromadendrene	Trace – 3
ladene	Trace – 3
δ-cadinene	Trace – 3
globulol	Trace – 1
viridiflorol	Trace – 1

Source: RIRDC and ATTIA (2007)

Australian tea tree oil is mainly produced by steam-distilling leaves of *M. alternifolia*. The form of this species varies from a small to medium sized tree to a large shrub with papery bark and a range of height from 4 to 14 metres (Wrigley and Fagg, 1993). The natural distribution of this species is largely in swamps and low-lying areas of the north coast of New South Wales (Wrigley and Fagg, 1993), extending to the 'Granite Belt' near Stanthorpe in south-east Queensland (Butcher, 1994; Doran *et al.*, 2006).

1.1.2 Tea tree oil constituents

The essential oil of *M. alternifolia* (Maiden and Betche) Cheel occurs in oil glands located adjacent to the leaf epidermis which are first apparent in immature leaves (Butcher, 1994; List *et al.*, 1995). The components of tea tree oil are terpene hydrocarbons, mainly monoterpenes, sesquiterpenes, and their associated alcohols

(Carson *et al.*, 2006). The first comprehensive analysis using gas chromatography-mass spectrometry of Australian tea tree oil was conducted by Swords and Hunter (1978), who identified 48 components in this oil with the major components being terpenes (terpinen-4-ol, γ-terpinene, 1,8-cineole, and *p*-cymene). This investigation also reported that tea tree oil contained viridiflorene. More recently gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS) analysis by Brophy *et al.* (1989) reported a total of 97 constituents in tea tree oil and corrected the viridiflorene structure assigned by Swords and Hunter (1978). They suggested that viridiflorene should have (*R*)-methyl configuration at C3. This investigation also initially revealed that *M. alternifolia* is the source of the sesquiterpenoids α-ylangene, bicyclogermacrene, palustrol, globulol, rosifoliol and spathulenol as complementary to monoterpenoids β-phellandrene, *trans*- and *cis*-sabinene hydrate, and *trans*-piperitol.

The constituents of tea tree oil as reported by several authors were reviewed by Southwell (1999) who concluded that there are up to 113 different volatile constituents in tea tree oil, including minor and trace components. The composition of tea tree oil may change during storage, with increasing *p*-cymene in place of declining levels of α- and γ-terpinene and terpinolene due to effect of light, heat, and exposure to air and moisture. The oil can be stored for 10-13 years (Brophy *et al.*, 1989; Southwell, 1999), with retention of the original quality, providing it is in dark, cool and dry storage conditions.

Table 1.2 Composition of the six chemotypes of *M. alternifolia*

Chemotype historic 'physiological' forms	1 "Type"	2 "Var D"	3 "Var C"	4 "Var A"	5 "Var B"	6
Compound						
terpinen-4-ol (HS-GC%)	22-40	<3	10-14	6-4	<1	<1
terpinen-4-ol (est. SD-GC%)	34-54	<5	16-19	16-20	<6	<4
terpinen-4-ol (Butcher *et al*., 1994)	na-42	1-2	15-20	na	na	Na
1,8-cineole (HS-GC%)	0-17	22-44	34-46	41-63	72-86	65-80
1,8-cineole (est. SD-GC%)	0-8	10-26	18-28	24-44	55-72	47-64
1,8-cineole (Butcher *et al*., 1994)	0-11	17-34	30-36	36-48	65-71	Na
terpinolene (HS-GC%)	2-6	41-60	16-24	0-3	<1	6-14
terpinolene (est. SD-GC%)	1-5	48-69	18-27	0-2	0-2	6-15
terpinolene(Butcher *et al*., 1994)	na	28-57	10-18	na	na	na

Source: Homer *et al.* (2000)

It has been reported that there are considerable variations in morphology and leaf oil concentration and composition of *M. alternifolia* at both intra- and inter- population levels. Butcher *et al.* (1994) identified five distinct oil chemotypes and a more recent investigation by Homer *et al.* (2000) revealed that six distinct oil chemotypes occur in this species, based on the relative proportions of three main components of the essential oil (1,8-cineole, terpinen-4-ol and terpinolene). These include a terpinen-4-ol type, a terpinolene type and four 1,8-cineole types (Table 1.2). The oil composition of *M. alternifolia* leaf oil is considered to be controlled by genetic rather than environmental factors (Homer *et al.*, 2000).

Tea tree oil with a high proportion of terpinen-4-ol (± 40%) and a low proportion of 1,8-cineole (± 3%) is the most favoured for commercial use in the tea tree oil industry (Baker, 1999; Doran *et al.*, 2006; Homer *et al.*, 2000; Lee *et al.*, 2002) due to the misconception that 1,8-cineole is an irritant to skin and mucous membranes (Carson *et al.*, 2006). Hence, during the industry boom period of the 1980s, tea tree oil with a lower content of 1,8-cineole was considered preferable and the cineole content of tea tree oil became an indicator of the oil quality (Davis, 2003). This misconception has been negated by several authors such as Hausen *et al.* (1999) and Southwell *et al.* (1997a, 1997b) who showed that higher proportions of 1,8-cineole do not cause irritation and are not detrimental to the efficacy of tea tree oil as long as the proportion of terpinen-4-ol in the oil stays high. However, the industry preference for low 1,8-cineole prevails to this day.

1.1.3 Uses of tea tree oil

Leaves of *M. alternifolia* were used medically by the Australian Aborigines for their wound healing and anti-inflammatory properties for centuries (Drury, 1989; Lassak and McCarthy, 1990). Scientific studies on the efficacy of tea tree oil against human infections were first initiated in the 1920s at the Museum of Technology and Applied Science in Sydney (Markham, 1999; RIRDC and ATTIA, 2007; Wrigley and Fagg, 1993). Recent investigations by Southwell *et al.* (1993) and Carson and Riley (1995) confirmed that the antimicrobial activities of this oil are principally attributed to the presence of terpinen-4-ol although other components appear to contribute

significantly to the antimicrobial activity. On the other hand, recent investigation by Cox *et al.* (2001) found that the presence of non-oxygenated terpenes (such as γ-terpinene and *p*-cymene) in tea tree oil can reduce the effectiveness of terpinen-4-ol by decreasing its aqueous solubility.

Minimum inhibitory concentration (MIC) and mycelial growth inhibition (MGI) have been recorded for the oil and numerous oil components against many bacteria, fungi and plant pathogens (Angelini *et al.*, 2008; Carson *et al.*, 2002; Carson and Riley, 1995; Cox *et al.*, 2001; Hammer *et al.*, 1997, 2000a, 2000b, 2002, 2003, 2004; Southwell *et al.*, 1997a; van Vuuren and Viljoen, 2007). A review of antimicrobial and other medicinal properties of tea tree oil by Carson *et al.* (2006) shows that at least 27 species of bacteria and 24 species of fungi have been reported to be susceptible to tea tree oil. Tea tree oil may also exhibit antiviral and antiprotozoal activities, however, this claim requires more scientific study (Carson *et al.*, 2006).

Tea tree oil is now used in a wide variety of products, either as pure oil or formulated into many kinds of value-added products as a preservative, or as an antifungal, antiseptic or antibacterial agent. Such products include shampoos and conditioners, soaps, bath oils, mouthwashes, toothpastes, deodorants, moisturisers, face cleansings and washes, hand washes, foot sprays and powders, shaving products, antiseptic creams, body lotions, sun blocks, lip balms, post-waxing treatments, acne creams and washes, tinea creams and powders, vaginitis creams and douches, burn creams and other health products and dog shampoos and other veterinary care products (Colton *et al.*, 2000; RIRDC and ATTIA, 2007; Southwell and Lowe, 1999; Wrigley and Fagg, 1993).

1.2 The Australian tea tree oil industry

1.2.1 The development of the Australian tea tree oil industry

The production of tea tree oil commenced in 1926, a year after the identification of the germicidal properties of tea tree oil which were reported as 11-13 times more powerful than phenol (carbolic acid) (Colton *et al.*, 2000; Davis, 2003). The natural stands around Grafton, Casino and Lismore (Davis, 2003) which occur mainly in the

Richmond and Clarence River valleys (Colton *et al.*, 2000) in the Northern Rivers District of New South Wales were the centre of production. The tea tree industry in Australia was mainly a cottage industry in its early stages (1930s-1940s) and, until the 1980s, Australian tea tree oil was obtained solely from natural stands. This period was called the 'bush industry' as the leaves were steam-distilled in primitive 'bush' stills to obtain the oil, and these were located near the natural stands being harvested (Colton *et al.*, 2000; Davis, 2003). During the first period of the industry, production levels varied from 2 to 20 tonnes per annum (Davis, 2003), sufficient to supply the demand of the oil including supplies of oil for the soldiers during World War II. Shortly after the end of the war, the industry went into decline as with the discovery of antibiotics and synthetic biocides they replaced the function of tea tree oil (McCartney, 2003; Wrigley and Fagg, 1993).

Expansion of the Australian tea tree oil industry from harvesting of natural stands to plantation production began in the 1980s when tea tree oil production increased in response to increasing demand from both domestic and export markets. Plantations had already started on a small scale in the late of 1970s (Colton *et al.*, 2000). The increasing demand for oil was stimulated by a worldwide outlook seeking to return to natural products (Baker, 1999; Colton *et al.*, 2000; Doran *et al.*, 2002; Wrigley and Fagg, 1993) combined with new marketing promotion and strategy both in Australian and the USA (Davis, 2003).

By the late 1980s, established plantations in New South Wales had expanded to more than 500 ha with an annual production at approximately 70 tonnes of oil. The industry grew more than ten times during the decade of the 1990s (Colton *et al.*, 2000). By 1991 the estimation of oil production was about 100-120 tonnes (Merry, 1991) and by 1993 plantations of tea tree had been extended from Taree/Port Macquarie in New South Wales to Dimbulah in far northern Queensland (Colton *et al.*, 2000; McCartney, 2003). From the second harvest onwards, the average annual industry oil yield was about 150 kg per hectare but with a very wide range of 100 to 500 kg/ha for planting densities of 25,000 - 35,000 plants per hectare (Davis, 2003).

By 1999-2000, the industry reached the real peak with about 5,000 ha of plantation and production of 650 tonnes of oil per annum (Colton *et al.*, 2000). However annual

production had declined by 2002, to about 405 tonnes of oil, representing 62% of total Australian essential oil production and 37% of their total value (McCartney, 2003). At its peak the industry involved about 300 growers and distillers (Davis, 2003). Currently, according to ATTIA (2006), the annual tea tree oil production is about 400-480 tonnes, derived from more than 4,000 ha of plantation resources.

1.2.2 Tea tree oil markets

More than 80% of the tea tree oil produced in Australia is exported to North America (especially the USA) and Europe, with the remainder used domestically and with a small proportion going to Asia (Colton *et al.*, 2000; McCartney, 2003). The price of this oil was relatively stable at about $2/kg in the early 'bush industry' (Colton *et al.*, 2000). In the early 1980s, the price was about $12 - $15/kg, it reached $25/kg in 1986 and by 1989/90 the oil price peaked at about $60/kg before stabilizing at about $45-55/kg during the 1990s (Colton *et al.*, 2000; Davis, 2003).

As a consequence of a substantial increase in plantation area during 1990-2000, production of oil was dramatically amplified. The inevitable effect of overproduction saw a substantial reduction of price from $45 - $55/kg in 1999 to about $12/kg in 2004/2005 (ATTIA, 2006; Davis, 2003), or well below the cost of production (±$20/kg) for most Australian growers (Colton *et al.*, 2000). A recession in the industry occurred during 2000-2006 which saw many growers leave the industry. Recovery was evident by 2007 and the price of oil held firm at $45-48/kg during September 2008 (EOPAA, 2008).

1.2.3 Silviculture of tea tree plantations

In a commercial plantation, tea tree is commonly established by seedlings as an intensively-managed row crop (Colton *et al.*, 2000). Trees are planted in a high density population to maximize biomass yield as leaf yield per hectare tends to increase with an increase in plant density (Small, 1981). As a close-spacing tolerant species, tea tree can be planted at high density without any adverse impact on plant growth, however, Small (1981) suggested that the optimum plant density exceeds 27,000 plants/ha. Colton and Murtagh (1999) noted that based on commercial

experience in New South Wales and Queensland, a population density of 35,000 plants/ha is likely to be optimal. To achieve this plant stocking, a plantation can be arranged in single rows 75 cm apart combined with 38 cm within-row spacing, 100 cm apart combined with 28 cm within-row spacing, or twin rows 38 cm apart on 150 cm bed. However, the most common planting layout is with a 1 metre between-row spacing (Colton and Murtagh, 1999; Colton *et al.*, 2000). The advantage of such a dense population is that full ground cover can be achieved very quickly after planting and harvest and thus providing a limited space for weeds to grow (Colton *et al.*, 2000). The choice of plant spacing and layout depends also on the size of machines to be used in the plantation i.e. tractors, mowers, cultivators, sprayers and harvesters.

Figure 1.1 A commercial tea tree plantation at 35 cm within-row spacing and 1 meter between-rows spacing (28,571 plants/ha) at Bungawalbin, NSW (Photo: Prastyono)

High yielding plantations are influenced by many agronomic factors and growing conditions such as a continuous supply of moisture during the growing season either from irrigation or rainfall, adequate and balanced nutrition either naturally provided in the soil or through fertilizer input, a minimum level of weed interference, and a minimum level of pest and disease attacks (Colton *et al.*, 2000). Irrigation and fertilizer are not a critical concern in tea tree plantations in Northern Rivers region of

New South Wales where the plantations are generally located in areas of alluvial soils that are reasonably fertile and have moisture retentive soils that receive a high annual rainfall (Colton *et al.*, 2000). However these become important issues when tea trees are grown in light, infertile, sandy soils combined with very high evapo-transpiration and inadequate annual rainfall as in the Mareeba-Dimbulah region of North Queensland (Colton *et al.*, 2000; Drinnan, 1997).

Another factor that limits growth and oil production of tea tree plantations is weed competition. Virtue (1999) noted that weed interference reduces oil yield through two mechanisms i.e. competition and allelopathy. A reduction of tea tree leaf yield by an average of 25% due to weed interference was reported by Virtue *et al.* (2000). Weed management in commercial plantations should include both preventive (i.e. prevent the movement of new weeds into and within the plantations and provide unsuitable conditions for weed establishment) and control techniques (i.e. application of herbicides, soil cultivation, mowing, grazing, mulching, growing a legume crop during the year before planting, and improving tea tree's competitiveness) (Virtue, 1999).

Several insect pests and diseases have been identified in tea tree plantations. However, the full pest complex of tea tree remains unknown and the magnitude of any pest attack depends on the weather conditions (Campbell and Maddox, 1999). Colton *et al.* (2000) noted that only a small number out of 100 insect species found in tea tree plantations are considered as significant pests by tea tree growers. The most common pests are pyrgo beetle (*Paropsisterna tigrina*), psyllids (*Trioza* spp.), and eriophyoid mites (*Eriophyoid* spp.), pasture scarabs (*Diphucephala lineata*), leaf hoppers, and African black beetles (Colton *et al.*, 2000). Colton *et al.* (2000) also described several diseases generally caused by various fungi found in tea tree plantations e.g. stem blight caused by *Dothiorella* sp., leaf drop caused by *Cylindrocladium* sp., charcoal root disease caused by *Macrophemena phaeseolina* or *Diplodia* sp., and leaf scab caused by *Elsinoe* sp.

Crops are ready to harvest once their canopies are fully developed and have reached their highest leaf yield. Crops are usually harvested by cutting their stem at about 15

cm above ground (Colton and Murtagh, 1999). The interval from planting to first harvest varies between plantation sites and growing conditions, however, 12 months after planting is typical (Colton and Murtagh, 1999; Colton *et al.*, 2000; Kernot, 1994). The subsequent harvest is generally after 8-12 months of coppice re-growth in North Queensland and about 12 months in the Northern Rivers region of New South Wales, and up to 15-24 months further south where growth is slower due to cooler temperature (Colton *et al.*, 2000). Trees from seedlings reach their mature levels of yield at third harvest (i.e. year three in NSW and year 2 in North Queensland) and yield, thereafter, tend to stabilise. First and second harvest yield are typically 50% and 75% of mature yield (Colton *et al.*, 2000).

1.3 The tea tree breeding programme

The dependency on natural stands as the source of oil declined as the massive expansion of tea tree plantations occurred during the first decade and a half of tea tree plantation development (1980 to mid-1990s). Yet, natural stands had a significant role in supplying seed for plantations although without any complex procedures for selecting seed trees and no certificate of origin. The consequence of this unsophisticated genetic sourcing is substantial variability of oil yield and oil quality in the resulting plantations (Baker *et al.*, 2007). The variation in oil yield both between and within natural stands (Butcher, 1994) and plantations of *M. alternifolia* (Davis, 2003), indicates considerable potential for improving productivity through breeding (Butcher *et al.*, 1996).

The average plantation production of 150 kg/ha per annum has long been considered below potential. The potential of tree breeding to provide progressive economic gains in oil yield and oil quality of *M. alternifolia* was recognised when a three-year tea tree breeding project (1993-1996) funded by RIRDC/ATTIA was initiated in May 1993 (Baker *et al.*, 2007; Doran *et al.*, 1997). This first phase of the breeding project was followed by two successive 5-year projects (Doran *et al.*, 2002; Baker *et al.*, 2007) and the current 3-year project. A series of provenance/progeny trials (Butcher *et al.*, 1996; Doran *et al.*, 1997) indicated from the high variability in leaf oil yield and composition, plant biomass production and coppicing, together with high

heritability for oil yield (0.67), moderate heritability for plant dry weight (0.25) and coppicing (0.27) that substantial improvement in any single trait could be achieve by selection and breeding.

To date, a realised genetic gain trial to compare the performance of three grades of improved seed (clonal seed orchard (ATTIA 2B), first generation seedling seed orchard (ATTIA 2A) and selected provenance) released to the industry by the breeding programme against industry standards (unimproved seedlots) revealed that the average improvements in yield of improved sources over industry standards were 83%, 55% and 43%, respectively. A superiority of improved seedlots over the industry standards in oil quality was also demonstrated through higher levels of terpinen-4-ol (higher by 4%) and lower levels of 1,8-cineole (Doran *et al.*, 2006).

1.4 Background to this study

In conjunction with the development of seed orchards as part of the breeding strategy and in order to capture increased gains in oil yield from the breeding programme, the development of elite clones is a focus of the programme (Baker *et al.*, 2007; Doran *et al.*, 2000). Cloning is a quick way to produce genetically identical replicates of trees possessing desirable characteristics (Frampton and Foster, 1993), to access and maintain genetic gain, and enables genetic gains achieved in tree breeding to be captured maximally (Evans and Turnbull, 2004).

The idea was supported by the evidence that *M. alternifolia* is suitable for mass vegetative propagation by stem cuttings and is therefore, a potentially viable option for maximising the capture of genetic gain in the production population (e.g. Sachs *et al.*, 1990 in the USA; and Prospectus, n.d., Whish, 1992 and Williams, 1995 in Australia). An attempt to develop commercially the potential of specific tea tree clones was undertaken by Oil Field Limited during 1997-1998, when about 3 million cuttings were raised and planted over more than 67 hectares in northern Queensland, Australia. It was claimed in the company Prospectus that the oil yield from their clonal plantations would be 2-3 times the average industry production of 150-200

kg/ha (Prospectus, n.d.). The company was placed in receivership before these claims could be verified.

Clones selected for their superior oil yielding capacity, ease of propagation and rootability are likely to be an appropriate option for establishing tea tree plantations. They show a superior oil yielding capacity throughout the life of the plantation, particularly in the first two harvest as they exhibit mature oil levels only reached by seedlings at the third harvest (Baker *et al.*, 2007). Another advantage for marketing is that the consistency of oil quality will be greater in clones than in oil from seedlings that can be highly variable (J. Doran[2] pers. comm., 2007).

There are major constraints in using clones to establish tea tree plantations, however, despite clearly defined advantages. These include growers' concern that the benefit/cost ratio does not favour clones due to clones being too costly compared to seedlings in the establishment phase (50 cents per propagule as opposed to 12 cents for seedlings). Another concern is that clones are not durable in the ground as a consequence of the absence of tap root so that the mortality rate during harvesting is higher (J. Doran pers. comm., 2007). Therefore, the aim of this study is to evaluate whether the use of clones in tea tree plantation establishment is a financially viable proposition.

1.5 Objectives of this study

The objectives of the study are:

1. To compare leaf biomass, oil concentration and oil quality of *Melaleuca alternifolia* plantings established from selected clones against that of seedlings grown from improved seed from a breeding programme,
2. To analyse the financial viability of tea tree plantations established using selected clones and so inform tea tree growers and breeders of the value of cloning to this industry.

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[2] Hon. Research Fellow CSIRO

The objectives were pursued by investigating two research topics, each of which has a number of research questions. The research topics and questions were:

1. Variation in growth and oil traits, biomass and oil production of tea tree plantations established using selected clones and improved seedlings at different spacings.
   1. Does spacing have a significant effect?
   2. Do clones differ from seedlings within each plant spacing?
   3. Are there any differences amongst the clones within each plant spacing?
   4. Is there an interaction between spacing and clones/seedlings?

2. Financial viability of tea tree plantations established using selected clones
   2.1 What are the differences in NPV and IRR of tea tree plantations established using selected clones and improved seedlings at each plant spacing?

1.6 How this study was conducted

The research questions were investigated using the materials and methods, summarized in Table 1.3.

Table 1.3 Research objectives and questions, research activities and methodologies

Research Topics and Questions	Research Activities*	Experimental Methodology*
1. Variation in growth and oil traits, biomass and oil production of tea tree plantations established using selected clones and improved seedlings at different spacings		
1.1. Does spacing have a significant effect on commercial traits for oil production?	Assess growth and oil traits, biomass and oil production of clones and seedlings included in the 2004 and 2006 clonal spacing trial (CST)	a. Measurement of tree height and leafiness score b. Observation of frost damage and flowering (2006 CST only) c. Sampling trees to investigate oil traits, biomass and oil production of clones and seedlings d. Statistical analyses
1.2. Do clones differ from seedlings within each plant spacing?	Assess growth and oil traits, biomass and oil production of clones and seedlings included in the 2004 and 2006 CST	a. Measurement of tree height and leafiness score b. Observation of frost damage and flowering (2006 CST only) c. Sampling trees to investigate oil traits, biomass and oil production of clones and seedlings d. Statistical analyses

Table 1.3 (continued) Research objectives and questions, research activities and methodologies

Research Topics and Questions	Research Activities*	Experimental Methodology*
1.3. Are there any differences amongst the clones within each plant spacing?	Assess growth and oil traits, biomass and oil production of clones and seedlings included in the 2004 and 2006 CST	a. Measurement of tree height and leafiness score b. Observation of frost damage and flowering (2006 CST only) c. Sampling trees to investigate oil traits, biomass and oil production of clones and seedlings d. Statistical analyses
1.4. Is there an interaction between spacing and clones?	Assess growth and oil traits, biomass and oil production of clones and seedlings included in the 2004 and 2006 CST	a. Measurement of tree height and leafiness score b. Observation of frost damage and flowering (2006 CST only) c. Sampling trees to investigate oil traits, biomass and oil production of clones and seedlings d. Statistical analyses
2. Financial viability of tea tree plantations established using selected clones		
1.5. What are the differences in NPV and IRR of tea tree plantations established using selected clones and improved seedlings at each plant spacing?	a. Collect data of estimated oil yield of tea tree plantation established using selected clones and improved seedlings b. Collect data of tea tree plantation establishment and operation costs from tea tree growers	a. Use the estimated oil yield of best three clones of the 2006 CST to predict oil yield of tea tree plantations established using clones b. Use the estimated oil yield of ATTIA 2B seedling in the 2002 yield trial to predict oil yield of tea tree plantations established using improved seedlings c. Comparison of NPV and IRR of tea tree plantations established using selected clones vs. improved seedlings.

* CST is Clonal Spacing Trial
ATTIA 2B is SSO1 (first generation seedling seed orchard est. 1994) seed

1.7 Organization of the thesis

This thesis is presented in 6 chapters:

Chapter 1 gives an introduction to Australian tea tree oil and its industry, the tea tree breeding programme, the background and objectives of this study and arrangement of this thesis.

Chapter 2 presents a review of literature pertaining to factors related to production of essential oils and biomass, financial analysis of tea tree plantations, and advantages and risks of clonal plantations.

Chapter 3 describes procedures to determine plant biomass (dry weights of key tree components) and oil traits of tea tree.

Chapter 4 describes the variation in growth and oil traits of tea tree plantations established using selected clones and improved seedlings at different spacings.

Chapter 5 determines the financial viability of tea tree plantations established using selected clones.

Chapter 6 summarizes the key results of Chapters 4 and 5, draws conclusions and explains the implications of this study for the tea tree breeding programme and commercial tea tree plantations.

Chapter 2 A review of literature pertaining to factors related to production of essential oils and biomass; financial analysis of tea tree plantations; and advantages and risks of clonal plantations

2.1 Introduction

Oil yield of foliar essential oil bearing species is highly dependent on leaf biomass and oil concentration of the leaf (Murtagh, 1999). These variables may vary among individual plants within species as well as between species and are influenced by environmental factors and plant silviculture. Variation in leaf biomass and leaf oil concentration of commercial oil bearing species will affect profitability and financial viability of plantations. An understanding of factors affecting production and composition of essential oil and production of biomass in a particular species will assist growers and tree breeders to maximise oil yield in plantations of the species.

Genetic gain achieved in tree breeding programmes can be maximally captured and used directly in commercial plantations by deploying vegetatively mass propagated, selected superior clones (Evans and Turnbull, 2004). Clonal plantations have long been practiced in forestry as clones provide several benefits compared to seedlings (Libby and Ahuja, 1993). However, there are also some issues with regard to problems and risks associated with the development of clonal plantations.

An understanding of the factors related to production of essential oil and biomass, financial analysis to estimate the feasibility and profitability of using clones in tea tree plantation establishment, and advantages, problems and risks of clonal plantations are fundamental to the interpretation of results of this study. These factors are reviewed in this chapter.

2.2 Factors related to production of essential oil

2.2.1 Genetic factors

One of the most important intrinsic factors affecting production of secondary plant products is genetics (Flück, 1963). Several experimental works have been carried out on the genetic factors that serve to regulate the production of terpenoids. Hefendehl and Murray (1972) and Murray *et al.* (1972) revealed that in *Mentha* species, the expression of terpenes are clearly controlled by a relatively simple genetic system. Studies of *Pinus* species, however, gave varied results. Several studies summarized in Harborne and Turner (1984) and Squillace *et al.* (1980) similarly proposed that the production of particular terpenes in several *Pinus* species is controlled by single genes, usually with a dominant/recessive pair of alleles. However, those conclusions are questionable because the majority of the studies of the composition of coniferous resin used proportions of individual terpenes which are not considered as independent variables, thus, leading to confused and inconsistent results (Birks and Kanowski, 1988). Based on a review of inheritance studies of coniferous resin, Birks and Kanowski (1988) concluded that there was no available evidence of monogenic control of coniferous resin production. This idea was in agreement with White and Nilsson (1984) who found that the monoterpene levels of *Pinus contorta* are strongly controlled by multiple genes.

The complexity of genes governing oil concentration and 1,8-cineole content of *E. camaldulensis* was also noted by Doran (1992). Recent studies of the oils of numerous *Eucalyptus* species and *M. alternifolia* using quantitative genetic methods have indicated that oil concentration and composition are moderately to strongly controlled by genetic factors (Doran, 2002; Doran *et al.*, 2002). Shelton *et al.* (2002) investigated the level of genetic control of monoterpene composition in the essential oil of *M. alternifolia* and concluded that chemical profile of the essential oil was strongly controlled by genetic factors although the actual details of inheritance remain unclear.

Genetic correlations (r_g) which are calculated as the correlation of the breeding values of two traits and express the extent to which these traits are influenced by the

same genes (Falconer, 1989) are important to predict the magnitude and direction of response in one trait to selection for another (Williams *et al.*, 2002). Where there are strongly positive genetic correlations between two traits, selection for one of the correlated traits will be effective in improving the other trait. On the other hand, it can be difficult to breed for two traits in the one population when there are strong negative genetic correlations between them (Doran, 2002).

There are several authors who reported correlations between plant growth and oil traits. Gershenzon (1984) noted that many species have a strong negative correlation between growth and production of secondary compounds. Butcher *et al.* (1996) reported negative genetic correlations between oil yield and growth traits in *M. alternifolia*. However, in a much more comprehensive study involving a much larger set of *M. alternifolia* families, Doran *et al.* (2002) showed that growth and oil traits were poorly correlated and selection for one was unlikely to affect the other. A study on a related species, *Melaleuca cajuputi* subsp. *cajuputi*, by Susanto *et al.* (2003) gave a similar result. Current strategies in breeding *M. alternifolia* assume that growth and oil traits are independent factors that can be improved concurrently by employing a combined selection index (Baker *et al.*, 2007).

Further examples of genetic correlation between growth traits and oil traits have been reported in *Eucalyptus* species. Negative correlations between leaf oil concentrations and growth traits: tree height, stem diameter, crown surface area and crown density in *Eucalyptus camaldulensis* were reported by Doran and Matheson (1994) and in *E. nitens* (Li, 1993). On the other hand, Harris (2002) reported a positive genetic correlation between tree basal area and leaf oil concentration (r_g=0.76) and 1,8-cineole concentration (r_g=0.36) in *E. radiata* subsp. *radiata.* Grant (1997) has also documented that there was a strong positive genetic correlation between oil yield per tree and leaf biomass (r_g=0.846) despite there being negative correlations between oil concentration in leaves and area of individual leaf (r_g=-0.295) and leaf biomass (r_g=-0.174) in *E. polybractea*. The negative correlation between oil concentration and leaf area is in agreement with other data (e.g. King *et al.*, 2006) which indicates that the larger-leaved form of *E. polybractea* produces less oil than the narrow-

leaved form (Doran, 2002) as there is a negative correlation between gland density and leaf area (King *et al.*, 2006).

2.2.2 Ontogeny and flowering

Oil accumulation in the plant organ, tissue and cells and infrequently its composition depends on the developmental phase of the plant *per se* (Sangwan *et al.*, 2001). There are four reasons for ontogenetic variations in essential oil. Flück (1963) showed that an increase/decrease of essential oils occurs in the following possible ways: the compound could be metabolized or translocated to other organs or lost by evaporation or resinification and thus change the ratio between the substance and other compounds present in the organ.

Southwell and Stiff (1989) investigated the change in monoterpenoids in *M. alternifolia* leaves linked to ontogenetic phases along branches and found that the percentages of sabinene, *cis*- and *trans*- sabinene hydrate decreased with leaf maturity with concomitant increases in the percentages of *p*-methanes, γ-terpinene, terpinen-4-ol and α-terpineol. The ontogenetic changes in leaf oil composition reported by these authors are consistent with the development of the oil gland population per leaf documented by List *et al.* (1995). List *et al.* (1995) concluded that these facts together with those of the structure of the mature oil gland and the lack of a diurnal fluctuation in oil yield, support the concept of a one-way developmental pathway for oil gland formation and oil content. The consequence of this phenomenon is that oil concentration should be static or decline after leaf maturation. Consistent with the List *et al.* (1995) hypothesis, Drinnan (1997), in a review of *M. alternifolia* cultivation in North Queensland, observed that oil concentration of flush material and young coppice growth (<2 months old) was 2-3% lower than that of mature material. He also reported that oil concentration tended to decrease when trees reached more than 12 months-of-age.

There are several studies of variation in oil concentration at various ontogenetic stages of *Eucalyptus* leaf (viz. cotyledons, seedling leaves, juvenile leaves, intermediate leaves and adult leaves). The oil concentration of seedling leaves is typically much lower than that of other stages. There appears, however, that there is

no general pattern in the comparison of oil concentration from juvenile, intermediate and adult leaves and it appears to be highly dependent on species (Doran, 1991). For instance, young leaves of *E. citriodora* frequently have a higher oil concentration than mature leaves (Weiss, 1997 *cited in* Sangwan *et al.*, 2001), and oil concentration from the leaves of the mature crown was less than that from leaves of coppice growth in *E. polybractea* (Barton *et al.*, unpublished data *cited in* Brooker *et al.*, 1988). In *E. nitens*, oil concentration in leaves of juvenile-intermediate regrowth was lower than that of adult leaves (Franich, 1986). Oil concentration of aged leaves was generally less than recently mature leaves (Doran, 2002), e.g. in *E. camaldulensis*, 1,8-cineole concentration increased concomitantly with increasing leaf age, reaching the highest level in fully expanded but non-lignified leaves but then declined to stable levels with leaf lignification (Doran and Bell, 1994). Consistent with this evidence, foliar oil of *E. radiata* was highest in fully expanded, non-lignified leaves (Kar, 2003). In contrast, conifers generally have the highest oil concentrations in immature foliage (e.g. in white spruce, Sinclair *et al.*, 1988). Investigations of the ontogenetic stages of herbaceous plants showed similar large variations of oil content and composition without a consistent pattern (e.g. *Erigeron canadensis*, *Daucus carota* and *Anethum graveolens*, Gora *et al.*, 2002).

Oil concentration and composition during the flowering stage also varies among species. For example, the oil of *Erigeron canadensis* reached the highest concentration during inflorescence formation and gradually decreased during and after blossoming (Gora *et al.*, 2002). The oil concentration of *Anethum graveolens*, however, declined during bud formation and later increased, reaching the highest concentration at flowering stage (Huopalahti and Linko, 1983). Similarly, the oil concentration of *Salvia officinalis* was found to be highest in the late flowering season. However, the proportion of leaf in flowering plants was lower than that of non-flowering plants, therefore, total oil yield of flowering plants was lower than that of plants in the vegetative stage (i.e. non-flowering plants) (Máthé Jr. *et al.*, 1992; Perry *et al.*, 1999; Perry *et al.*, 1996). Another variation of oil concentration was found in Japanese mint (*Mentha arvensis*) which reaches its highest oil and menthol level during flower bud initiation stage (Duriyaprapan *et al.*, 1986).

There is only limited published data on the effect of flowering on oil traits in *M. alternifolia* as the plants are usually harvested before reaching reproductive maturity. Butcher (1994) noted that oil concentration of *M. alternifolia* declined immediately before flowering. In contrast, Drinnan (1997) observed that oil concentration was not significantly affected by the flowering/fruiting season.

2.2.3 Oil glands

Essential oils in *M. alternifolia* occur in the oil glands located adjacent to the epidermis which are equally distributed on both sides of a leaf (Butcher, 1994; List *et al.*, 1995). They are first apparent in immature leaves, with the number per leaf increasing as the leaf expands, to reach a maximum prior to the leaf being fully expanded (List *et al.*, 1995). List *et al.* (1995) suggested that the oil gland density appears to be under some degree of genetic control as the variation within a plant was less than that between plants from the same seed source. List *et al.* (1995) found that the oil concentration was not correlated with oil gland density. Therefore, they suggested that variation in oil gland size may account for variations in oil concentration.

The evidence that there was no correlation between oil concentration and oil gland density in *M. alternifolia* is in agreement with the report of very poor correlation between oil gland densities and total concentration of four major monoterpenes in *E. camaldulensis* (Doran, 1992). However, in contrast, King *et al.* (2006) detected that both gland density and total gland volume were positively correlated with oil content in *E. polybractea*.

2.2.4 Seasonal and diurnal changes

Several studies have been carried out to determine the variation in essential oil concentration and composition over time. The response to seasonal changes appears to vary between individual trees, chemical form (Simmons and Parsons, 1987) and species (Li, 1993). Murtagh (1991b) proposed that the oil concentration of *M. alternifolia* is likely to be subject to a number of overlapping cycles. A seasonal cycle causes a fluctuation according to season, being highest in summer (November-

May) and lowest in late winter/early spring (September-October) (Drinnan, 1997; Murtagh, 1999; Williams and Home, 1988), whilst water supply can cause fluctuations on a monthly scale (Murtagh, 1988). The fluctuations in the composition of major components were minor and likely not related to seasonal change in leaf oil concentration (Murtagh and Smith, 1996). More recent research by de Figueiredo (2006) also confirmed that oil concentration and composition of this species, planted in South Africa, varied throughout the season. However, the reason why this fluctuation takes place in *M. alternifolia* has yet to be well understood. Whether it is attributable to the leaf age distribution and specific leaf area or other factors is still unknown (Murtagh, 1999). Seasonal variation in oil concentration also occurs in other myrtaceous species with sub-epidermal oil glands [e.g. *E. camaldulensis* (Doran *et al.*, 1995), *E. kochii* and *E. plenissima* (Brooker *et al.*, 1988), *E. polybractea* (Milthorpe et al., 1994) and *E. radiata* (Kar, 2003)].

A daily variation of oil concentration in *M. alternifolia* trees was observed by Murtagh and Etherington (1990), possibly due to the different levels of metabolic activity or oil losses through stomata or the cuticle (Murtagh, 1989, 1991b). However the pattern of daily variation was not consistent between days or between sites (Murtagh and Etherington, 1990). To elucidate this result, List *et al.* (1995) observed the functional anatomy of oil glands and found that the oil loss from the leaf between modified epidermal cells that capped mature oil glands is more likely than loss through stomata. The oil loss occurs when the turgidity of the epidermal gland cap cells changes (e.g. due to changes in leaf water potential). The change in turgor and shape of the epidermal cells may affect the effectiveness of steam distillation. Therefore, List *et al.* (1995) concluded that the daily variation of oil concentration noted by Murtagh and Etherington (1990) appeared to be attributable to the efficiency of the steam distillation process rather than Murtagh's (1988) hypothesis that it was related to the minimum temperature of the preceding night.

Trends in diurnal variation in oil concentration of *M. alternifolia* are not entirely clear. List *et al.* (1995) and Curtis (1996) found no significant variation in oil concentration due to diurnal factors. Murtagh and Baker (1994), however, revealed significant diurnal variations in oil concentration and concluded that they were

associated with changes in water vapour pressure deficit of the atmosphere. Murtagh (1999) also proposed a double pool conceptual model for oil storage (i.e. one pool represents a stable storage, while the second pool has a more variable concentration) to explain the rapid recovery in oil concentration following a short-term loss of oil in *M. alternifolia*. The new oil is possibly obtained either from direct synthesis or interconversion from other chemical compounds (Murtagh, 1999).

Murtagh (1988) suggested also that oil formation in taxa with glandular hair reservoirs (including *Myrtaceae*) may be more affected by environmental factors than other taxa with schizogenous ducts [e.g. *Pinus*]. In an earlier review, Flück (1963) noted that diurnal variation in essential oil was undoubtedly proven in plants with glandular hairs, e.g. *Salvia officinalis*, but there was no significant variation in plants with excretory glands [or schizogenous ducts], e.g. *Pinus silvestris*. This is because of the difference in the anatomy of the oil bearing structure leading to the possibility of oil losses through evaporation and resinification. Oil loss from glandular hairs is easier, as they are covered by only a very thin lipophilic cuticle, whereas the excretory gland is protected by a thick-walled epidermis and even-walled fibrous hypodermis (Flück, 1963).

In addition, Brooker *et al.* (1988) reported that neither time of day nor position in the crown resulted in significant change in oil content in *E. kochii* and *E. plenissima*, species which have sub-epidermal oil glands. Another research in Myrtaceae by Leach and Whiffin (1989) found that diurnal variation in essential oil of *Angophora costata* is minimal. They found that only two out of 58 compounds present in the oils of this species showed significant diurnal variations.

2.2.5 Environmental factors

Flück (1963) noted that the most important extrinsic factors affecting the production of secondary plant products are climate (i.e. precipitation, temperature and radiation) and soils (i.e. physical, chemical and microbiological factors). The effects of environmental factors on the concentration and composition of essential oils are likely to involve a complex mixture of variables and the response to such factors varies both within and between species (Doran, 1991).

Water stress

The response to water stress varies both within and between species (Holtzer *et al.*, 1988). Water stress tends to decrease oil production in trees (e.g. *Pinus, Abies* and *Pseudotsuga*) (Gershenzon, 1984). Doran and Bell (1994) found that prolonged drought stress reduced oil production in young leaves of *E. camaldulensis*.

In contrast, Gershenzon (1984) reported that many species of herbs and shrubs (e.g. *Marjorana hortensis, Menta piperita,* and *Satureja douglasii*) respond to water stress by slowing growth while continuing to produce secondary metabolites, leading to higher oil concentrations. More recent studies on herbaceous plants such as *Mentha arvensis* by Misra and Srivastava (2000) and *Satureja hortensis* by Baher *et al.* (2002) have confirmed the report by Gershenzon (1984). Charles *et al.* (1990) and Simon *et al*. (1992) noted that water stress induced alterations in oil accumulation appear mainly due to the effect of water stress on plant growth and differentiation. This is because the oil gland density of plants under conditions of stress is higher due to the reduction in leaf area.

Few studies have documented the effects of water stress on oil concentration of *M. alternifolia.* List *et al.* (1995) and Drinnan (1997) reported that a short term water stress (8 days to 2 weeks) had no effect on oil concentration and oil composition. Drinnan (1997) observed that a prolonged period of water stress (1-3 months) reduced oil concentration in *M. alternifolia* when it was planted in a light sandy soil. He also found that the highest oil concentrations occurred when soils were continuously moist but not saturated. In contrast, Murtagh (1991a) reported that *M. alternifolia* planted in moisture retentive soils in an irrigation area increased water content but without a corresponding change in oil concentration.

Light and fertilizer

The effects of light and fertilizer on the oil concentration are hard to define. Both in herbaceous and woody plants results have been inconsistent. Several studies on herbaceous plants reported that the oil concentration in *Juniperus horizontalis* increased by increasing light intensity while fertilizer inputs reduced oil

concentration (Fretz, 1976). On the other hand, Hashemi *et al.* (2008) documented that N fertilization up to 60 kg/ha could increase oil concentration of *Cuminum cyminum* but oil concentration decreased at the higher levels of N fertilization. Similarly, Farooqi *et al.* (1999) reported that the oil concentration per unit tissue weight of *Menta* spp. was higher in the plants exposed to sunlight for a short period than those exposed for longer periods.

An inconsistent effect of fertilizer on oil concentration has also been reported in Myrtaceae such as *Eucalyptus*. Application of nitrogen and potassium fertilizer increased leaf production and leaf oil concentration in *E. radiata* (Kar, 2003). However, Milthorpe *et al.* (1994) found an inconsistent effect of fertilizer on oil production of *E. polybractea.*

There has been very little research into the effect of light and fertilization on oil concentration of *M. alternifolia*. Drinnan (1997) reported that the oil concentration of nutrient deficient plants was 1.5-2.5% lower than that of healthy plant. On the other hand, List *et al.* (1995) revealed that nitrogen and phosphorous fertilizer and light levels appeared to have no effect on both leaf oil concentration and oil composition. These inconsistent results suggest caution in acceptance of any generalised relationship between oil concentration and either fertilizer input or light intensity.

Temperature and humidity

Production of secondary plant products is often influenced by temperature (Flück, 1963). The effect of temperature on oil concentration of *M. alternifolia* was examined by Curtis (1996) who found that oil concentration was more than doubled when temperature was increased from 15/10°C (day/night temperatures) to 30/25°C, with a rate of gain of 1.27 mg/g/^{o}C. However the oil concentration was less at a temperature of 35/30°C. Other research by Murtagh and Smith (1996) showed that an increase in the mean temperature over 3 months before harvesting increased the oil concentration at a rate of 1.02 mg/g/^{o}C. Curtis (1996) also noted that oil concentration was principally related to leaf age and the minimum temperature of the morning preceding harvest. The oil concentration of 100 day-old leaves increased from 42 to 50 mg/g when the minimum temperature decreased from 20 to 10°C. The

older leaves (300 days) were not affected by the temperature change. The oil concentration is also likely to be affected by relative humidity, and was greater when the daytime relative humidity was high (Colton *et al.*, 2000; Drinnan, 1997).

2.3 Factors related to biomass production

M. alternifolia is adaptable to a wide range of soil types, yet it requires specific climate and soil conditions to produce a consistently high yield (Colton and Murtagh, 1999). A highly productive commercial plantation can be achieved when it is planted on a site which mimics its natural conditions of damp soil in humid, sub tropical areas of northern New South Wales (Colton *et al.*, 2000). The productivity of plantations depends on the biomass yield and oil concentration in the leaves. The total production of biomass of *M. alternifolia* is determined by various factors including temperature, water availability, plant density and month of harvest (Murtagh, 1996).

2.3.1 Temperature

M. alternifolia grows optimally when the temperature is between 16°C and 35/30°C (day/night temperature) (Colton *et al.*, 2000). Curtis (1996) revealed that in a controlled environment, the leaf emergence rate increased from 0.1/d to 2.1/d as temperature increased from 15/10°C to 35/30°C, but the rate of increase began to slow at the higher temperatures. Murtagh (1996) found that the proportion of leaf in twig on a dry weight basis was influenced by air temperature as growth of leaves was more likely to be restricted by cool conditions than was the growth of fine stems.

2.3.2 Water stress

The effect of water stress on biomass production is associated with moisture availability in the subsoil (Murtagh, 1999). Water stress in *M. alternifolia* starts when the soil is dried to less than 69% of total available water content (Murtagh, 1996). The distinct effect of water stress on plant growth occurs in the post-flush stage of growth (Murtagh, 1999). Prolonged water stress reduced growth rates and caused extensive defoliation of the trees (Drinnan, 1997). In Murtagh's (1996) findings,

water stress occurring in spring reduced biomass to an average 24% of the optimum yield. However, Small (1981) found no clear association between biomass yield of *M. alternifolia* and annual rainfall. The reduction of biomass production, height, and branching due to water stress has also been reported in herbaceous oil-bearing plants (e.g. *Mentha arvensis*, Misra and Srivastava, 2000).

2.3.3 Planting density

Small (1981) compared the response of *M. alternifolia* to plant spacing (i.e. 1.22, 0.61 and 0.305 m within-row by 1.22 m between-row spacing) and found that this species is tolerant to close spacing, with leaf yield per hectare increasing concomitantly with increasing plant density. However, he found that the leaf fresh weight per tree increased quadratically with increased plant spacing and the tree weight decreased by 41% when the plant population was increased fourfold. This is most likely because the high plant density induces small branches, slow diameter growth, a low degree of stem taper, and rapid upward retreat of the bases of the live crowns (Smith *et al.*, 1997). Consistent with Small's (1981) findings, Macdonald (n.d.) reported that *M. alternifolia* trees planted at plant stocking of 13,300 plants per hectare in the Fraser Coast region of Queensland were stronger, healthier and had a denser leaf mass than plants at higher stockings.

A significant quadratic relationship between plant density and biomass production on a dry weight basis of *E. polybractea* and *E. kochii* was also found by Milthorpe *et al.* (1998). This result came from a trial comparing 5 planting densities (between 2,000 and 9,000 plants/ha) which was harvested annually. An indirect effect of higher plant density is that, combined with optimal growth conditions, the crop trees will have a competitive advantage over weeds (Virtue *et al.*, 2000). This helps prevent biomass yield losses.

2.3.4 Other effects

Total plant biomass of *M. alternifolia* is affected by month of harvesting, being highest when plants are harvested annually between July and October. This is probably because the most efficient stage of coppice growth (4-6 month after

harvest) was matched with the optimum growing conditions of high soil moisture and warm temperature (Virtue *et al.*, 2000) over January-March and December-April respectively (Murtagh, 1999). Virtue (1999) also suggested that harvesting plantations in late spring or summer will provide optimal growth conditions to accelerate shoot emergence and growth of *M. alternifolia*. The effect of month of harvest on coppicing ability and leaf yield of *E. radiata* was investigated by Kar (2003) who revealed that the highest leaf production of 12-month old coppice was achieved when trees were harvested in summer due to vigorous shoot growth in the following spring.

Fertilizer applications generally affect oil yield by enhancing the amount of biomass yield per unit area (List *et al.*, 1995; Sangwan *et al.*, 2001). Kar (2003) reported that application of nitrogen and potassium fertilizer effectively increased biomass of *E. radiata* by increasing leaf : wood ratio. Drinnan (1997) also documented that *M. alternifolia* grew quicker when small amounts of fertilizers (i.e. nitrogen, potassium and phosphorous) were regularly applied.

Another factor than can hamper growth of *M. alternifolia* during both establishment and the annual regrowth cycle is competition for water, nutrients and-or light by weeds (Virtue, 1999). Virtue *et al.* (2000) found that the weed interference could reduce leaf yield of coppice by an average of 25%.

2.4 Financial analysis of tea tree plantations

Net present value (NPV) and internal rate of return (IRR) can be used to determine whether a project is financially acceptable (Perkins, 1994). Campbell and Brown (2003) define NPV as the difference between the discounted present value of future benefits and the discounted present value of future costs. While IRR is the discount rate at which the NPV equals zero. Generally, a project with NPV more than or equal to zero and IRR more than or equal to a real discount rate is accepted as financially viable. The situation is different when selecting the best project to be accepted among mutually exclusive projects. A project is preferable when its NPV and IRR are higher than other projects. However, in some cases, the NPV and IRR decision-rules can end up with a conflicting result because of the ‘switching’ phenomenon

which allows changes in the ranking of the projects. Because of the possibility of switching, Perkins (1994) and Campbell and Brown (2003) suggested that the decision-rule for mutually exclusive projects, such as is the case in this study, is to give preference to the project with the highest NPV.

There are a number of financial modelling approaches for estimating costs, cashflows and financial returns of tea tree plantations, such as reported by Reilly (1991), Hinton (1994), Hinton (1999) *cited in* Colton *et al.* (2000) and Agtrans Research (2001) *cited in* Doran *et al.* (2002). Generally, tea tree plantations with a productive life expectancy of 15-20 years or more are highly profitable and have good internal rates of return at oil prices of $40-45 with average oil yields of 170-220 kg/ha (Colton *et al.*, 2000). Details of previous financial modelling of tea tree plantations are given below. It should be noted, however, that assumptions used in these financial analyses are now dated as there have been significant changes in costs over the years. Caution is advised when interpreting the results of these analyses as a consequence.

Reilly (1991) classified tea tree production systems into three categories: highly mechanized large scale plantations with installed steam distillation; smaller scale plantations with suspended or immersed charge, low pressure distillation, and; natural stands using a bush still. A hypothetical case study of a 75 ha plantation was used to represent the first farm category. Some assumptions were used in this analysis, i.e. capital cost of $684,500; year 1 planting and running costs of $407,250; year 2 to 10 running costs of $200,800; overhead cost for running a plantation of $12,000 per year; plant depreciation of $21,684 per annum. An interest rate of 12% was charged ($38,496 per annum); planting density of 30,000 plants/ha; the plantation was mature and at full production from year 2 onwards, and had an expected productive life of 10 years. The profit and returns from tea tree plantations from this simulation were dependent on the yield and price of oil, as summarised in Table 2.1. The plantation was profitable when the oil prices and oil yields exceeded $40/kg and 100 kg/ha respectively.

A 15 ha plantation was chosen for the second case study by Reilly (1991) and, as with the previous case study, some scenario-specific assumptions were used in this

simulation. Smaller plantations were generally a secondary venture, hence, the value of land was slightly less than in the previous simulation and equipment was mainly second hand and shared with other enterprises. The planting density was also reduced to 20,000 plants/ha. Therefore, the capital costs were lower than the previous model i.e. $207,000. Planting and running costs for year 1 were $70,660 while running cost for the subsequent years was $35,750. Other costs which included overheads, depreciation of plant and interest charged for plant at 12% were $4,000, $9,525, and $17,640 per year respectively. As in the previous hypothetical plantation of 75 ha, the 15 ha plantation was considered profitable when the oil prices exceeded $40 and oil yields were greater than 100 kg/ha. However, the 75 ha plantation had a slightly higher return on capital than the 15 ha one (Reilly, 1991).

Table 2.1 Profit ($) and return on capital (%) at 1991 costs for a 75 ha tea tree plantation for various yields and oil prices (Reilly, 1991)

Oil yield (kg/ha)	Oil price ($/kg)				
	20	30	40	50	60
100	-164,905 (-7.6)	-89,905 (0.9)	-14,905 (5.9)	60,095 (12.7)	135,095 (19.5)
150	-90,905 (0.9)	21,595 (9.3)	134,095 (19.5)	246,595 (29.7)	359,095 (39.9)
200	-14,905 (5.9)	135,095 (19.5)	285,095 (33.1)	434,095 (46.7)	585,095 (60.3)
250	60,095 (12.7)	247,595 (29.7)	435,095 (46.7)	622,595 (63.7)	810,095 (80.7)

Note: Figures in the parentheses indicate return on capital (%)

Different scenarios for tea tree plantation establishment were employed by Hinton (1994) to estimate the profitability of tea tree plantations on irrigated farms in the Mareeba-Dimbulah region of northern Queensland. Three 10 ha hypothetical or model farms based on their different harvesting and distilling regimes (viz. contract harvesting and distilling; purchase harvester and on-farm distilling plant, and; costs of harvester and on-farm distilling plant shared with another farm) were used. The 10 ha farm was chosen as this size represented the “living area” for a family unit in this region. The following assumptions were used in the analyses: the establishment costs were $164,590, $246,590 and $188,090 for farm 1, 2 and 3 respectively; tree planting density of 30,000 plants/ha; estimated oil yield of 175 kg/ha/harvest, which was derived from 0.7% of 25,000 kg leaf fresh weight, from year 3 onwards; 50 and 80% of full production was derived from year one and two respectively; farm gate oil

price of $50/kg; rotation time of every eight months after the first harvest at twelve months; expected productive tree life of 10 years; and real discounted rate of 6%. It was revealed that the third farm scenario was the most profitable with an internal rate of return (IRR) of 24.33% in comparison to 11.78% and 17.68% for farms 1 and 2. Plantation outlays were recovered by the end of years 9, 7 and 5 for farms 1, 2 and 3 respectively (Figure 2.1). The returns to capital and management cost were $10,261, $27,808, and 36,495 for farms 1, 2 and 3 respectively.

A sensitivity analysis to show the effect of oil price and oil yield changes on profitability was also carried out. The break-even price for tea tree oil production from farms 1, 2 and 3 were $46, $38 and $34/kg respectively. Assumed oil yield was 175kg/ha/harvest while break-even oil yields were 158, 140 and 123kg/ha/harvest from farms 1, 2 and 3 respectively, assuming an oil price of $50/kg (Hinton, 1994).

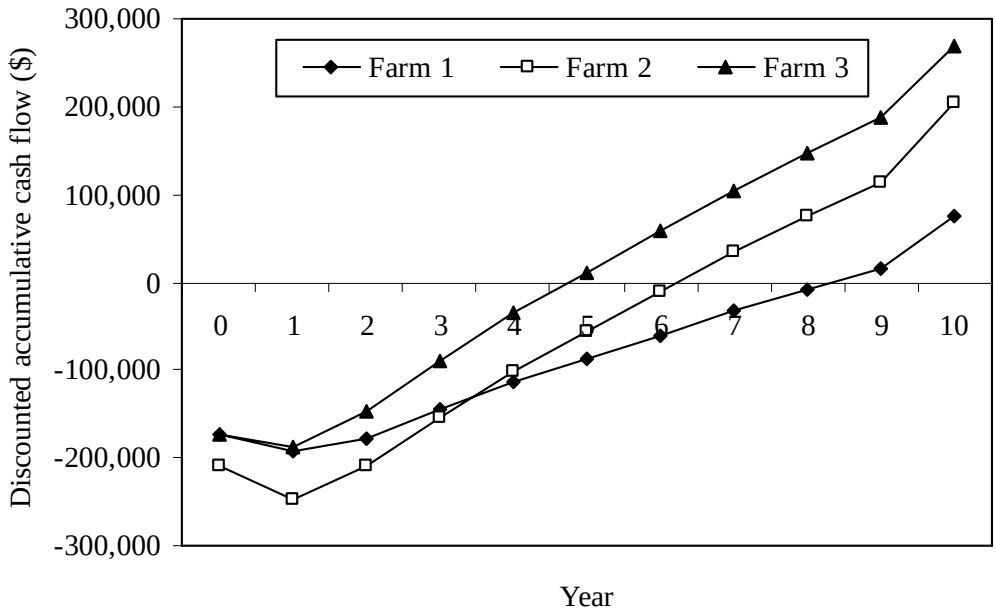

Figure 2.1 Discounted accumulative cash flow for model 10 ha tea tree farms in northern Queensland at 1994 prices (Hinton, 1994)

In a later study, Hinton (1999) *cited in* Colton *et al.* (2000) estimated that in a 20 ha irrigated tea tree plantation in northern Queensland (farm model 2 of the previous scenario), the capital and establishment cost was $504,000 or $25,200/ha and annual running costs were approximately $2,350/ha. The break-even oil price for the plantation at 1999 costs was $34.80, assuming an oil yield of 250 kg/ha (Hinton, 1999 *cited in* Colton *et al.*, 2000). In comparison, in the Northern Rivers district of

NSW, the establishment costs (without land cost) for a minimum 25 ha plantation in 2000 totalled about $7,000 - 9,000/ha, while the annual operational costs were approximately $2,500 - 3,500/ha. The break-even price for average oil yields of 170-220kg/ha was $25 (Colton *et al.*, 2000).

Five scenarios around a 100 ha tea tree plantation have been used by Agtrans Research (2001) *cited in* Doran *et al.* (2002) to demonstrate a commercial investment analysis. These included a commercial plantation using improved seeds and clonal materials from the RIRDC/ATTIA tea tree breeding programme (i.e. seed from selected provenance with gains of 30% over oil yield of industry standards of 140 kg/ha (192.4 kg/ha), improved seeds with gains of 60% (236.8 kg/ha), 90% (281.2 kg/ha), 120% (325.6 kg/ha) and clonal materials with gains of 150% (370 kg/ha). These investments assumed a seedling and cutting price of $0.10 and $0.37 respectively. Total establishment costs of seedling-based plantations were $12,768/ha (38,000 plants/ha) while clone-based plantations were $20,068 (30,000 plants/ha). Annual running costs were assumed equal between them in both non mature and mature yield, i.e. $2,746 and $3,233/ha respectively. The proportion of oil yield in year 1 and year 2 were 50% and 75% of mature yield. Trees were expected to be productive for 15 years. It was estimated that the break even oil price of the five plantation scenarios at 10% discount rates were $27, $22, $19, $16 and $17 respectively. An oil-price sensitivity analysis is summarized in Table 2.2.

Table 2.2 Result of price sensitivity analysis

Criteria	Price ($/kg)	Farm 1 (30%)	Farm 2 (60%)	Farm 3 (90%)	Farm 4 (120%)	Farm 5 (150%)
NPV ($)	25	- 310,817	460,069	1,230,956	2,001,842	2,042,729
	30	357,284	1,282,348	2,207,412	3,132,475	3,327,539
	35	125,386	2,104,627	3,183,386	4,263,109	4,612,350
B/C	25	0.8 to 1	1.4 to 1	2 to 1	2.6 to 1	2 to 1
	30	1.3 to 1	2 to 1	2.7 to 1	3.5 to 1	2.7 to 1
	35	1.8 to 1	2.7 to 1	3.5 to 1	4 to 1	3.3 to 1
IRR (%)	25	6	15	22	29	23
	30	14	23	31	38	31
	35	21	30	39	47	37

The anticipated gains in productivity through use of different levels of improved germplasm are given in brackets. B/C is benefit:cost ratio. Farm 5 (150% gain) is the clonal plantation model. Source: Agtrans Research (2001) *cited in* Doran *et al.* (2002)

The assumption of the proportion of mature oil yield of clones in year 1 and 2 employed by Agtrans Research (2001) *cited in* Doran *et al.* (2002) was considered to be an under estimate as clones give higher oil concentration compared with seedlings at the first harvest (i.e. 12 months from planting). This is most likely related to their physiological maturity, where clones exhibit mature oil levels from planting that will only be reached by seedlings at the third harvest (Baker *et al.*, 2007).

It is clear that growing tea tree as a commercial plantation is reasonably costly but can provide high profits and good internal rates of return. The profitability of this venture is very sensitive to changes in oil yield and oil price.

2.5 Clonal plantations

2.5.1 Development of clonal plantations

The techniques of cloning forest trees and deploying clonal plantations of forest trees have been in existence for hundreds or even thousands of years (Libby and Ahuja, 1993). Examples are in sugi (*Cryptomeria japonica*) in Japan (Ohba, 1993) and poplars (*Populus* spp.) and willows (*Salix* spp.) in many European, Asian and Mediterranean countries (Zsuffa *et al.*, 1993). Clonal forestry using grafting was proposed by G. Anderson in 1906 in Sweden followed by other countries i.e. Germany and Denmark in the early 1920s, but was not adopted because of cost and knowledge considerations (Libby and Ahuja, 1993). Afforestation using Chinese fir (*Cunninghamia lanceolata* (Lamb.) Hook) stump cutting has been widely practised in southern China for the past 800 years. However, this tradition has been gradually replaced with seedling-based forestry since 1957 (Minghe and Ritchie, 1999).

Steckling, that uses rooted cuttings that are sufficiently developed to plant, has been used for reforestation of yellow-cedar in British Columbia since the 1970s (Russell, 1993). A rising interest in clonal forestry in the tropics began in the early 1970s particularly for short rotation, fast growing species for supplying demand of pulp and paper industries. The shift from use of seedlings to rooted cuttings for establishing eucalypt plantations started in the late 1970s (Evans and Turnbull, 2004). The potential enhancement of production from clonal eucalypt plantations was first

realised by Aracruz Forestal S.A in Brazil. Since then, the clonal propagation method has been applied to other tropical species such as pines, teak, *Gmelina arborea*, *Acacia mangium* and its hybrids with *A. auriculiformis* and many other species (Evans and Turnbull, 2004). Clonal forestry of *Eucalyptus* species and its hybrids is recognised as the most successful clonal forestry program in tropical and subtropical regions (Libby and Ahuja, 1993; Zobel, 1993).

2.5.2 Advantages of clonal plantations

The advantages of vegetative propagation for commercial plantations compared to seedlings are worthy of note. Potential advantages of clonal forestry were highlighted by Libby (1985) and can be summarized as follows: (1) the ability to rapidly capture a greater proportion of the additive and non-additive genetic variation than can be achieved by conventional breeding programmes, (2) the ability to identify and provide clones that are well adapted to a particular site, and the possibility of attaining specific and optimal deployment of sets of clones, (3) the elimination of inbred individuals from production plantations, (4) the mass production of valuable but expensive genotypes obtained through hybridization or biotechnology, (5) the ability to use maturation states other than juvenile, (6) the possibility of using "correlation breakers", to capture two or more favourable characteristics which are usually negatively correlated, (7) the ability to select and utilize greater genetic diversity than is normally found in a single progeny, (8) the greater simplicity of managing of hedge-orchards than of managing seed-orchards, (9) the shorter period between selection and production, compared to seed orchards, (10) the ability to programme planting sequences and increasing plantation productivity by reducing negative competitive interaction, and (11) the rapid deployment of increasingly superior clones passing through multiple trait selection programmes.

Such advantages have also been discussed by Evans and Turnbull (2004) i.e. overcoming inadequate seed supply, uniformity of trees in terms of growth and yield, maximum capture of the genetic gains achieved from tree breeding, multiplication of hybrids, adaptation to specific sites, resistance to particular diseases and reducing wood production cost. The most important advantage of clonal plantations in

supporting a tree breeding programme is that genetic gains achieved in tree breeding can be maximally captured by cloning and directly used in operational plantations (Evans and Turnbull, 2004). Thi is a result of the direct passing-on of the desired characteristics in the selected, genetically improved trees (ortets) to their offspring (ramets) (Frampton and Foster, 1993; Nikles, 2004). For instance, a significant improvement of pulpwood productivity of eucalypt plantations from 5.9 air-dried t/ha/yr to 10.9 t/ha/yr has been achieved by Aracruz Celulose SA in Brazil through establishing plantations using clones produced as the result of a selection and breeding programme (Campinhos, 1999).

2.5.3 Problems and risks in developing clonal plantations

It has been considered that while clonal plantations promise potential advantages as previously discussed, some risks are attached in adopting them (Burdon and Aimers-Halliday, 2003; Leakey, 2004a). Problems involved in developing clonal plantations have been discussed by Evans and Turnbull (2004) and Burdon and Aimers-Halliday (2006). Burdon and Aimers-Halliday (2006) classified such risk into three categories: propagation failure; underperformance of successfully propagated clonal material due to cultivar decline or inadequate clonal evaluation, and; delayed clonal failure.

Propagation failure includes complete failure and difficult or costly propagation that hampers some clones from being used commercially (Burdon and Aimers-Halliday, 2006). Failure in achieving successful propagation of desired clones due to different levels of rooting ability can result in some promising genotypes being excluded from the clonal programme (Evans and Turnbull, 2004). This may lead to a narrowing of the genetic base of deployed material, therefore, increasing risk of delayed clonal failure (Burdon and Aimers-Halliday, 2006). Maturation or physiological aging of a plant has been identified as an agent of rooting difficulty in many species generally amendable to being vegetatively propagated (Bonga and von Anderkas, 1993; Burdon and Aimers-Halliday, 2006; Evans and Turnbull, 2004; Greenwood and Hutchinson, 1993). In addition, some species are difficult to vegetatively propagate even from juvenile material such as *Acacia mearnsii*, *Eucalyptus globulus* and *E. nitens* (Evans and Turnbull, 2004). Rates of propagation failure are highly dependent

on species, genetic groups within species and the mode of propagation technique or clonal storage technologies (Aimers-Halliday and Burdon, 2003; Burdon and Aimers-Halliday, 2003). Maturation has been a particular problem in developing clonal plantations for most *Pinus* species (Burdon and Aimers-Halliday, 2006). However, this problem is less serious in some hardwood species e.g. poplars, aspens and willows (Zsuffa *et al.*, 1993) and most eucalypt species (Eldridge *et al.*, 1994).

There are some ways to deal with the adverse effects of plant ageing, including rejuvenation by coppicing, serial propagation of cuttings over several generations, repeated grafting and micropropagation to improve rooting ability of plant material from mature or old trees (Bonga and von Anderkas, 1993; Eldridge *et al.*, 1994); delaying maturation in juvenile material by hedging (i.e. regular severe pruning of seedlings or stecklings) (Bolstad and Libby, 1982); retaining juvenility of clones through cool storage of organ/tissue, cryopreservation and somatic embryogenesis plus storage (Eldridge *et al.*, 1994) and involving active countermeasures, for example developing more reliable and economic propagation and clonal storage (Aimers-Halliday and Burdon, 2003).

Cultivar decline or inadequate clonal evaluation of successfully propagated clonal material can lead to underperformance of deployed propagules (Burdon and Aimers-Halliday, 2006). Cultivar decline can be expressed by other manifestations of maturation problems such as poor growth, early flowering, some differences in wood properties, poor root systems, slow diameter growth and plagiotropic growth where propagules show branch-like behaviour rather than normal erect form (Bentzer, 1993; Bonga and von Anderkas, 1993; Burdon and Aimers-Halliday, 2006; Eldridge *et al.*, 1994; Greenwood and Hutchinson, 1993). Inadequate evaluation of clones reduces genetic gain to be captured by clones, particularly when genotype-site interaction is substantial (Burdon and Aimers-Halliday, 2006). Therefore, clonal evaluation across multiple sites is required to evaluate the magnitude of rank changes among clones at various sites so as to facilitate optimal deployment of available clones (Frampton and Foster, 1993).

The most widely publicised misgiving of the clonal plantation is categorised as delayed clonal failure, because the perception is that a large biologically-uniform

stand will be at risk from biotic (i.e. pests and diseases) and climatic (such as wind, drought, frost, and snow) damage (Burdon and Aimers-Halliday, 2006; Leakey, 2004a). There are multiple examples where monoclonal plantations have proven to be at high risk of serious damage or even complete failure due to pests, diseases or other hazards, e.g. outbreak of stem-canker and leaf-rust on poplar clone I-214 in Yugoslavia (Kleinschmit *et al.*, 1993). Another example is the severe damage to clonal plantations of poplar in Australia during 1972 and 1973 due to outbreak of leaf-rust (Palmberg, 1978 *cited in* Bishir and Roberds, 1999). It has been considered that the most obvious disadvantage of clonal forestry is a possible reduction of genetic diversity (Kleinschmit *et al.*, 1993). Libby (1985) noted, however, that clonal forestry that deliberately selects highly productive unrelated clones will more effectively maintain genetic diversity rather than traditional plantations of related seedlings from a seed orchard/stand. Another common apprehension of clonal plantations is susceptibility to wind throw due to an absence of tap roots in rooted cuttings (Leakey, 2004a). However, Leakey (2004b) concluded that the lack of a tap root is not the primary reason for tree instability, as in fact not every mature tree grown from seed has a tap root. The most substantial factor in relation to wind stability of trees is the ability to form “sinker” roots. The problem of roots lacking the ability to form sinkers can be avoided by selecting only easily propagated plants that form multiple roots combined with conditions and techniques which ensure the rapid formation of a radially-arranged and vigorous root system (Leakey, 2004b).

2.5.4 Clonal deployment

A successful clonal plantation depends on the development of appropriate clones and the deployment of the clones (Foster and Bertolucci, 1994). The high risk of deploying a single clone over a large area must be considered by plantation managers. The options are then to choose whether a plantation will be an intimate mixture of several clones, mosaics of different clones in monoclonal blocks (Burdon and Aimers-Halliday, 2006; Evans and Turnbull, 2004; Foster and Bertolucci, 1994; Lindgren, 1993) or different subsets of the deployed clones in mixture (Burdon and Aimers-Halliday, 2006) or even mixtures of clones and seedlings (Kleinschmit *et al.*, 1993).

Theoretical considerations lead to mosaics of monoclonal blocks being considered unfavourable, however, several experiments on mixed clone plantations have largely failed to support the expected superiority of this mode (Foster and Bertolucci, 1994). For instance, Zobel (1992) reported that about 20% of *Eucalyptus grandis* clones in a 20-clone mixture used in plantation establishment were suppressed and often died before harvest due to different patterns of growth among them. Burdon and Aimers-Halliday (2006) have presented several issues which should be considered in selecting a clonal deployment strategy, i.e. epidemiological considerations, nature and significance of competitive interactions between individual trees and logistics of salvage harvesting.

There are several reasons for planting clones in a mosaic of single clone blocks. For example, establishing, tending and harvesting costs are lower than that of mixed clonal plantations; less silviculture expertise is required (Lindgren, 1993) and surveillance of the growth and health of clones and replacement of those with low performance is easier to carry out (Evans and Turnbull, 2004; Lindgren, 1993). The choice of monoclonal block size depends on species and location (Evans and Turnbull, 2004). However, 10-20 hectares blocks are most preferred for practical operational reasons (Zobel, 1992). For instance, clonal plantations of *Eucalyptus urophylla x E. grandis* hybrids in the Congo involve 15-20 clones in blocks of 20-50 ha of each clone (Leakey, 2004a). This deployment method is likely to be most appropriate for short rotation plantations (Kleinschmit *et al.*, 1993). As within-block genetic diversity is narrow, this leads to greater vulnerability of individual blocks to pest and disease attack and other risks associated with lack of genetic diversity, Arbez (2001) suggested several ways to reduce such risks such as decreasing the rotation length, limiting the plantation area and the period of use of a given clone, and increasing the number of commercially available tested clones.

When clones of long rotation plants are deployed, unlike in the case of short rotation plants that are selected for their performance over only a fraction of the normal rotation, it is possible that unforeseen problems may occur later in the rotation. Therefore, using intimate clonal mixtures or mixtures of clones and seedlings is probably the safest option (Kleinschmit *et al.*, 1993). A mixture of clones provides

several potential advantages: greater stability in available environment, less risk of pest and disease attack, and yield might be increased as diverse genotypes can exploit different part of the site (Evans and Turnbull, 2004).

Several aspects need to be considered to decide the optimum number of clones to use in a commercial plantation to protect against catastrophic failure while at the same time achieving stand uniformity with high yield and ease of management (Bishir and Roberds, 1999). Such considerations are related to species, length of rotation, environment, genetic make up, variation and adaptability of the clones (Evans and Turnbull, 2004; Zobel, 1992), the fraction of the initial plants remaining at harvest, the intensity of the system and status of the clones, and whether they are well known and high-ranking (Lindgren, 1993). Lindgren (1993) has suggested that the optimum number of clones to be used in commercial plantations remains uncertain although some authors have suggested an optimal number based on theoretical deductions (Libby, 1982; Hühn, 1985, 1986a, 1986b, 1986c).

Mathematical models of risk were employed by Libby (1982) who proposed the following options: a mosaic of several unrelated clones in small monoclonal plantations. This is frequently the best strategy, particularly when many hazards are present; a large mixture of clones which is as safe as a seedling plantation but will give lower genetic gains compared to use of fewer clones; a mixture of 7-25 unrelated clones is likely to be optimal when planting density and subsequent silviculture ensure damage or mortality levels are acceptable to management; a mixture of 2-3 clones is likely to be the worst strategy; and a mixture of a relatively small number of unrelated clones of different species would be the safest choice providing they are well-matched species.

Mathematical models of risk suggest that, in particular circumstances, a large number of clones provide greater risk than a smaller number (Hühn, 1986b; Libby, 1982). Roberds and Bishir (1997) presented theoretical arguments that using more than 40 clones is not required as deploying 30 to 40 unrelated clones in plantations provides an equivalent protection against catastrophic loss to a larger number of unrelated clones. In addition, Bishir and Roberds (1995, 1997) suggested that risk can decrease, remain relatively constant, or even increase as the number of clones

increases. A more recent study by these authors (Bishir and Roberds, 1999) indicated that the choice of number of clones being used in a plantation depends on the level of risk that can be accepted by plantation managers, intensity of pest attack, level of clonal resistance to attack and gene frequencies associated with susceptible alleles.

Chapter 3 Procedures to determine tree biomass and oil traits of tea tree

3.1 Introduction

The main aim of commercial *Melaleuca alternifolia* (Maiden and Betche) Cheel plantations is to maintain or enhance tea tree oil production in terms of quantity and quality. To achieve these enhancements, particularly sought after during times of low prices and growing competition, better-quality germplasm should be used at establishment. Genetically improved seeds from a tea tree breeding project have been released to the industry since 2001. However, to provide highly improved germplasm with superior oil yield and oil quality, the breeding programme previously described in Chapter 1 has developed elite clones as a means to capture improvements in oil production.

The clonal trials established by this project have been evaluated in this study to determine their biomass production and oil characteristics. Evaluation of these variables is needed to determine firstly oil yield as this depends on three principal components (i.e. yield of biomass harvested, the proportion of leaf in the total biomass and the oil concentration in the leaves) (Colton *et al.*, 2000). Secondly, there is a need to know the quality of tea tree oil produced as this will effect its marketability. This is determined by the combination of the oil's physical constants (i.e. refractive index, optical rotation, specific gravity, and solubility in alcohol) and chemical composition for compliance with the particular standard of tea tree oil or market requirement (Southwell, 1999), as described in Chapter 1.

In this chapter, the three procedures, biomass (tree component) dry weight, extraction of leaf oil using solvent techniques, and gas chromatographic analysis used to determine oil yield and oil quality are described. A flowchart of the procedures is given in Figure 3.1. All procedures were undertaken at the Essential Oil Unit of Wollongbar Primary Industry Institute (NSW Department of Primary Industry) from November 2007 to January 2008 for the evaluation of the 2006 clonal spacing trial and from July 2008 to August 2008 for the 2004 clonal spacing trial.

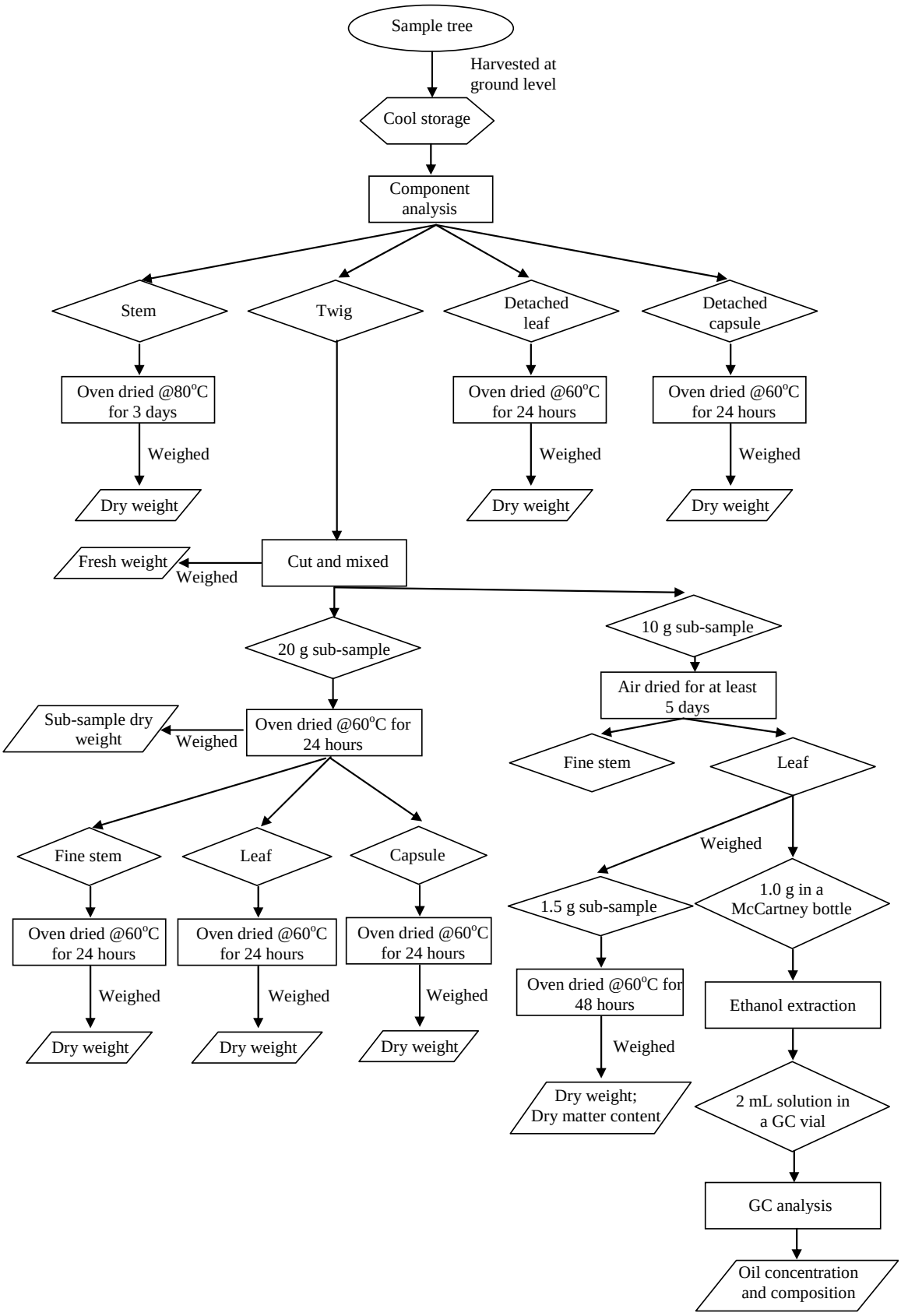

Figure 3.1 Flowchart of the procedures to determine tree biomass and oil characteristics of tea tree

3.2 Procedures to determine biomass dry weight

3.2.1 Introduction

In this study, biomass refers to the above-ground mass of the tree. Tea tree oil occurs largely in oil glands within the leaf (Butcher, 1994; List *et al.*, 1995). There are a number of factors contributing to leaf yield (i.e. total biomass, proportion of twig in the total biomass and proportion of leaf in the twig). It is, therefore, useful to divide biomass into three components (i.e. main stem, fine stem, and leaf) to verify the proportions and correlations among them. Stems are defined by their diameter, with main stems being greater than 2.5 mm while fine stems are 2.5 mm or less and carry most of the leaf. The combination of the fine stem and the leaf components was referred to as twig by Murtagh (1996). This classification then was implemented by Doran *et al.* (2006) with a slightly modified criterion for the fine stem component (i.e. ≤ 3 mm diameter instead of ≤2.5 mm diameter).

Murtagh (1996) showed that the leaf yield of tea tree was strongly correlated with the total yield of biomass (with a correlation coefficient of 0.94), yet, the proportion of leaf in twig and proportion of twig in the total biomass also influenced the total yield of biomass. In Murtagh's (1996) findings, the proportion of leaf in twigs on a dry weight basis varied from 0.58 to 0.71 which was influenced by air temperature as growth of leaves was more likely to be restricted by cool condition than was the growth of fine stems. Whilst the proportion of twig in total biomass was affected by the total tree weight (i.e. it decreased as the total biomass increased). It should be noted, however, that Murtagh's research was conducted on plants raised from seedlings and not from stem cuttings as is the case here.

3.2.2 Materials and methods

To determine biomass, selected trees were harvested by cutting the main stem near ground level, bagged and then stored in a cool room to minimise oil losses due to respiration or volatilization prior to component analysis (Murtagh and Curtis, 1991). These authors documented that *M. alternifolia* foliage can be stored up to 13 days

after harvesting without oil losses or changes in oil composition, provided it is not heated during storage.

In this study, each selected tree was divided into 3 main components: main stem (>3mm diameter); twig (fine stem ≤3mm diameter, attached leaf and, for some trees, seed capsules) and; leaf. A fourth component, an additional capsule component, was added if there were capsules present on the sampled tree (Figure 3.1). Component analysis was completed within 2 weeks after harvest. To obtain dry weights of the key tree component, the main stems were oven-dried at 80°C for 48 hours (to a constant weight) while detached leaf and capsule were dried at 60°C for 24 hours (to a constant weight), and then weighed. The twig component was cut into smaller pieces (approximately 40 mm in length) and mixed. This mixed twig was immediately weighed and 2 sub-samples taken (about 20g to estimate the proportion of leaf in twig and about 10g to determine the oil concentration and composition).

The 20g twig sub-sample was then oven-dried at 60°C for 24 hours (to a constant weight), and weighed. After drying, leaf and or capsule was detached from the fine stem. These components were redried and weighed. Consequently, the total dry weight of each component and the total biomass dry weight of the sampled tree could be calculated using the following formulas:

$$L_{TOT}DW = LDW + \left[\left(\frac{L_{SS}DW}{T_{SS}DW}\right) X \left(\frac{T_{SS}DW}{T_{SS}FW} X\, T_{TOT}FW\right)\right] \text{ ..... (Equation 3.1)}$$

$$FS_{TOT}DW = \left[\left(\frac{FS_{SS}DW}{T_{SS}DW}\right) X \left(\frac{T_{SS}DW}{T_{SS}FW} X\, T_{TOT}FW\right)\right] \text{ ..... (Equation 3.2)}$$

$$C_{TOT}DW = CDW + \left[\left(\frac{C_{SS}DW}{T_{SS}DW}\right) X \left(\frac{T_{SS}DW}{T_{SS}FW} X\, T_{TOT}FW\right)\right] \text{ ..... (Equation 3.3)}$$

$$BIO_{TOT}DW = L_{TOT}DW + FS_{TOT}DW + C_{TOT}DW + SDW \text{ ..... (Equation 3.4)}$$

Where $L_{TOT}DW$= Leaf total dry weight; LDW= Leaf component dry weight; $L_{SS}DW$= Leaf dry weight in the twig sub-sample; $T_{SS}DW$= Twig sub-sample dry weight; $T_{SS}FW$= Twig sub-sample fresh weight; $T_{TOT}FW$= Twig total fresh weight; $FS_{TOT}DW$= Fine stem total dry weight; $FS_{SS}DW$= Fine stem dry weight in the twig sub-sample; $C_{TOT}DW$= Capsule total dry weight; CDW= Capsule component dry weight; $C_{SS}DW$= Capsule dry weight in the twig sub-sample; $BIO_{TOT}DW$= Total biomass dry weight of the sampled tree; SDW= Stem component dry weight.

The subsequent procedures for the 10 g sub-sample are presented in the following section under the heading of Extraction by ethanol.

Figure 3.2 Tea tree components: main stem (A), twig (B), leaf (C) and capsule (D) (Photo: Prastyono)

3.3 Analytical methods for the evaluation of tea tree oil

3.3.1 Introduction

To obtain an accurate oil sample from essential oil-bearing plants, the examination should be focused on only the plant organ that contains the oil (Wish and Williams, 1996). As the essential oil of tea tree is mainly in the leaves (Butcher, 1994; List *et al.*, 1995), evaluation of oils in this study was mainly focused on this organ.

A rapid and accurate solvent extraction method of *Eucalyptus* leaf oil was developed by Ammon *et al.* (1985a) for the GC analysis of terpenes components which later was used by several authors, such as Doran (1992) to evaluate leaves of *Eucalyptus*

camaldulensis and Brooker *et al.* (1988) for determining cineole content of *Eucalyptus kochii* and *E. plenissima*. The relatively similar extraction technique for the *M. alternifolia* (Maiden and Betche) Cheel leaf oil was implemented by Southwell and Stiff (1989) and Brophy *et al.* (1989).

3.3.2 Solvent extraction and Gas Chromatography procedure

Extraction by ethanol

Solvent extraction is an alternative method to steam distillation to extract oil from leaves (Baker *et al.*, 2000; Brophy *et al.*, 1989; Murtagh, 1999). Although solvent extraction has never been seriously considered to obtain tea tree oil on an industry scale, this alternative method has been found to be very useful on a laboratory scale which contributed to an understanding of tea tree leaf chemistry (Southwell, 1999). Oil resulting from solvent extraction has different levels of chemical components to oil obtained from the steam distillation process which is generally accepted by industry (Baker *et al.*, 2000; Southwell, 1999). The demerits of using solvent extraction to obtain oils of tea tree leaves, such as the cost of processing and the scale of operation, may make it impractical for use by the industry (Southwell, 1999).

On a laboratory scale, the advantage of this method is that a smaller sample (single leaf to at least 5g) can be used while steam distillation needs a larger sample size (Murtagh, 1999). In addition, the conversion of precursor compounds in flush leaves (sabinene, *cis*-sabinene hydrate, and *trans*-sabinene hydrate) to the major constituents (terpinen-4-ol and γ-terpinene) as occurs during steam distillation can be avoided by solvent extraction (Southwell and Stiff, 1989). A micro-extraction method which enables the oil from a single tea tree leaf (1-10mg of dry weight) to be extracted and analysed using gas chromatography or gas chromatography-mass spectrometry was employed in research by Brophy *et al.* (1989) and Southwell and Stiff (1989). This allowed an examination for the first time of ontogenetical changes in monoterpenoids of *M. alternifolia* leaf from the apex to the base of the branch (Southwell and Stiff, 1989) and evaluation of leaf oil of *M. alternifolia* seedlings (Russell and Southwell, 2003a, 2003b).

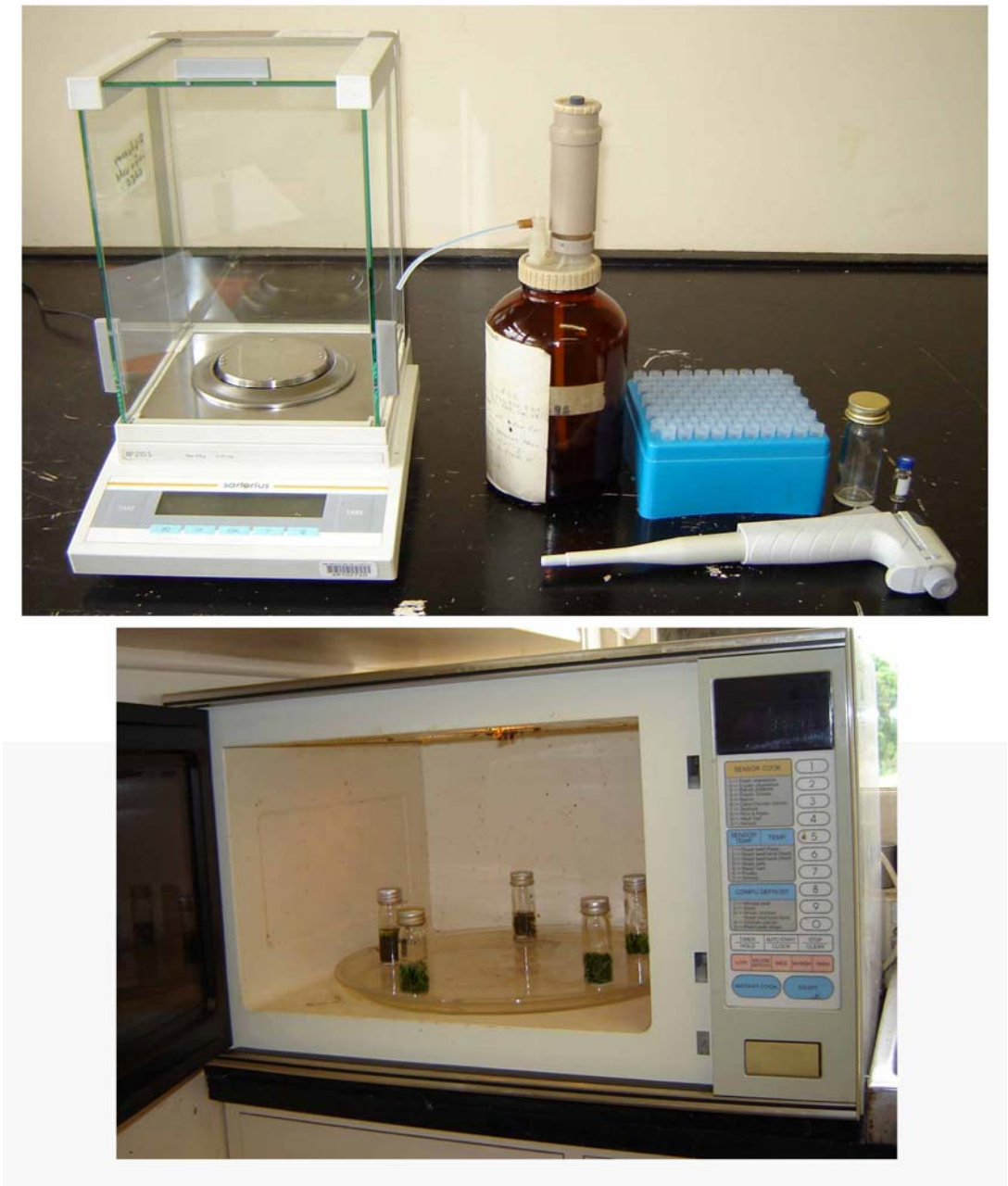

Figure 3.3 Equipment used in the ethanol extraction method (Photo: Prastyono)

A microwave-assisted dry method for extracting essential oils was initiated by Craveiro *et al.* (1989) for leaves of *Lippia sidoides.* It was found that the oil extracted from a 5 minute microwaving method was qualitatively identical to that derived from 60-90 minutes steam distillation. The microwave-assisted ethanol extraction of tea tree leaves was first employed by Southwell *et al.* (1995) who found that 10 seconds of microwave irradiation (700 W) reduced extraction time from 30 hours of extraction at room temperature (20°C) to only 1 hour for a 1 mg sample. A study by Baker *et al.* (2000) concluded that for air-dried tea tree leaf (1 g), the optimum time

ensuring complete oil extraction was 25 seconds of microwave pre-treatment with the leaves in solution allowed to stand for further 3 days.

Baker *et al.* (2000), compared oil recovered from tea tree leaves by ethanol extraction and steam distillation and found that oil recovered from steam distillation was 12-18% lower than that from ethanol extraction technique. The distilled oil also had lower levels of sesquiterpenoids with higher amounts of terpinen-4-ol and 1,8-cineole than ethanol extracted oil. They also confirmed that oil derived from the distillation process was equivalent to oil from the industry process while extracted oil was relatively identical to 'in-situ leaf oil composition'. Therefore they concluded that both solvent and steam distillation techniques are suitable for tea tree oil analysis. However, the solvent extraction method is more practical to implement in a tree breeding project where large numbers of leaf samples are evaluated.

Materials and methods

The 10g sub-sample taken from the mixed twig component (Figure 3.2) was placed in a labelled paper bag and air-dried for at least 5 days prior to extraction. Leaf was separated from the fine stem and 1.0g of leaf then placed into a McCartney bottle for determination of oil concentration and composition. The remaining leaf was oven-dried (60°C for 2 days) to determine leaf dry matter content.

To the leaf sample in the McCartney bottle, 12mL of an extraction solution (ethanol with 0.22% tetradecane as an internal standard) were added, weighed and bottle capped. The capped bottle samples were then heated in a microwave oven for 25 seconds and left to stand for 3 days allowing for full extraction oil (Baker *et al.*, 2000). Afterwards, bottles were shaken to mix the solution and left to stand for about 4 hours, enabling any solid materials in the liquid solution to settle prior to transfer of 2mL of the solution into a vial for gas chromatographic analysis.

Gas Chromatography analysis of ethanol extracts

Gas-liquid Chromatography (GLC) or simply Gas Chromatography (GC) has been used to evaluate *Eucalyptus* oil since the 1960's (Doran, 1992) and similarly the first published report on the GC examination of tea tree oil was in 1966 (Southwell,

1999). The oil for quantitative determination of tea tree oil by GC analysis can be either from stem distillation or ethanolic extraction process. The ethanolic extraction is, however, more expedient than the conventional steam distillation as the extracted oil delivers solutions that is suitable for a direct injection into gas chromatograph for the qualitative determination of tea tree oil (Brophy *et al.*, 1989; Southwell and Stiff, 1989). The addition of a known weight internal standard (e.g. *n*-tetradecane) in the solvent extraction enables concentration of oil in a leaf can be determined by GC analysis of the extracted oil (Baker *et al.*, 2000; Southwell, 1999).

Materials and methods

GC analysis in this study was accomplished using a Shimadzu GC-14B Series instrument equipped with a flame ionization detector (FID) at 300°C (Figure 3.4 (A)). The GC was fitted with capillary column Altech AT-35 (60m length x 0.25mm diameter), with a 0.25 μm film thickness and was operated under the following conditions: hydrogen carrier gas with flow rate 1 ml/minute; injector temperature at 250°C; detector temperature at 300°C; column temperature was programmed to start at 60°C (isothermal for 3 min), then rising at 9°C/min to 240°C, then held isothermal for 7 min; injection, 1 μl of extract solution; split ratio, 25:1; and column pressure, 100 kpa. Samples were loaded using an AOC-1400 auto sampler from a 100-vial carousel. To obtain a correction factor value of oil concentration and terpinen-4-ol content, vials of standard oil were placed in the carousel between every 10 vials of tea tree leaf extract. Run time was approximately 30 minutes for each sample. GC was integrated with a computer with Delta Solution 5.5 software to process the results from the GC analyses.

Typically about 50-90 components were detected from the oil of leaf sample as there is variation in leaf oil composition of *M. alternifolia* (1999) (e.g. see chromatogram in Figure 3.4 (B). Components were manually identified from the chromatogram by comparison of their retention time with those of pure compounds run as reference standards. Retention times of principal components of tea tree oil, 1,8-cineole, terpinen-4-ol and its precursors (sabinene, *trans*- and *cis*- sabinene hydrate) and internal standard (*n*-tetradecane), were recognized and peak area of each component

and total peak area of all components (includes internal standard) were then entered into an Excel Spreadsheet.

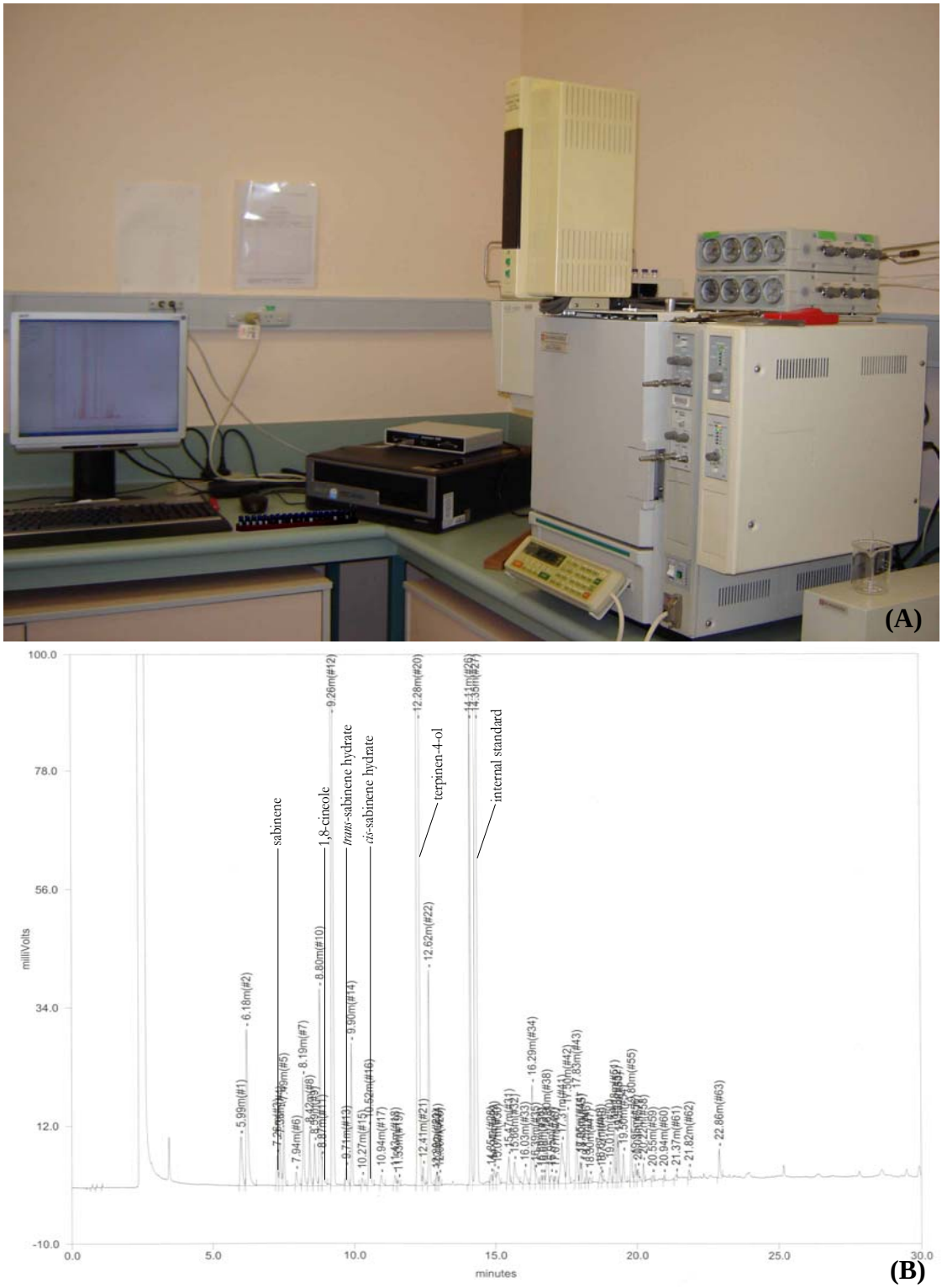

Figure 3.4 Gas Chromatography instrument (A) and example of chromatogram of solvent extract of *M. alternifolia* leaf sample (B) (Photo: Prastyono)

The proportion of each component from the oil was calculated by dividing its peak area with peak area of oil (i.e. total peak area of all components minus peak area of internal standard) and was expressed as a percentage. Southwell and Stiff (Butcher *et al.*, 1994) and Southwell (1989) noted that sabinene, *cis*- and *trans*- sabinene hydrate which are only present in leaves of flush growth are converted to terpinen-4-ol and γ-terpinene as the leaf matures in the tree or during the steam distillation process. However, the proportion of terpinen-4-ol and γ-terpinene formation from these constituents is not clear. Therefore, in this study, it is assumed that sabinene, *trans*- and *cis*- sabinene hydrate are entirely converted to terpinen-4-ol.

In this study, oil concentration was calculated as the weight of oil (in mg) per gram of leaf oven-dry weight (mg/g ODW) using the following equation:

$$\mathbf{O_s = [((A_{tot} - A_{is}) / (A_{is} \times W_{is})) / W_s] / C / RRF \times 100} \quad \text{…. (Equation 3.5)}$$

Where O_s = Oil concentration of sample (leaves) in mg/g leaf oven-dry weight; A_{tot} = Total peak area; A_{is} = Peak area of internal standard; W_{is} = Internal standard weight (in mg); W_s = Sample (leaves) oven-dry weight in the extraction solution (in g); C = Correction factor (in this case, 100 - 104.21); and RRF = Relative response factor of the column to tea tree oil (in this case, 0.891)

3.3.3 Dry matter determination of leaf samples

Oil yield which is typically given on a per unit area basis is derived from the mathematical combination of leaf biomass on a given area and oil concentration in the leaf. Oil concentration is the amount of oil derived from leaves on a per unit weight basis (2003). Because there is considerable variation of water content within fresh leaf, the best way to express oil concentration is on a leaf dry weight basis, even though fresh leaves are usually used in the steam distillation process (Murtagh, 1999). Hence, Murtagh (Murtagh and Smith, 1996) recommended that the standard unit for oil concentration in tea tree should be milligrams of oil per gram of leaf dry weight (mg/g DW). This approach should be implemented in any comparative study of oil production of individual trees such as in the calculation of genetic parameters or studies of seasonal variation (Murtagh, 1999).

A simple and rapid method for determining water content of *Eucalyptus* leaves using the solvent extraction method followed by Karl Fisher titration was described by

Ammon *et al.* (Doran, 1992). Although this method is undoubtedly accurate, the equipment used in this procedure is expensive. A method based on oven-drying of duplicate leaf samples to those taken for ethanol extraction is an alternative method for determining the water content of foliage samples. Even though this method is less accurate than Karl Fisher titration, it is simple and practical to apply (Ammon et al., 1985b). In this study, the oven-drying method was used to determine the dry matter content of tea tree leaf samples.

Materials and methods

To determine the dry matter content of leaf samples for oil extraction, sub-samples were used. From the 10g twig sample a 1.0g sample of leaf was used for extraction while the remaining separated air dried leaf (about 1.5g) was weighed placed into a metal tin and oven-dried at 60°C for 2 days. The dry matter contents of leaf samples were obtained by dividing leaf oven-dried weight by air-dried weight. This dry matter content value was then used as a multiplier for determining the dry matter content of the extracted leaf sample.

3.4 Conclusions

All procedures to determine tree biomass and oil traits of tea tree were based on procedures which have been implemented by previous researchers in their studies on tea tree cited throughout this chapter. There was no new procedure conducted in this study.

The determination of tree biomass (dry weight of key tree components) of tea tree by dividing the tree into 3 or in particular cases 4 components was found to be useful and practicable. Ethanolic extraction of leaves to obtain oil of the leaves gave solution that was ready to be injected into GC for quantitative determination of the major component of tea tree. This method is, therefore, more convenient and practicable than the steam distillation method. However, the different chemical composition between oil obtained from ethanol extraction and steam distillation should be understood.

Chapter 4 Variation in growth and oil traits of *Melaleuca alternifolia* plantations established using clones and seedlings at different spacing

4.1 Introduction

Expanding clonal tree plantations has been the aim of plantation forest managers in several tropical countries for about the last 25 years. Clonal forestry offers some advantages over use of seedlings as discussed in the Chapter 2. One such advantage is in tree improvement where the genetic gains achieved by breeding can be captured efficiently by cloning and used directly in operational plantations (Evans and Turnbull, 2004). Therefore, it is possible that oil yield of commercial plantations of tea tree can be maximised by deploying clonal material of the best selected trees from a breeding programme. Several attempts have been made, either on a small research scale or on a commercial scale such as by Oil Fields Limited in northern Queensland (Prospectus, n.d.), to demonstrate the potential of clones to boost oil yield in tea tree plantations. However, none of the past clonal plantations of tea tree have survived long enough to be successfully evaluated.

A common constraint in developing clonal plantations is associated with the higher production costs of clonal plants. However, current propagation technology has been proven to reduce propagation costs to levels relatively similar to seedling production in *Eucalyptus* species (Evans and Turnbull, 2004). In the case of tea tree, the higher oil yields of clones compared with seedlings has been suggested as a favourable offset to the higher cost of establishment (Doran *et al.*, 2002). The Australian tea tree breeding programme has been working on testing this hypothesis since the first phase of the programme commenced in 1993. Their aim has been to identify and make available to industry superior oil producing clones suitable for mass vegetative propagation to allow industry to evaluate the potential of clonal tea tree plantations. Earlier attempts failed for various reasons but twenty elite clones have now been selected and two field trials established in 2004 and 2006. These field trials have been evaluated in this research.

4.1.1 The first suite of elite clones

Selections of suitable *M. alternifolia* clones for mass vegetative propagation from the progeny trial established in 2000 (PT1), the controlled-cross progeny plots established in 2000 (CC1) and plants in the Wollongbar breeding arboretum were initiated in 2001. There were three levels of screening processes to select the best trees to be cloned, i.e. growth performance, oil characteristics, and rootability as stem cuttings. Initially, a total of 100 and 35 best trees from PT1 and CC1 respectively were selected based on their superior height and leafiness scores. The next stage of screening was to rank the initial selections based on their oil characteristics (oil concentration, 1,8-cineole and terpinen-4-ol levels). Only trees with an oil concentration of more than 50 mg/g, a 1,8-cineole content of total oil of less than 3.5% and a terpinen-4-ol content of more than 37% were included in the rooting ability test. A total of 81 trees (40 trees from PT1, 21 trees from CC1, 20 trees from the Wollongbar breeding arboretum were initially selected, however, only 72 trees made it through to inclusion in the rooting trial. Ultimately, 12 clones with the following mean characteristic were selected from the trial: mean oil concentration of 92.6 mg/g, 0.8% 1,8-cineole and 39.1% terpinen-4-ol content and 88.9% strike rate as a rooted stem cutting. These selected clones were then mass propagated at Toolara nursery in 2003. Two clones, however, failed to grow well at the nursery, leaving 10 clones for inclusion in the 2004 clonal trial at Bungawalbin, NSW (Baker *et al.*, 2007).

4.1.2 The second suite of elite clones

Another suite of elite clones were selected from the 2002 progeny trial (PT2), the controlled-cross progeny plots established in 2002 (CC2) and the 2002 yield trial (YT2). The selection processes were started in July 2003 with the development of a primary selection index based on the growth performance of progeny in the three trials. The subsequent screening processes were the same as those used in selecting the first suite of elite clones. A total of 10 best clones were selected from the second screening processes with the means of oil concentration, 1,8-cineole and terpinen-4-ol percent of total oil and rooting ability 73.9mg/g, 1.2%, 36.7% and 93.4% respectively. Ortets of these clones were transported to Toolara nursery in 2005 for

mass propagation. Ramets were then used to establish further clonal field trials in 2006 at Bungawalbin, NSW (Baker *et al.*, 2007).

4.1.3 Clonal trials

In the first trial, ten clones from the first suite of elite clones were used to establish a clonal yield trial while 3 of these clones were also used to establish a spacing trial. Both were planted adjacent to one another in September 2004 at Bungawalbin, NSW. The 10 best clones from the second suite of elite clones were planted in a second set of clonal spacing and yield trials in October 2006 in an area nearby the 2004 clonal trials.

The purpose of establishing clonal yield trials was to evaluate the performance of selected clones in a commercial plantation setting compared to improved seedlots from the breeding programme. The clonal spacing trials were established to evaluate if improved off-paddock oil yield from using selected clones enabled growers to reduce planting density and associated establishment costs while still delivering increased oil production (Baker *et al.*, 2007).

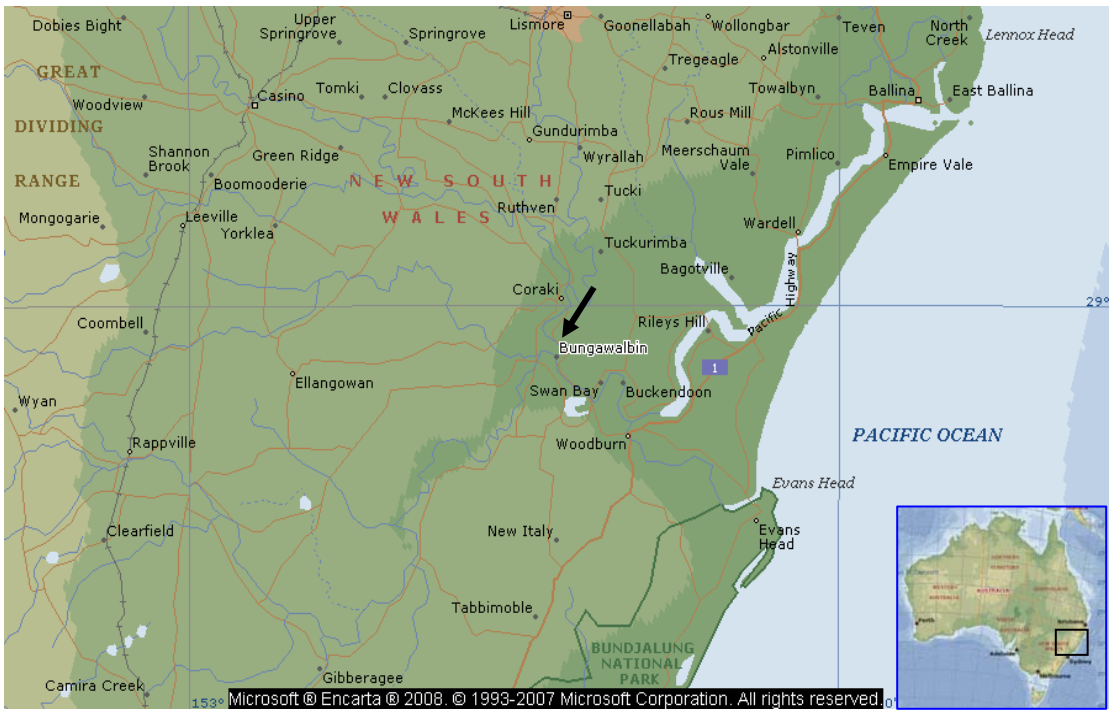

Figure 4.1 Location of the 2004 and 2006 *M. alternifolia* clonal spacing and yield trials at Bungawalbin, NSW

4.2 Objectives

In this study, only the clonal spacing trials were evaluated because of time and cost constraints. The aim was to determine the variation of growth and oil traits of *M. alternifolia* propagated as rooted cuttings of selected clones and seedlings from improved seedlots planted at different spacings in trials established in 2004 and 2006 at Bungawalbin, NSW. The growth traits assessed were tree height, leafiness, frost damage, and dry weights of tree components affecting oil yield. The oil traits determined were foliar oil concentration (mg/g ODW) and percentage of 1,8-cineole and terpinen-4-ol in oil extracts. Oil yields of the clones and seedlings at different plant spacings were derived from the measures of leaf biomass and oil concentration.

4.3 Materials and methods

This study is based on the spacing trials of *M. alternifolia* clones and seedlings at Bungawalbin (Figure 4.1) planted in 2004 and 2006 by the RIRDC/ATTIA tea tree breeding project managed by NSW Department of Primary Industry.

4.3.1 Field trials

2004 Clonal spacing trial

The trial was established in September 2004 at Bungawalbin to evaluate the performance of 3 clones of the first suite of elite clones and 2 seedlots (ATTIA 2A, ATTIA 2B) over 3 different spacings (Baker *et al.*, 2007). Table 4.1 gives information about the origin of these clones and seedlots.

The trial was designed as a split-plot design which comprised 3 main-plot treatments (30, 45 and 60 cm within-row spacing and 1 m between-row spacing) and 5 sub-plot treatments (clone 1, clone 2, clone 3, seedling 1 and seedling 2) with 4 replicates (Figure 4.2). Each plot consists of 2 rows by 10 plants and each replicate has 300 plants. Therefore, the total number of plants in the trial was 1200. There is an edge buffer comprising of one row of seedlings totalling 264 plants. Extrapolation of these

spacings to a per hectare stocking gives 33,333 plants/ha for spacing 1 (30 cm), 22,222 plants/ha for spacing 2 (45 cm) and 16,667 plants/ha for spacing 3 (60 cm).

Table 4.1 Details of the origin of clones and seedlots included in the 2004 clonal spacing trial

Treatment number	Variety number	Source number	Source origin	Family number
1	Clone 1	C9	PT1	22
2	Clone 2	C11	PT1	24
3	Clone 3	C30	PT1	60
4	Seedling 1	ATTIA 2A	SSO1	Bulked
5	Seedling 2	ATTIA 2B	CSO1	Bulked
Buffers	Seedling 1	ATTIA 2A	SSO1	Bulked

Source: Baker *et al.* (2007)

ATTIA2A : SSO1 seed
ATTIA2B : CSO1 seed
CSO1 : first clonal seed orchard est. 1995
PT1 : progeny trial est. 2000
SSO1 : first generation seedling seed orchard est. 1994

B	B	B	B	B	B	B	B	B	B	B	B	B	B
B	5	2	1	5	2	3	3	4	1	2	3	5	B
B	4	5	3	3	1	2	4	5	2	4	4	1	B
B	2	3	4	4	3	5	1	2	4	1	2	3	B
B	3	1	5	2	4	1	5	1	3	3	5	2	B
B	1	4	2	1	5	4	2	3	5	5	1	4	B
B	B	B	B	B	B	B	B	B	B	B	B	B	B
	45 cm	60 cm	30 cm	60 cm	30 cm	45 cm	60 cm	45 cm	30 cm	45 cm	60 cm	30 cm	
	Replicate 1			Replicate 2			Replicate 3			Replicate 4			

Figure 4.2 Split plot design of the 2004 clonal spacing trial at Bungawalbin, NSW, showing replicate, within-row spacing and treatment number. The trial is surrounded by a single-row buffer (B) (Source: G. Baker[3], pers. comm., 2007)

Note: Plan is not to scale as plot area varies depending on spacing.

[3] Tea tree breeding project officer, NSW DPI Wollongbar

This trial was assessed in September 2005 and September 2006 for growth (height and leafiness). Leafiness was subjectively scored from 0.5 (sparse canopy) to 5 (bushy canopy with no gaps and good leaf/fine stem retention). The results indicated significant differences between varieties in growth characteristics (Baker *et al.*, 2007). Despite the survival rate in the trial being 96% in the first assessment, the J-rooting problem that occurred in this trial was predicted to reduce the trees' ability to develop a normal root system and lead to reduced survival over time (Baker *et al.*, 2007). Clones were J-rooted because of an inappropriate repotting technique applied by a local nursery when planting was delayed and clones were over-wintered in the nursery.

2006 Clonal spacing trial

The 2006 clonal spacing trial was established to evaluate the performance of 10 clones of the second suite of elite clones over the same three spacings compared in the 2004 trial. It was planted in October 2006 at Bungawalbin nearby the 2004 trials. Two improved seedlots were included in this trial as controls. Details of the origin of clones and seedlots included in the 2006 clonal spacing trial are presented in Table 4.2.

Table 4.2 Details of the origin of clones and seedlots included in the 2006 clonal spacing trial

Treatment number	Variety number	Source number	Source origin	Family number
1	Clone 1	C39	PT2	36
2	Clone 2	C52	PT2	88
3	Clone 3	C56	PT2	108
4	Clone 4	C57	PT2	110
5	Clone 5	C64	YT2	128
6	Clone 6	C66	YT2	128
7	Clone 7	C67	YT2	128
8	Clone 8	C68	YT2	128
9	Clone 9	C70	CC2	12
10	Clone 10	C71	CC2	117
11	Seedling 1	ATTIA 2B	CSO1	Bulked
12	Seedling 2	ATTIA 2B	CSO1	Bulked
End Buffers	Clone 9	C70	CC2	12
Side Buffers	Seedling 2	ATTIA 2B	CSO1	Bulked

Source: G. Baker, pers. comm. (2007)

ATTIA2B : CSO1 seed
CC2 : controlled-cross progeny plots est. 2002
CSO1 : first clonal seed orchard est. 1995
PT2 : progeny trial est. 2002
YT2 : yield trial est. 2002

The trial planted during October 2006 was arranged in a split-plot design and comprises 3 main-plot treatments (30, 45 and 60 cm within-row spacing and 1 m between rows) and 12 sub-plot treatments (clone 1, clone 2, clone 3, clone 4, clone 5, clone 6, clone 7, clone 8, clone 9, clone 10, seedling 1 and seedling 2) with 4 replications (Figure 4.3). Each plot consist of 2 rows by 10 plants, thus each replicate has 720 plants. Therefore, the total number of plants in the trial is 2880 plants with 240 plants as a buffer. Extrapolation of stockings to a per hectare basis is identical to the 2004 trial i.e. 33,333 plants/ha for spacing 1 (30 cm), 22,222 plants/ha for spacing 2 (45 cm) and 16,667 plants/ha for spacing 3 (60 cm).

B	6	2	5	12	9	4	11	3	7	8	1	10	8	4	6	12	11	2	7	5	10	3	9	1	B
B	12	4	11	9	3	1	2	8	10	5	6	7	2	9	7	4	1	5	12	3	11	6	8	10	B
B	5	8	7	2	10	11	1	12	6	4	9	3	3	5	11	10	7	12	9	6	1	8	2	4	B
B	7	3	6	10	5	2	9	4	1	11	12	8	12	10	8	2	6	3	4	1	7	9	11	5	B
B	1	11	4	3	8	6	7	10	12	9	2	5	7	11	3	1	9	8	10	2	4	5	6	12	B
B	9	10	8	1	12	7	5	6	2	3	4	11	1	6	9	5	10	4	8	11	2	12	3	7	B

45 cm 60 cm 30 cm 60 cm 30 cm 45 cm 60 cm 45 cm 30 cm 45 cm 60 cm 30 cm

Replicate 1 Replicate 2 Replicate 3 Replicate 4

Figure 4.3 Split plot design of the 2006 clonal spacing trial at Bungawalbin, NSW, showing replicate, within-row spacing and variety number. The trial was surrounded by a single-row buffer (B) but only the end buffers are shown on the plan (Source: G. Baker, pers. comm., 2007)

Note: Plan is not to scale as plot area varies depending on spacing.

4.3.2 Growth assessment and sampling method

Growth assessments were undertaken on 23-24 October 2007 for the 2006 clonal spacing trial prior to the first harvest when trees were 12 months old and on 5-6 June 2008 for the 2004 clonal spacing trial prior to the third harvest when coppice was at age 18 months after second harvest. All surviving trees in both trials were assessed for height and leafiness score (subjective 6-point score from 0.5 [sparse canopy] to 5

[bushy canopy with no gaps and good leaf/fine stem retention]) while frost damage score (subjective 4-point score of 1 [heavy frost damage] to 4 [no damage]) and flowering score (0 for flowering trees and 1 for non-flowering trees) were determined only in the 2006 clonal spacing trial. Due to practical considerations, a sampling scheme based on the results of analyses of growth data was used to estimate dry weights of key tree components associated with off-paddock oil yield and oil traits of the clones and seedlings in the trials.

Sampling scheme for the 2006 clonal spacing trial

Frequency distributions were constructed for height (5 classes) and leafiness score (5 classes) for each variety at spacings 1 and 3 (Table 4.3). Spacing 2 was not sampled due to cost and time considerations. By ignoring this spacing, more trees could be sampled of the other two spacings. Analysis of variance of growth traits of clones showed that there was a significant variation among them. Therefore, the sampling strategy to estimate dry weight of key tree components and oil traits was to sample more clones with fewer trees sampled to represent each of them. The eight best clones were selected and 7 trees of each clone were chosen randomly from the frequency distribution of height and leafiness score classes. Since the two-improved seedlots included in this trial are from the same origin i.e. CSO1 and there was notable variability among trees, a total of 20 trees were selected randomly from the two-improved seedlots. The sampled trees were taken from spacings 1 and 3 and were randomly distributed amongst replicates. In this way, a total of 152 trees were sampled and harvested and tree components (stem, fine stem, leaf and capsule) and oil traits (oil concentration and percentage of 1,8-cineole and terpinen-4-ol of total oils were determined.

Table 4.3 Sample of matrix of height/leafiness score classes of clone 1 at spacing 1 in the 2006 clonal spacing trial. The number of trees in each class is shown.

Height class (cm)	Leafiness score class				
	0.5-1.0	1.5-2.0	2.5-3.0	3.5-4.0	4.5-5.0
25-56					
57-88					
89-120			2		
121-152		3	39	2	
153-184		2	28	2	

Sampling scheme for the 2004 clonal spacing trial

An identical sampling scheme to the 2006 clonal spacing trial was applied to assess the 2004 clonal spacing trial. Analyses of variances of growth traits indicated that there were no significant differences both between- and within- groups of varieties (clones vs. seedlings). Therefore 10 trees were selected randomly from each variety of spacing 1 and spacing 3 and were randomly distributed amongst replicates. Hence, a total of 100 sample trees were harvested from this trial.

Laboratory procedures to determine dry weights of tree component (plant biomass) and oil traits of tea tree are described in Chapter 3.

4.3.3 Methodology to determine statistical significance of variation in plant growth, biomass and oil traits

The raw field and laboratory data were initially logged into an Excel spreadsheet and sorted into a format compatible with GenStat. Screening outlier data was performed prior to analyses of variance using GenStat Discovery Edition Release 4.24DE (VSN International LTD, 2005). In this case, data transformation was not required. The analyses of variance are based on the following linear model (Williams *et al.*, 2002):

$$\boldsymbol{Y_{ijk} = \mu + \rho_i + S_j + \eta_{ij} + V_k + S.F_{jk} + \varepsilon_{ijk}} \quad \text{(Equation 4.1)}$$

where: Y_{ijk} is the plot means of the kth family in the jth spacing and i^{th} replicate; μ represents the overall mean; ρ_i represents the deviation from μ of the ith replicate; S_j represents the deviation from μ of the jth spacing; η_{ij} represents the residual of main plot; V_k represents the deviation from μ of the kth variety; $S.F_{jk}$ represents the interaction of the jth spacing and k^{th} variety; and ε_{ijk} represents the sub-plot residual.

The traits examined were survival rate (%), plant height (cm), leafiness score (0.5-5), frost damage score (1-4) in the 2006 trial only, flowering score (0 and 1) in the 2006 trial only, predicted stem mass (g oven dry weight-ODW), predicted fine stem mass (g ODW), predicted leaf mass (g ODW), predicted total mass (g ODW), leaf oil concentration (mg/g ODW), 1,8-cineole (%), terpinen-4-ol (%) (refers to terpinen-4-ol plus its precursors) and estimated oil production (g). Growth traits (height,

leafiness score, frost damage score and flowering score) data were converted into plot means and were used subsequently as input for performing analysis of variance. Predicted biomass (tree component dry weight) of stem, fine stem, leaf, and total tree component and estimated oil yield, data were converted into plot totals and used as input to analyses of variance to account for different survival rates among varieties within each plot.

4.3.4 Statistical methodology to estimate dry weights of tree component and oil yield

General linear models (GLM) were used to fit the dry weight of stem, twig, leaf, capsule and total biomass to the design structure and the linear terms of height, leafiness, frost damage, and occurrence of flowers. GLMs were performed separately for each variety group at each plant spacing. Multiple linear regression analyses were performed involving common linear terms of height and leafiness scores to predict dry weights of tree components for each variety group at each spacing, based on the following model (Kenkel, 1995):

$$Y_i = \beta_0 + \beta_1 x_{i1} + \beta_2 x_{i2} + e_i \quad \text{(Equation 4.2)}$$

where: Y_i is the response value of ith variable; β_0 is a constant coefficient; β_1 is the coefficient of the first explanatory variable x_1 (height); x_{i1} represent the ith observation on the explanatory variable x_1; β_2 is the coefficient of the second explanatory variable x_2 (leafiness score); x_{i2} represent the ith observation on the explanatory variable x_2; and e_i is a random error of ith variable.

The appropriate regression equations for each clone at each spacing were used to predict dry weights of tree components for all trees in the trial. Estimated oil production per plant was obtained by multiplying the predicted leaf mass by the average of oil concentration of each variety. Total dry weights of key tree components and estimated oil production per plot were the summation of values for each variate of the surviving trees within each plot. Extrapolation to a per hectare basis of the predicted dry weights of tree component and oil yield was calculated by multiplying plot total value of each variate by number of plots required to cover a hectare i.e. 1,666.67 plots/ha and 833.33 plots/ha for spacings 1 and 3 respectively.

4.4 Results

4.4.1 Variation in growth traits of clones and seedlings included in the 2006 clonal spacing trial at 12 months from planting

There were notable variations in survival and tree growth traits, i.e. height (cm), leafiness score, frost damage score and flowering score, between spacings, variety groups (clone and seedling) and among varieties within variety group in the 12 month assessment of the 2006 clonal spacing trial of *M. alternifolia* (Table 4.4). Least significant differences at P=0.05 level for each variate are given from the analyses of variance (Table 4.5).

The seedling group were significantly poorer in performance compared to the clonal group for survival, height, leafiness and frost damage at all spacings except for the leafiness score at spacing 3 which was slightly higher than the average of the clones, however this different was not significant. The common occurrence of flowers on most clones in the first 12 months of planting is possibly an undesirable characteristic of deploying clones in place of seedlings as it might lead to reduced oil yield due to lowered oil concentration during flowering (Butcher, 1994).

Table 4.4 Means of growth traits of the clones and seedlings included in the 2006 clonal spacing trial at age 12 months from planting

Variety Group	Variety Number	Survival (%)			Height (cm)			Leafiness score (0.5-5)			Frost damage score (1-4)			Flowering score (0-1)		
		Spac.1	Spac.2	Spac.3	Spac.1	Spac.2	Spac.3	Spac.1	Spac.2	Spac.3	Spac.1	Spac.2	Spac.3	Spac.1	Spac.2	Spac.3
Clone	1	97.5	100	100	150.5	148.4	146.8	2.74	3.01	3.20	3.31	2.98	2.80	0.99	0.99	0.98
	2	97.5	100	100	124.7	117.7	115.0	3.70	3.91	4.05	3.15	3.10	3.01	0.81	0.98	0.91
	3	98.8	100	100	124.8	121.3	115.8	3.64	3.66	3.49	3.03	2.98	2.84	0.63	0.65	0.51
	4	100	98.8	97.5	116.3	109.3	109.8	3.17	3.45	3.32	2.39	1.91	1.73	0.06	0.05	0.01
	5	100	100	98.8	123.7	114.5	113.9	3.78	4.23	4.47	3.09	3.06	3.08	0.43	0.59	0.72
	6	100	98.8	100	131.0	120.5	117.6	4.49	3.62	4.13	2.90	3.20	2.80	0.30	0.38	0.48
	7	100	98.8	96.3	132.7	131.2	131.6	3.08	3.25	3.37	2.96	3.05	2.87	0.64	0.75	0.81
	8	100	97.5	100	134.6	126.0	129.6	3.20	3.42	3.51	2.93	2.87	2.85	0.13	0.11	0.18
	9	98.8	100	100	123.8	116.6	120.1	3.47	4.03	4.10	3.15	2.80	2.80	0.94	0.76	0.90
	10	98.8	100	98.8	124.7	118.7	115.8	2.98	3.22	3.47	3.52	3.14	3.05	0.95	0.91	0.94
	mean	99.1	99.4	99.1	128.7	122.4	121.6	3.43	3.58	3.71	3.04	2.910	2.78	0.59	0.62	0.64
Seedling	11	97.5	95.0	98.8	113.4	112.9	102.7	3.21	3.37	3.48	2.99	2.62	2.83	0.00	0.00	0.00
	12	97.5	97.5	96.3	112.2	107.5	109.4	3.31	3.19	3.51	2.67	2.74	2.65	0.00	0.00	0.00
	mean	97.5	96.3	97.6	112.8	110.2	106.1	3.26	3.28	3.50	2.83	2.68	2.74	0.00	0.00	0.00
l.s.d (P=0.05)		*3.77*	*3.77*	*3.77*	*9.30*	*9.30*	*9.30*	*0.38*	*0.38*	*0.38*	*0.33*	*0.33*	*0.33*	*0.17*	*0.17*	*0.17*

Survival in the trial was very high with an overall average of more than 99% for clones and more than 96% for seedlings. Analysis of variance of survival (Table 4.5) shows that only variety group had significant effect on plant survival while spacing and variety within group and interaction between them did not have any effect on survival. Analyses of variance of growth traits (Table 4.5) shows that there were significant to highly significant differences between spacing, variety groups and varieties within group in all growth traits except for flowering score which did not differ significantly between spacing. Neither interaction between variety groups and spacing nor interaction between varieties within group and spacing caused any significant differences in the growth traits assessed.

Table 4.5 Analyses of variance of survival, height, leafiness score, frost damage and flowering score of variety groups for the 2006 clonal spacing trial at age 12 months from planting

Source of variation	df	Survival (%)		Height (cm)		Leafiness score (0.5-5)		Frost damage score (1-4)		Flowering score (0-1)	
		ms	vr	ms	vr	ms	vr	ms	vr	ms	vr
Replicate	3	7.581	1.05	33.62	0.76	0.22058	3	0.10832	1.96	0.02098	1.37
Spacing	2	0.000	0.000ns	668.13	15.18**	1.58683	21.55**	0.64198	11.60**	0.02893	1.89 ns
Group	1	90.312	12.50**	4230.17	96.09**	0.75602	10.27*	0.51923	9.38*	7.46846	486.63**
Spacing*Group	2	5.000	0.69ns	27.21	0.62ns	0.09597	1.3 ns	0.07181	1.3 ns	0.00441	0.29 ns
Variety within Group	10	1.854	0.26ns	1155.40	26.25**	1.49478	20.30**	1.21102	21.88**	1.20575	78.56**
Spacing*Variety within Group	20	6.792	0.94ns	26.24	0.60 ns	0.07861	1.07 ns	0.08521	1.54 ns	0.02103	1.37 ns
Residual	105	7.224		44.02		0.07363		0.05536		0.01535	

** statistically significant at P < 0.001; * at P < 0.05; ns = statistically not significant at P < 0.05

Varieties from the clone group differed significantly among themselves for all growth traits at all spacings except for flowering score which was not affected by spacing (Table 4.6). There was no significant difference in all growth traits due to the interaction between spacing and variety. Varieties from seedling group, however, did not differ significantly in any growth traits assessed at any of the 3 spacings (ANOVA is not shown).

Table 4.6 Analyses of variance of height, leafiness score, frost damage and flowering score of varieties within the clone group for the 2006 clonal spacing trial at age 12 months from planting

Source of variation	df	Height (cm)		Leafiness score (0.5-5)		Frost damage score (1-4)		Flowering score (0-1)	
		ms	vr	ms	vr	ms	vr	ms	vr
Replicate	3	37.70	0.88	0.09293	1.25	0.05875	1.07	0.02677	1.46
Spacing	2	603.03	14.07**	1.54227	20.68**	0.67120	12.27**	0.03323	1.82ns
Variety	9	1283.89	29.95**	1.66064	22.27**	1.33473	24.40**	1.33972	73.30**
Spacing*Variety	18	20.65	0.48ns	0.08292	1.11ns	0.08344	1.53ns	0.02333	1.28ns
Residual	87	42.87		0.07457		0.05470		0.01828	

** statistically significant at P < 0.001; * at P < 0.05; ns = statistically not significant at P < 0.05

4.4.2 Variation in oil traits of clones and seedlings included in the 2006 clonal spacing trial at age 12 months from planting

There was a significant difference in oil concentration between clones and seedlings at 12 months from planting at the two spacings assessed (1 and 3) (Table 4.7). Least significant differences at P=0.05 level of each variate are included from the analyses of variance (Table 4.8). Overall, clones from the second suite of elite clones provided by tea tree breeding programme gave on average 44% and 55% greater oil concentration than seedlings at spacing 1 and 3 respectively. Oil concentration of clones averaged 91.6 mg/g ODW at spacing 1 and 86.69 mg/g ODW at spacing 3 compared to seedlings that averaged 63.6 mg/g ODW and 55.77 mg/g ODW from spacing 1 and 3 respectively. Consistency in 1,8-cineole content was a feature of each clone compared to greater variability amongst seedling stock.

The International Standard applying to tea tree oil requires that the oil should be comprised of 30 percent or more of terpinen-4-ol, the main indicator of antimicrobial activity, and 15 percent or less of 1,8-cineole (International Standard Organisation, 1996). However, due to the misconception that 1,8-cineole is an irritant to skin and mucous membranes, the current market requires the oils' 1,8-cineole content to be 3% or lower and terpinen-4-ol content to be higher than 36% (Colton *et al.*, 2000; Davis, 2003). Oils from both clones and seedlings had desirable levels of terpinen-4-ol (>40%) and 1,8-cineole (3% or lower).

Table 4.7 Means of oil traits of the clones and seedlings included in the 2006 clonal spacing trial at age 12 months from planting

Variety Group	Variety Number	Oil concentration (mg/g ODW)		1,8-cineole (% of total oil)		Terpinen-4-ol (% of total oil)	
		Spac.1	Spac.3	Spac.1	Spac.3	Spac.1	Spac.3
Clone	1	82.14	78.00	1.63	1.62	43.31	42.88
	2	93.57	87.16	0.50	0.52	42.23	42.66
	3	95.78	88.91	2.69	2.69	42.03	42.07
	5	94.75	91.93	1.57	1.55	43.02	43.93
	6	95.95	92.47	0.79	0.53	43.54	43.96
	7	89.28	82.33	2.39	2.46	42.09	41.58
	8	92.37	86.98	3.25	2.81	41.79	41.63
	9	88.91	85.75	0.49	0.51	44.29	43.76
	mean	91.59	86.69	1.66	1.59	42.79	42.81
Seedling	11	68.00	58.25	1.78	2.82	42.54	41.81
	12	57.56	54.13	2.92	1.79	41.62	41.95
	mean	62.78	56.19	2.35	2.31	42.08	41.88
l.s.d (P=0.05)		*7.40*	*7.40*	*0.97*	*0.97*	*1.43*	*1.43*

Analysis of variance of oil concentration, 1,8-cineole and terpinen-4-ol were carried out on the sampled trees. Oil concentration was influenced by plant spacing, being greater at the closer spacing while the contents of 1,8-cineole and terpinen-4-ol were not affected. Oil traits were significantly affected by variety group and variety within group but were not affected by interaction between spacing and variety within group.

Table 4.8 Analyses of variance of oil traits of variety groups (clones and seedlings) in the 2006 clonal spacing trial at age 12 months from planting

Source of variation	df	Oil concentration (mg/g ODW)		1,8-cineole (% of total oil)		Terpinen-4-ol (% of total oil)	
		ms	vr	ms	vr	ms	vr
Spacing	1	1335.69	25.81**	0.1655	0.19 ns	0.131	0.07 ns
Group	1	25141.17	485.84**	10.6064	12.00**	17.449	9.08*
Spacing*Group	1	61.80	1.19ns	0.0057	0.01ns	0.567	0.30 ns
Variety within Group	8	327.68	6.33**	12.6148	14.27**	10.262	5.34**
Spacing*Variety within Group	8	20.77	0.40 ns	1.4812	1.68 ns	1.168	0.61 ns
Residual	131	51.75		0.8839		1.921	

** statistically significant at P < 0.001; * at P < 0.05; ns = statistically not significant at P < 0.05

Varieties from the clone group differed significantly among themselves for all oil traits (Table 4.9) while varieties from the seedling group differed only minimally

among themselves in terms of oil concentration and terpinen-4-ol content (Table 4.10). The interaction between spacing and variety did not affect any oil traits for both the clone and seedling groups. Analysis of variance (data is not shown) was also performed to evaluate the effect of flowering to oil concentration. The result indicates that there was no significant difference in oil concentration between flowering trees and non flowering trees.

Table 4.9 Analyses of variance of oil traits of varieties within clone group for the 2006 clonal spacing trial at age 12 months from planting

Source of variation	df	Oil concentration (mg/g ODW)		1,8-cineole (% of total oil)		Terpinen-4-ol (% of total oil)	
		ms	vr	ms	vr	ms	vr
Spacing	1	673.37	23.72**	0.1766	1.70 ns	0.012	0.01 ns
Variety	7	304.17	10.72**	14.4150	138.74**	11.531	10.51**
Spacing*Variety	7	10.29	0.36ns	0.1068	1.03 ns	0.966	0.88 ns
Residual	96	28.38		0.1039		1.097	

** statistically significant at P < 0.001; * at P < 0.05; ns = statistically not significant at P < 0.05

Table 4.10 Analyses of variance of oil traits of varieties within seedling group for the 2006 clonal spacing trial at age 12 months

Source of variation	df (mv)	Oil concentration (mg/g ODW)		1,8-cineole (% of total oil)		Terpinen-4-ol (% of total oil)	
		ms	vr	ms	vr	ms	vr
Spacing	1	647.6	5.59*	0.007	0.00 ns	0.775	0.19 ns
Variety	1	508.9	4.39*	0.032	0.01 ns	1.472	0.35 ns
Spacing*Variety	1	95.8	0.83 ns	11.304	3.74 ns	2.630	0.63 ns
Residual	35(1)	115.8		3.024		4.182	

* statistically significant at P < 0.05; ns = statistically not significant at P < 0.05

4.4.3 Variation in predicted dry weights of tree component and estimated oil yield of clones and seedlings included in the 2006 clonal spacing trial at age 12 months from planting

The generalized linear model analyses showed that there was no significant interaction between growth traits and varieties, indicating that the regression lines were parallel for the varieties within each group at each spacing. It is also showed that height and leafiness score had significant effects on biomass but the frost damage score and flowering score did not influence the dry weights of the key tree components. Thus, multiple linear regression analyses were performed involving

common linear terms in height and leafiness scores to predict dry weights of the key tree components for each variety group at each spacing (Table 4.11). Capsule dry weight of the tree could not be predicted by this method and was ignored in this study. Although capsules contain some oil (varies from 4.28 mg/g ODW of mature capsules to 14.44 mg/g ODW of immature capsules), they would not have any significant impact on total oil yield as the proportion is very small (on average 0.6% of total dry weight of plant) and plants would normally be harvested annually before reaching reproductive maturity.

Table 4.11 Summary of the linear regression analyses of variety groups included in the 2006 clonal spacing trial at age 12 months from planting

Response variate	Spacing	Group	Fitted terms	Estimate value	t statistic (d.f=53)	Variance accounted for (%)
Stem (g)	1	Clone	Constant	-348.9	-6.66**	63.4
			Height (cm)	2.799	9.45**	
			Leafiness score	39.30	4.82**	
		Seedling	Constant	-137.2	-2.24*	39.5
			Height (cm)	1.339	3.72*	
			Leafiness score	20.7	2.04	
	3	Clone	Constant	-392.9	-6.74**	68.5
			Height (cm)	3.452	10.89**	
			Leafiness score	41.93	4.74**	
		Seedling	Constant	-106.4	-2.36*	62.2
			Height (cm)	1.386	5.76**	
			Leafiness score	14.91	1.73	
Fine stem (g)	1	Clone	Constant	-102.9	-5.50**	57.6
			Height (cm)	0.814	7.70**	
			Leafiness score	16.76	5.76**	
		Seedling	Constant	-60.2	-3.55*	60.9
			Height (cm)	0.5172	5.21**	
			Leafiness score	10.84	3.87*	
	3	Clone	Constant	-136.8	-4.83**	52.7
			Height (cm)	1.166	7.57**	
			Leafiness score	19.39	4.51**	
		Seedling	Constant	-52.2	-2.12*	54.8
			Height (cm)	0.657	5.01**	
			Leafiness score	8.61	1.83	
Leaf (g)	1	Clone	Constant	-253.1	-4.88**	54.1
			Height (cm)	2.010	6.87**	
			Leafiness score	46.89	5.82**	
		Seedling	Constant	-143.8	-2.89*	50.6
			Height (cm)	1.278	4.39**	
			Leafiness score	24.62	3.00*	
	3	Clone	Constant	-415.1	-6.23**	62.3
			Height (cm)	3.048	8.41**	
			Leafiness score	70.1	6.92**	

Table 4.11 (continued) Summary of the linear regression analyses of variety groups included in the 2006 clonal spacing trial at age 12 months from planting

Response variate	Spacing	Group	Fitted terms	Estimate value	t statistic (d.f=53)	Variance accounted for (%)
Leaf (g)	3	Seedling	Constant	-128.9	-2.41*	52.9
			Height (cm)	1.345	4.72**	
			Leafiness score	26.8	2.62*	
Total mass (g)	1	Clone	Constant	-709	-6.45**	65.5
			Height (cm)	5.666	9.40**	
			Leafiness score	103	6.20**	
		Seedling	Constant	-341	-2.93*	52.3
			Height (cm)	3.135	4.59**	
			Leafiness score	56.2	19.3*	
	3	Clone	Constant	-950	-7.29**	70.3
			Height (cm)	7.743	10.91**	
			Leafiness score	130.8	6.61**	
		Seedling	Constant	-288	-2.72*	64.4
			Height (cm)	3.388	6.02**	
			Leafiness score	50.3	2.49*	

**statistically significant (P<0.001)

The appropriate regression equations for each variety group at each spacing, as presented in the Table 4.11, were used to predict components of dry weight for all trees in the trial. Estimated oil yield per plant was obtained by multiplying predicted leaf mass and average of oil concentration for each variety at each plant spacing. These predicted values of tree components and the estimated oil yields were then converted into plot totals to be used as data input in analyses of variances. Means of plot totals of predicted tree components of dry weight and estimated oil yield are presented in Figure 4.4. Least significant differences at P=0.05 level of each variate are included from the analyses of variance (Table 4.12).

Table 4.12 Analysis of variance of plot totals for predicted dry weights of tree components and estimated oil production per plot of clones and seedlings included in the 2006 clonal spacing trial at age 12 months from planting

Source of variation	df	Predicted stem mass/plot (g ODW)		Predicted fine stem mass/ plot (g ODW)		Predicted leaf mass/plot (g ODW)		Predicted total mass/plot (g ODW)		Estimated oil production/ plot (g)	
		ms	vr	ms	vr	ms	vr	ms	vr	ms	vr
Replication	3	195975	1.26	23364	1.07	173370	1.03	1.039E+06	1.16	1390	1.13
Spacing	1	10560492	68.14**	2966011	135.42**	22097575	131.61**	9.364E+07	104.28**	104298	84.68**
Group	1	38011724	245.25**	4830986	220.56**	56783203	338.19**	2.571E+08	286.35**	746494	606.08**
Spacing*Group	1	1256542	8.11*	58766	2.68 ns	1392806	8.30*	6.700E+06	7.46*	14207	11.53*
Variety within Group	8	1012667	6.53**	69358	3.17*	425118	2.53*	3.398E+06	3.78*	4033	3.27*
Spacing*Variety within Group	8	266067	1.72ns	41701	1.90 ns	321954	1.92 ns	1.607E+06	1.79 ns	2858	2.32*
Residual	57	154993		21903		167905		8.980E+05		1232	

** statistically significant at P < 0.001; * at P < 0.05; ns = statistically not significant at P < 0.05

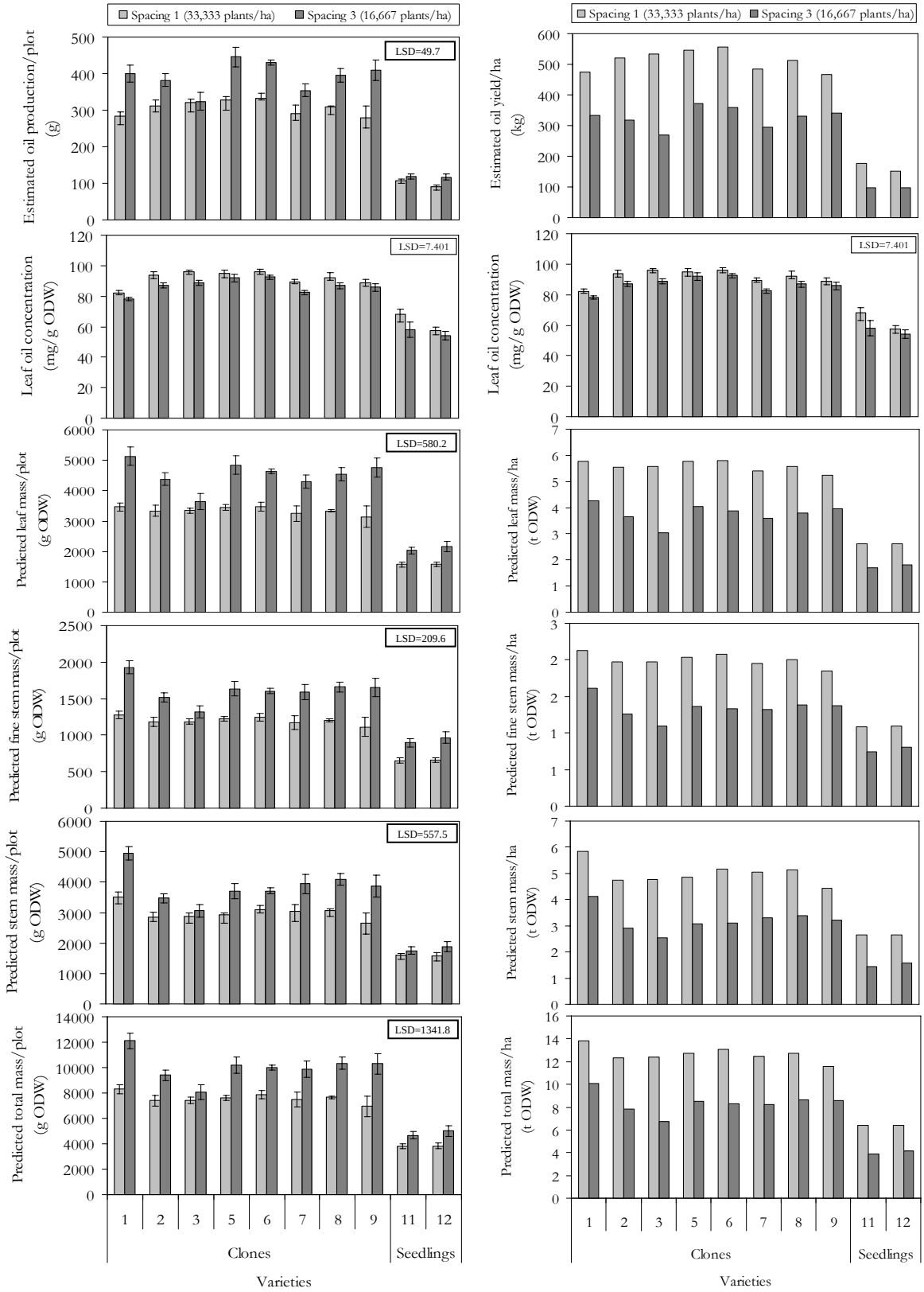

Figure 4.4 Leaf oil concentration, predicted dry weights of key tree components and estimated oil production per plot (g/plot) (left) and extrapolation to per hectare value (e.g. kg of oil/ha) (right) of the clones and seedlings included in the 2006 clonal spacing trial at age 12 months from planting

Table 4.13 Analysis of variance of plot totals of predicted dry weights of tree components and estimated oil production per plot of variety within the clone group included in the 2006 clonal spacing trial at age 12 months

Source of variation	df	Predicted stem mass/plot (g ODW)		Predicted fine stem mass/ plot (g ODW)		Predicted leaf mass/plot (g ODW)		Predicted total mass/plot (g ODW)		Estimated oil production/ plot (g)	
		ms	vr	ms	vr	ms	ms	vr	ms	vr	ms
Replication	3	258933	1.46	32500	1.36	251164	1.30	1.438E+06	1.41	1916	1.30
Spacing	1	11613910	65.70**	2718556	113.76**	22394829	116.01**	9.629E+07	94.2**	117075	79.25**
Variety	7	1154521	6.53**	78519	3.29*	482882	2.50*	3.865E+06	3.78*	4568	3.09*
Spacing*Variety	7	301137	1.70ns	47131	1.97 ns	365695	1.89 ns	1.822E+06	1.78 ns	3235	2.19 ns
Residual	45	176781		23898		193048		1.021E+06		1477	

** statistically significant at P < 0.001; * at P < 0.05; ns = statistically not significant at P < 0.05

Table 4.14 Analysis of variance of plot totals of predicted dry weights of tree components and estimated oil production per plot of variety within the seedling group included in the 2006 clonal spacing trial at age 12 months

Source of variation	df	Predicted stem mass/plot (g ODW)		Predicted fine stem mass/ plot (g ODW)		Predicted leaf mass/plot (g ODW)		Predicted total mass/plot (g ODW)		Estimated oil production/ plot (g)	
		ms	vr	ms	vr	ms	ms	vr	ms	vr	ms
Replication	3	48477	0.80	7363	0.54	34565	0.57	238649	0.65	114.1	0.57
Spacing	1	203124	3.35 ns	306221	22.30*	1095552	18.05*	4049593	10.98*	1430.3	7.12*
Variety	1	19690	0.33 ns	5229	0.38 ns	20773	0.34 ns	127488	0.35 ns	286.6	1.43 ns
Spacing*Variety	1	20580	0.34 ns	3693	0.27 ns	15771	0.26 ns	108771	0.30ns	218.7	1.09 ns
Residual	9	60572		13730		60707		368670		200.9	

* statistically significant at P < 0.05; ns = statistically not significant at P < 0.05

Spacing, variety groups and varieties within group had significant effects on predicted dry weights of tree components and estimated oil yield both in clones and seedlings (Table 4.12). Response of clones and seedlings to different plant spacings are shown in Figure 4.4. Generally, on a per plot basis, dry weights of all tree components and estimated oil yield increased concomitant with increase of plant within-row spacing from 30 cm (33,333 plants/ha) to 60 cm (16,667 plants/ha). Clones appeared more responsive to spacing than seedlings. Clones gave an increase of predicted stem and leaf dry weights ranging from 28% to 35% as plant within row spacing increased from 30 cm (33,333 plants/ha) to 60 cm (16,667 plants/ha) compared to increases in seedlings which ranged from 14% to 33%. However, the increase of fine stem was higher in the seedlings than in the clones, averaging 34% and 42% for clones and seedlings respectively. Consequently, the increase of estimated oil production per plot which is the function of predicted leaf mass and

average of leaf oil concentration of the variety was also higher at the wider spacing (28% greater for clones and 19% for the seedlings).

On a per hectare basis (Figure 4.4), as the number of plants per hectare increased (16,667 plants/ha cf. 33,333 plants/ha) while plant within row spacing decreased (60 cm within-row spacing cf. 30 cm within-row spacing), predicted stem, fine stem, and leaf mass increased 56%, 49% and 48% for clones and 63%, 48% and 45% for seedlings. Therefore, estimated oil yield also increased in parallel with the increase of leaf yield. Plantations established using clones were predicted to have a leaf yield of about 5.585 tonnes ODW/ha and 3.779 tonnes ODW/ha from spacing 1 and 3 respectively at first harvest. With the mean of oil concentration at first harvest of 91.60 mg/g ODW at spacing 1 and 86.69 mg/g ODW at spacing 3, clones gave an oil yield of 511.7 kg/ha and 327.1 kg/ha at spacing 1 and 3 respectively. In comparison, plantations from seedlings were predicted to produce a leaf yield, leaf oil concentration and oil yield of about 2.625 tonnes ODW, 63.60 mg/g ODW, 164.75 kg/ha and 1.749 tonnes ODW, 55.77 mg/g ODW and 98.13 kg/ha from the first harvest at spacing 1 and spacing 3 respectively.

Varieties (individual clones) among the clonal group showed different performance in predicted dry weights of tree components and estimated oil production per plot (Table 4.13). In contrast, there was no difference between varieties within the seedling group (Table 4.14) as the two-seedlots are from the same origin, i.e. CSO1 (Table 4.2). Overall, the clones from the second suite of elite clones provided by the tea tree breeding programme gave 113% and 116% greater predicted leaf mass per plot and 211% and 233% greater estimated oil production per plot than improved seedlings from spacing 1 and spacing 3 respectively. On a per hectare basis by extrapolation of the data, the superiority of clones over seedlings was 73% and 113% for predicted leaf mass and 108% and 211% for estimated oil yield per hectare from spacing 1 and spacing 3 respectively (Figure 4.4).

4.4.4 Variation in growth traits of coppices of the clones and seedlings included in the 2004 clonal spacing trial at age 18 months after second harvest

Due to the various degrees of J-rooting that occurred in the cuttings planted in this trial their survival has declined over time. The average of survival in this, the third assessment of the trial, was much lower (i.e. 60%) than in the first assessment (i.e. 96%) (Baker *et al.*, 2007) and this was considered to be mainly due to this problem. There are substantial differences in survival amongst the clones and seedlings in this trial while height and leafiness score are remarkably similar between the different varieties (Table 4.15). Least significances differences at P=0.05 level of each variate are given from the analysis of variance (Table 4.16).

Table 4.15 Means of growth traits of the coppices of the clones and seedlings, by variety group, included in the 2004 clonal spacing trial at age 18 months after second harvest

Variety Group	Variety Number	Survival (%)			Height (cm)			Leafiness score (0.5-5)		
		Spac.1	Spac.2	Spac.3	Spac.1	Spac.2	Spac.3	Spac.1	Spac.2	Spac.3
Clone	1	47.5	62.5	56.2	147.2	150.3	148.2	3.66	3.81	3.98
	2	61.2	56.2	72.5	139.0	129.1	133.9	4.18	4.20	4.43
	3	71.2	57.5	51.2	151.4	117.2	134.6	3.82	3.51	4.20
	mean	60.0	58.7	60.0	145.9	132.2	138.9	3.89	3.84	4.20
Seedling	4	83.8	72.5	82.5	138.6	141.3	136.9	3.84	4.15	4.34
	5	83.8	88.8	88.8	145.5	132.1	136.6	3.72	4.14	4.44
	mean	83.8	80.6	85.7	142.1	136.7	136.8	3.78	4.14	4.39
l.s.d (P=0.05)		*21.19*	*21.19*	*21.19*	*19.96*	*19.96*	*19.96*	*0.57*	*0.57*	*0.57*

Analyses of variance of survival, height and leafiness score of the coppices (Table 4.16) has shown that there is a highly significant difference in survival between clones and seedlings while survival among varieties within each groups was not significantly different. Moreover, there was no significant difference in survival between different spacings. Clones were slightly inferior in performance than seedlings for height and leafiness score, however, these differences did not reach statistically significant difference among varieties, group and spacing. On the other hand, there was significant differences in leafiness score due to different spacing and among varieties both within clone and seedling groups.

Table 4.16 Analyses of variance of survival, height and leafiness score of the coppices of clones and seedlings included in the 2004 clonal spacing trial at age 18 months after second harvest

Source of variation	df	Survival (%)		Height (cm)		Leafiness score (0.5-5)	
		ms	vr	ms	vr	ms	vr
Replicate	3	518.2	2.35	1781.7	9.11	0.4470	2.76
Spacing	2	40.4	0.18ns	543.4	2.78ns	1.0105	6.25*
Group	1	8122.5	36.82**	3.0	0.02ns	0.2462	1.52ns
Spacing*Group	2	16.9	0.08ns	93.2	0.48ns	0.2181	1.35ns
Variety within Group	3	238.9	1.08ns	552.6	2.82ns	0.5135	3.18*
Spacing*Variety within Group	6	350.3	1.59ns	283.9	1.45ns	0.0702	0.43ns
Residual	42	220.6		195.6		0.1617	

** statistically significant at P < 0.001; * at P < 0.05; ns = statistically not significant at P < 0.05

4.4.5 Variation in oil traits of the coppices of clones and seedlings included in the 2004 clonal spacing trial at age 18 months after second harvest

There was notable variation in oil concentration and oil composition among the varieties. Oil concentration of clones was generally less than seedlings with an average of 75.68 mg/g ODW and 75.59 mg/g ODW from the spacing 1 and 3 respectively while oil concentration of seedlings averaged 81.32 mg/g ODW and 76.47 mg/g ODW from spacing 1 and 3 respectively (Table 4.17). Despite the apparent small differences, variation in oil composition did reach significance in some cases (see Tables 4.18 and 4.19). As expected clones were very consistent in their 1,8-cineole content and substantially less variable in this regard than seedling treatments.

Table 4.17 Means of oil traits of the coppices of the clones and seedlings included in the 2004 clonal spacing trial at age 18 months after second harvest

Variety Group	Variety Number	Oil concentration (mg/g ODW)		1,8-cineole (% of total oil)		Terpinen-4-ol (% of total oil)	
		Spac.1	Spac.3	Spac.1	Spac.3	Spac.1	Spac.3
Clone	1	75.11	77.95	2.155	2.052	40.57	40.44
	2	79.39	78.50	0.418	0.412	40.32	40.22
	3	72.53	70.32	1.621	1.588	38.76	39.93
	mean	75.68	75.59	1.40	1.35	39.88	40.20
Seedling	4	80.17	79.76	2.684	1.141	38.28	39.62
	5	82.46	73.18	1.938	1.947	38.54	38.98
	mean	81.32	76.47	2.31	1.54	38.41	39.30
l.s.d (P=0.05)		*6.59*	*6.59*	*0.725*	*0.725*	*1.27*	*1.27*

Table 4.18 Analyses of variance of oil traits of the coppices of clones and seedlings included in the 2004 clonal spacing trial at age 18 months after second harvest

Source of variation	df	Oil concentration (mg/g ODW)		1,8-cineole (% of total oil)		Terpinen-4-ol (% of total oil)	
		ms	vr	ms	vr	ms	vr
Spacing	1	88.70	1.65ns	3.4135	5.24*	8.375	4.17*
Group	1	276.28	5.14*	6.5645	10.09*	32.292	16.09**
Spacing*Group	1	130.16	2.42ns	2.9281	4.50*	2.005	1.00ns
Variety within Group	3	205.11	3.82*	9.7118	14.92**	5.067	2.52ns
Spacing*Variety within Group	3	86.49	1.61ns	1.9596	3.01*	2.464	1.23ns
Residual	88	53.76		0.6509		2.008	

** statistically significant at P < 0.001; * at P < 0.05; ns = statistically not significant at P < 0.05

Table 4.19 Analyses of variance of oil traits of the coppices of varieties within clone group included in the 2004 clonal spacing trial at age 18 months after second harvest

Source of variation	df	Oil concentration (mg/g ODW)		1.8-cineole (% of total oil)		Terpinen-4-ol (% of total oil)	
		ms	vr	ms	vr	ms	vr
Spacing	1	0.03	0.00ns	0.09768	8.40*	1.588	0.93ns
Variety	2	287.64	7.22*	14.56727	1252.16**	7.448	4.38*
Spacing*Variety	2	34.22	0.86ns	0.01227	1.05ns	2.697	1.59
Residual	53	39.82		0.01163		1.701	

** statistically significant at P < 0.001; * at P < 0.05; ns = statistically not significant at P < 0.05

Although trees planted at the wider spacing were more likely to have lower oil concentration, analysis of variance of this trait shows that these differences were not statistically significant at P<0.05 (Table 4.18). The content of 1,8-cineole and terpinen-4-ol within the clonal group was also unexpectedly affected by plant spacing. The difference of oil composition between clone and seedling group was negligible. Varieties within the clonal group differed significantly among themselves in terms of oil concentration and composition (Table 4.19) while varieties in the seedling group did not differ significantly (ANOVA is not shown).

4.4.6 Variation in predicted dry weights of key tree components and estimated oil production per plot and oil yield per hectare of the coppice of the clones and seedlings included in the 2004 clonal spacing trial at age 18 months after second harvest

To predict dry weights of key tree component of trees in the trial, identical procedures to those used in the 2006 clonal spacing trial were applied. Unlike the previous data of the 2006 clonal spacing trial where data transformation was not required, transforming data to log natural (ln) was performed on the data from this trial. In this way, coefficient of determination (R^2), the proportion of variability in a data set that is accounted for by a statistical model, was higher than that without data transformation. The appropriate regression equations to predict each tree component at each planting space are summarised in the Table 4.20. The natural logs of the predicted tree components were then back transformed by performing an antilogarithm of a natural log function.

Table 4.20 Summary of the linear regression analyses of the coppice of the clones and seedlings included in the 2004 clonal spacing trial at age 18 months after second harvest

Response variate	Spacing	Fitted terms	Estimate value	t statistic (d.f=46)	Variance accounted for (%)
Ln Stem (g)	1	Constant	-9.85	-5.89**	84.4
		Ln Height (cm)	2.646	7.42**	
		Ln Leafiness score	1.282	8.12**	
	3	Constant	-8.67	-6.42**	85.0
		Ln Height (cm)	2.181	6.96**	
		Ln Leafiness score	2.177	7.84**	
Ln Fine stem (g)	1	Constant	-8.71	-4.89**	83.6
		Ln Height (cm)	2.266	5.97**	
		Ln Leafiness score	1.518	9.03**	
	3	Constant	-6.45	-4.86**	81.3
		Ln Height (cm)	1.704	5.54**	
		Ln Leafiness score	2.025	7.43**	
Ln Leaf (g)	1	Constant	-8.33	-4.86**	83.6
		Ln Height (cm)	2.318	6.35**	
		Ln Leafiness score	1.399	8.65**	
	3	Constant	-7.43	-5.40**	79.8
		Ln Height (cm)	2.117	6.64**	
		Ln Leafiness score	1.626	5.75**	
Ln Total mass (g)	1	Constant	-7.93	-4.78**	84.7
		Ln Height (cm)	2.433	6.88**	
		Ln Leafiness score	1.381	8.82**	
	3	Constant	-6.47	-5.26**	84.7
		Ln Height (cm)	2.028	7.12**	
		Ln Leafiness score	1.903	7.53**	

**statistically significant (P<0.001)

Table 4.21 Analysis of variance of plot totals for predicted dry weights of key tree components and estimated oil production per plot of the coppice of clones and seedlings included in the 2004 clonal spacing trial at age 18 months after second harvest

Source of variation	df	Predicted stem mass/plot (g ODW)		Predicted fine stem mass/plot (g ODW)		Predicted leaf mass/plot (g ODW)		Predicted total mass/plot (g ODW)		Estimated oil production/plot (g)	
		ms	vr	ms	vr	ms	vr	ms	vr	ms	vr
Replication	3	2091816	2.24	778846	2.08	2271834	2.24	14746337	2.21	12281	2.12
Spacing	1	5607637	6.02*	3430018	9.18*	9050706	8.91*	52786354	7.92*	45337	7.84*
Group	1	9799386	10.51*	4123612	11.03*	9522227	9.37*	68336212	10.25*	70662	12.22*
Spacing*Group	1	485208	0.52ns	309069	0.83ns	505206	0.50ns	3892919	0.58ns	1015	0.18ns
Variety within Group	3	652334	0.70ns	384367	1.03ns	603082	0.59ns	4868840	0.73ns	4679	0.81ns
Spacing*Variety within Group	3	1209906	1.30ns	441400	1.18ns	1152888	1.13ns	8050789	1.21ns	7522	1.30ns
Residual	27	932153		373753		1016111		6664453		5783	

*statistically significant at P < 0.05; ns = statistically not significant at P < 0.05

Plot total means and an extrapolation to a per hectare basis of the predicted tree components and estimated oil yield are presented in the Figure 4.5. Least significant differences at P=0.05 level for each variate are included from the analyses of variance in Table 4.21. As a logical consequence of lower survival, clones gave lower total predicted dry weights of key tree components, oil production per plot and estimated oil yield per ha than seedlings. Analyses of variance of the predicted values (Table 4.22) showed that there were significant differences of all variates due to spacing and variety group. Conversely, varieties within groups are not significantly different among themselves.

On a per plot basis, the measured characteristics of all variates increased as a response to an increase in growing space provided by increasing within-row spacing from 30 cm to 60 cm. Overall, seedlings responded better to a more growing space than clones as indicated by greater proportional increases. Predicted stem, fine stem and leaf mass per plot of seedlings increase an average 36%, 44%, and 42% respectively with greater space to grow compared to clones with an average of 26%, 33% and 36%. On the other hand, the estimated oil production per plot of clones increased 46% compared to 43% for seedlings. On a per hectare basis though, and as a direct result of reduced numbers of plants when planting space increases, predicted

stem, fine stem and leaf mass of clones dropped by 36%, 33% and 32% respectively while seedlings declined only moderately averaging 32%, 28% and 29%.

Extrapolation to per hectare values from plot totals for the clones was considered unfair because of the J-rooting problem. To compensate for this, another extrapolation to a per hectare basis was calculated by multiplying the mean value of individual trees by the number of plants in a hectare, i.e. 33,333 plants/ha for spacing 1 and 16,667 plants/ha for spacing 3, and by employing the average survival rate of the seedlings at each plant spacing assuming that clones would have a survival rate at the same levels of seedling if they are in a normal condition. This extrapolation is shown in the Figure 4.5-C.

Figure 4.5-C shows that by using the second extrapolation data, clones are expected to give predicted dry weights of stem, fine stem, leaf and oil yield on average 5.50 t/ha, 3.58 t/ha, 5.75 t/ha and 435.15 kg/ha from plantations at spacing 1 and 3.41 t/ha, 2.32 t/ha, 3.79 t/ha and 287 kg/ha from plantations at spacing 3. Meanwhile, seedlings are predicted to give predicted dry weights of stem, fine stem, leaf and oil yield on average 5.52 t/ha, 3.51 t/ha, 5.64 t/ha and 460.15 kg/ha and 3.59 t/ha, 2.43 t/ha, 3.85 t/ha and 295.83 kg/ha from spacing 1 and 3 respectively.

Those values are much higher than the first extrapolation values (Figure 4.5-B) where clones are predicted to have dry weights of stem, fine stem, leaf and oil yield on average 3.43 t/ha, 2.22 t/ha, 3.57 t/ha and 270 kg/ha from spacing 1 and 2.19 t/ha, 1.48 t/ha, 2.43 t/ha and 184.2 kg/ha form spacing 3. Whilst, seedlings are expected to give predicted dry weights of stem, fine stem, leaf and oil yield on average 4.74 t/ha, 3.02 t/ha, 4.85 t/ha, 395 kg/ha and 3.22 t/ha, 2.18 t/ha, 3.45 t/ha, 264.2 kg/ha from spacing 1 and 3 respectively.

Another tree component that can potentially contribute to oil yield in commercial tea tree plantations is fine stem as there was some oil content in both mature and immature fine stem ranging from 0.66 to 2.18 mg/g ODW for mature fine stem and 14.51 to 32.30 mg/g ODW for immature fine stem. However, in this study, the proportion of each category was not evaluated and the oil yield from this component was not included in the estimation of oil yield. Oil yield throughout this thesis is exclusively derived from leaves.

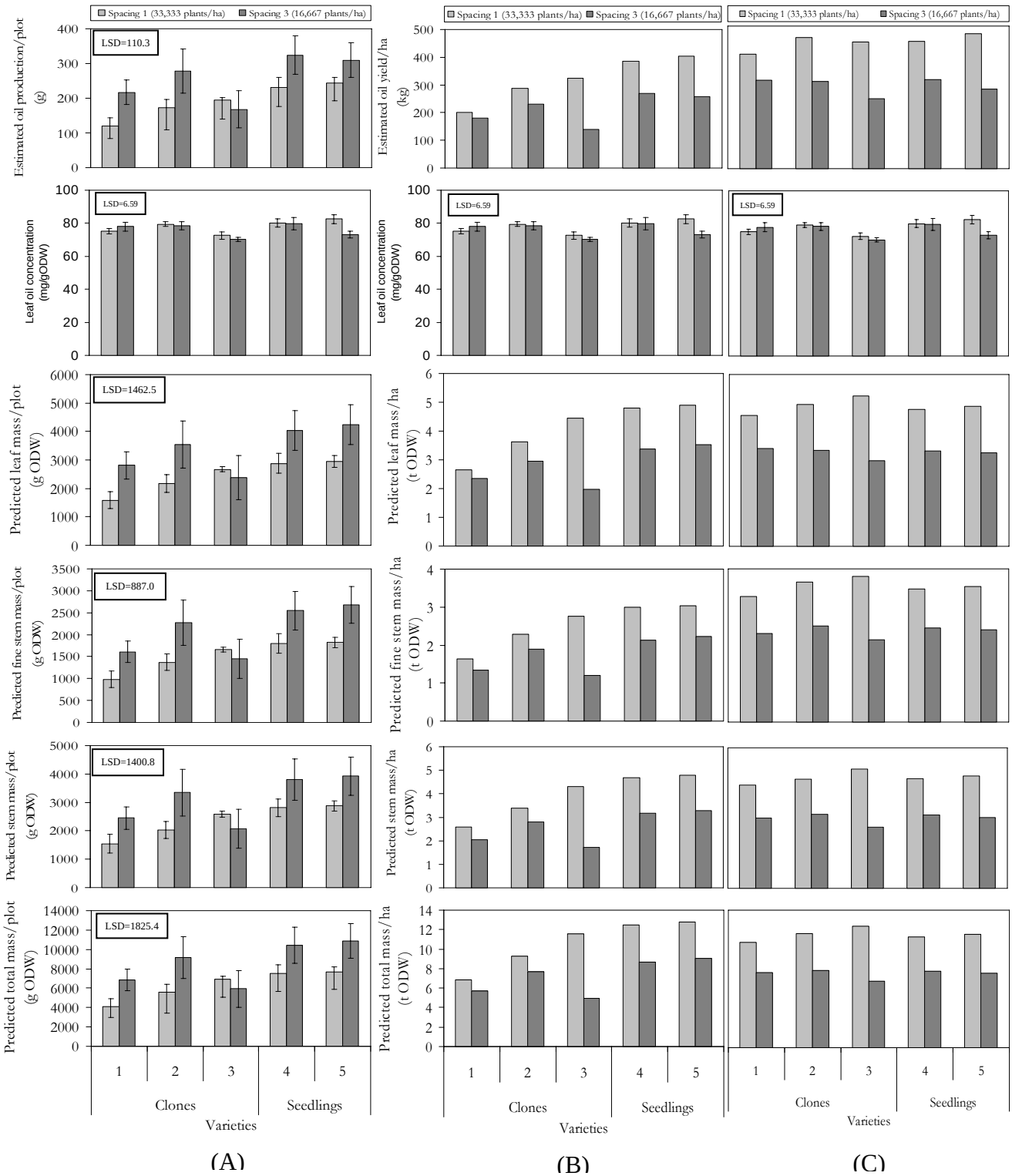

Figure 4.5 Leaf oil concentration, predicted dry weights of key tree components and estimated oil production per plot (g/plot) (A) and extrapolation to per hectare value (e.g. kg of oil/ha) (B and C) of the clones and seedlings included in the 2004 clonal spacing trial at age 18 months after second harvest

Note: Graph (B) is an extrapolation to per hectare values from plot totals, and (C) is an extrapolation to per hectare values from plot means

4.5 Discussion

4.5.1 Variation in growth traits of clones and seedlings

Remarkable differences in growth traits between cutting-raised plants (clones) and seedling-raised plants (seedlings) and within each group and between plant spacings were demonstrated in the first 12 month from planting i.e. trees in the 2006 clonal spacing trial (CST). The same effect was not seen in the coppice at age 18 months after second harvest i.e. trees at the 2004 CST. The extent of the differences in growth traits between clones and seedlings in the 2006 CST was most likely influenced by the fact that clones were older and bigger than the seedlings at planting (G. Baker, pers. comm., 2008). Consequently, when the trial was assessed at age 12 months from planting, clones showed substantially better performance than seedlings in survival, plant height, leafiness score and frost damage at all spacings. Therefore the influence of planting stock quality must be considered when comparing the growth traits of clones and seedlings in the 2006 CST. Later comparisons of growth traits of coppice after harvest would probably be more reasonable as the clones and seedlings start from relatively similar circumstances.

Growth traits of coppice of tea tree after the second harvest in the 2004 CST were not affected by variety group and plant spacing. It should be noted, however, that there were a number of extraneous sources of variation in the 2004 CST i.e. J-rooting, edge effects and flood effects. Growth of the coppice in this trial was most likely affected by external influences. Therefore, it is reasonably risky to draw a conclusion about the effect of plant variety and spacing on coppice growth traits from this trial. J-rooting may account for the lower survival of clones in that they could not develop an adequate root system. The logical consequences of this phenomenon are that nutrient uptake from the roots will not be optimal and trees can not develop a strong ‘sinker’ root. It is also possible that these particular clones are not suitable for this site. Libby (1985) and Evans and Turnbull (2004) recommended that well adapted clones to a particular site should be used to maximize the advantages of clonal plantations. Therefore, a multiple site clonal trial should be established before the selected clones are deployed to commercial plantations to investigate clone-by-environment interactions as the rank of clones might be change

across various site (Frampton and Foster, 1993). This knowledge is necessary to efficiently deploy clonal material (Frampton and Foster, 1993).

It is a common misunderstanding that rooted cuttings may in some way have root systems that are inferior to those of seedlings and consequently that cutting-raised plants are more susceptible to wind throw (Leakey, 2004a), or have a higher mortality following harvesting as in the case in the tea tree industry (J. Doran[4] pers. comm., 2007). Leakey (2004b), however argued that the lack of a tap root is not the fundamental reason for tree instability, as in fact not every mature tree grown from seed has a tap root. The most substantial factor in relation to wind stability of trees is the ability to form "sinker" roots. The problem of roots lacking the ability to form sinkers can be avoided by selecting only easily propagated plants that form multiple roots combined with conditions and techniques which ensure the rapid formation of a radially-arranged and vigorous root system (Leakey, 2004b). The J-rooting occurring at the 2004 CST was certainly a man made problem which was due to mismanagement in the nursery i.e. poor repotting techniques. There was no evidence of a similar problem in the clones included in the 2006 CST which were not repotted in the nursery. Although evidence of cutting-raised plant instability throughout several harvesting cycles is not available, the fact that clones in the 2006 CST had nearly 100% survival in the first 12 months from planting indicates that these clones had effective root systems.

M.alternifolia trees planted at a plant stocking of 13,300 plants per hectare in the Fraser Coast region of Queensland by Macdonald (n.d.) were found to be stronger, healthier and have a denser leaf mass than plants at higher stockings. In this study, there was no evidence that trees at a wider spacing i.e. 16,667 plants per hectare were healthier as the survival rate of trees was not affected by plant spacing both in the 2006 and 2004 CST. A similar behaviour of leafiness score was also demonstrated by trees in the 2006 CST and coppice of clones and seedlings in the 2004 CST. Leafiness score was significantly influenced by plant spacing for both clones and seedlings. Trees at wider spacings typically have a higher leafiness score as they are given more space and there is less competition for light.

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[4] Hon. Research Fellow CSIRO

4.5.2 Variation in oil traits of clones and seedlings

The oil concentration of cutting and seedling-raised plants at 12 months after planting was influenced by plant spacing, with the higher oil concentration being demonstrated by plants at the narrower spacing. The most likely explanation for this is that the young plants given the additional space are placing more resources towards growth than developing secondary products such as essential oils (J. Doran pers. comm., 2008). This result was confirmed in the coppice of the 2004 CST, where trees at the narrower spacing gave moderately higher oil concentration than those at the wider spacing, although, in this case the difference in oil concentration between the two spacings was not statistically significant. This result conflicts with those from a long term study by Small (1981) who found that plant spacing had no effect on oil concentration of *M. alternifolia* over 7 harvesting periods. Further examples of an absence of plant density effect on leaf oil concentration were found in blue mallee (*E. polybractea*) and oil mallee (*E. kochii*) (Milthorpe *et al.*, 1998).

The higher oil concentration of clones over seedlings at the first harvest (i.e. 12 months from planting) is most likely related to their physiological maturity, through which clones exhibit oil levels from planting that will only be reached by seedlings at the third harvest (Baker *et al.*, 2007; Colton *et al.*, 2000). Several studies in *Eucalyptus* species also showed that the oil yield of seedling leaves is typically much lower than that of other stages i.e. juvenile leaves, intermediate leaves and adult leaves (Doran, 1991). Sangwan *et al.* (2001) found that generally oil accumulation in the plant organs, tissue and cells, and infrequently its composition, depends on the developmental phase of the plant *per se*. Besides this ontogenetical factor, as outlined in Chapter 2, oil concentration is also dependent on genetic factors (Flück, 1963).

The first and second suite of elite clones provided by the RIRDC/ATTIA tea tree breeding project were selected from superior trees which averaged an oil concentration of >92.6 mg/g and >73.9 mg/g for the first and second suite of elite clones respectively. Their oil concentrations are higher than the average of mature oil concentration of progeny of CSO1 (ATTIA 2B, 72.3 mg/g). As outlined in Chapter 1, there are several advantages of cloning. Such advantages include a quick way to

produce genetically identical replicates of trees possessing desirable characteristics (Frampton and Foster, 1993), to access and maintain genetic gain, and to enable genetic gains achieved in tree breeding to be efficiently captured (Evans and Turnbull, 2004). Therefore, it was expected that the oil concentration of clones in the 2006 CST would be higher than that of the improved seedlings used as controls.

On the other hand, an anomaly happened in the 2004 CST where oil concentrations of the three clones studied were lower than the seedlings controls (Table 4.17). This result was confirmed in the 2004 Clonal Yield Trial (data not shown) where average oil concentrations of the 10 clones in this trial were much lower than those of their ortets (75.6 mg/g cf. 92.6 mg/g). In addition, the oil concentration of seedlings (ATTIA 2A -a seedlot of the SSO1- and ATTIA 2B –a seedlot of the CSO1) at two different spacings in the 2004 CST were much higher than their corresponding concentrations in the well established 2002 yield trial. In the 2002 trial their mature oil concentrations were 60.4 mg/g and 72.3 mg/g for ATTIA 2A and ATTIA 2B respectively (Baker *et al.*, 2007; Doran *et al.*, 2006).

Due to the fact that the assessment was carried out in early winter and that there was an inconsistent pattern between clones and seedlings, this anomaly in oil concentration was most likely not associated with seasonal fluctuation in oil concentration as has been reported by several authors. Drinnan (1997), Murtagh (1999) and Williams and Home (1988) have recorded a distinct seasonal pattern with oil concentration in *M. alternifolia* being highest in summer (November-May) and lowest in late winter/early spring (September-October). More recent research by de Figueiredo (2006) also confirmed that oil concentration and composition of this species, planted in South Africa, varied throughout the season.

It is important to note in interpreting these results that a number extraneous sources of variation are operating in the 2004 CST and these may contribute to this inconsistency rather than seasonal fluctuation. Because of this, care should be exercised in drawing any conclusions and recommendations from this trial.

The composition of tea tree oil determines its quality. The key determinants of tea tree oil quality are 1,8-cineole and terpinen-4-ol. They were not affected by plant spacing at age 12 months from planting in the 2006 CST in both clones and

seedlings. On the other hand, in the coppice of clones at 18 months after the second harvest in the 2004 CST, these compounds were affected by plant spacing whereas there was no effect on seedlings. Meanwhile, Small (1981) found that plant spacing had no effect on composition of *M. alternifolia* over 7 harvesting periods. This inconsistent result indicates that caution should be taken in concluding whether the variation in oil composition is due to plant spacing or other factors. This inconsistent result, however, is of little relevance as the quality of oils from the two clonal spacing trials was excellent and above the levels specified by the International Standard and current market requirement for tea tree oil. Hence, the variation of oil compositions either due to plant spacing or plant variety is irrelevant in terms of the economic value of the oils.

The priority concern then is how to obtain a higher leaf oil concentration of plants in tea tree plantations as it, along with leaf yield, are the key variables for maximizing oil yield of tea tree plantations. The choice of plant stocking per hectare and plant variety of plantations are then of foremost significance in determining the profitability of a tea tree venture. The options and their projected financial profiles are described in Chapter 5 of this thesis.

4.5.3 Variation in predicted dry weights of key tree components and estimated oil yield of clones and seedlings

In this study, at age 12 months from planting, predicted dry weights of key tree components (leaf, stem and fine stem) per hectare were influenced by plant spacing both for cutting- and seedling-raised plants, with higher yields per hectare being obtained at the narrower plant spacing. Leaf yield (ODW/ha), the most important biomass component that directly influences oil yield, increased linearly, by about 48% for clones and 45% for seedlings when plant density increases twofold from 16,667 plants/ha (plantation at spacing 3) to 33,333 plants/ha (plantation at spacing 1). This was accompanied by stem yield increases of 56% and fine stem yield increases of 49% in clones and increases of 63% and 48% by seedlings for stem and fine stem yield respectively. Coppice of tea tree at age 18 months after second harvest also showed a similar behaviour (Figure 4.5-C). Assuming the clones have a survival rate as high as seedlings, clones in the 2004 CST are expected to give an

increase of leaf, stem and fine stem on average of 54%, 61% and 54% respectively while yields from seedlings are predicted to increase by 47%, 54% and 44%.

These results are consistent with earlier research by Small (1981), who found that the leaf yield of *M. alternifolia* was highest in the densest plantation (26,908 plants/ha) and concluded that the species is 'tolerant to close spacing'. A significant quadratic relationship between plant density and biomass production of *E. polybractea* and *E. kochii* on a dry weight basis was also found by Milthorpe *et al.* (1998) by evaluating a trial testing 5 planting densities (between 2,000 and 9,000 plants/ha) which was harvested annually. This indicates that maximizing leaf yield may be achieved by maximizing the number of plants per hectare within the constraints imposed by the productivity of the site. The planting density of about 35,000 plants/ha which is currently used by most tea tree growers in New South Wales (Colton *et al.*, 2000) appears to be appropriate both for cutting- and seedling-raised plants. However, plant stockings for clonal plantations of tea tree may be modified after considering establishment cost e.g. cost of rooted cuttings, oil concentration of clones, projected longevity of the crop and management strategies.

Extrapolation to oil yield per hectare, assuming a per hectare plant stocking of 33,333 plants/ha and 16,667 tress/ha with plant survival of 99.1%, gives 511.7 kg/ha and 327.1 kg/ha for cutting-raised plants of the eight best clones from the second suite of elite clones. The two best performing clones, in terms of oil yield, in the 2006 CST were clone 5 (C64) and clone 6 (C66) that performed consistently at both narrow and wide spacings. Clone 3 (C56) showed better performance at the narrow spacing and clone 9 (C70) performed better at the wider spacing. However clone 3 contained 2.69% of 1,8-cineole with 42% of terpinen-4-ol as opposed to clone 9 which contained 0.5% of cineole and 44% of terpinen-4-ol which is preferred by the market.

Meanwhile, ATTIA 2B seedlings were predicted to give 164.8 kg/ha and 98.1 kg/ha for first harvest with survival rate of 97.5% and 96.3% from the first and second plant stocking respectively. These yields are considered to be below potential. There is a high probability that the extent of the differences in oil yields of cutting- and seedling-raised plants from the first harvest in the 2006 CST are exaggerated because

of the factors previously described. The factors were planting stock quality i.e. clones were older and bigger than the seedlings at planting and differences in oil concentration i.e. clones exhibit mature oil levels from planting that will only be reached by seedlings at the third harvest. Oil yield of seedling-raised plants of ATTIA 2B growing in the well established 2002 yield trial (Baker *et al.*, 2007; Doran *et al.*, 2006) are considered to be more representative. In this trial, where first harvest was at 16 months, ATTIA 2B at 30,000 plants/ha gave an oil yield of 258 kg/ha at first harvest (Doran *et al.*, 2006).

The substantial different in leaf yield between the 2006 CST and the 2002 yield trial, 2.625 tonnes ODW cf. 4.203 tonnes ODW, accounts for the different levels of oil yield between the progeny of CSO1 in the two trials as there were only moderate differences in foliar oil concentration (63.6 mg/g cf. 61.6 mg/g). Two factors are thought to be influencing this result. The first of these is plant age. In the 2006 CST, plants were harvested at age 12 months from planting while in the 2002 yield trial, plants were about 16 months from planting. Secondly, there was weed competition in the 2006 CST which can hamper growth of plants in the trial as weeds compete with plants for water, nutrients or light (Virtue, 1999). This competition can reduce leaf yield of coppice by an average of 25% (Virtue *et al.*, 2000).

The oil yield of clones in the 2004 CST was estimated to be on average 435.15 kg/ha and 287 kg/ha whereas seedlings were estimated to give 460.15 kg/ha and 295.83 kg/ha from plantation spacings of 1 and 3 respectively. The oil yields of third harvest of clones at both plant stockings in this trial were significantly lower than those of first harvest of clones in the 2006 CST. Meanwhile, the estimated oil yield of the two seedling treatments in this trial was higher than that of the average oil yield of these same seedlots in the 2002 yield trial of 282.7 kg/ha, at similar levels of survival and equivalent upper stocking of 30,000 plants/ha. This is evidence, therefore, of an unrepresentative estimation of mature oil yield. Because the yield data for clones and seedlings derived from the 2004 CST are considered unrepresentative, neither conclusions nor recommendation can be drawn from this trial.

4.6 Conclusions

Maximising oil yield of tea tree plantations is achieved by maximizing leaf oil concentration and leaf yield of the plantation (Murtagh, 1999). In this study, both leaf oil concentration and leaf yield were found to be higher at a denser plant stocking. Therefore, it can be concluded that the maximum oil yield can be achieved by planting tea tree at a closer plant spacing i.e. 33,333 plants/ha instead of 16,667 plants/ha.

The better performance in growth traits of clones over seedlings in the 2006 CST was presumably associated with the fact that there was different quality of planting stock quality at planting. The higher oil concentration of clones over seedlings in this trial is most likely related to their physiological maturity, where clones exhibit mature oil levels from planting that will only be reached by seedlings at the third harvest. Meanwhile, extraneous sources of variation i.e. J-rooting, edge effects and flood effects influenced growth and oil traits of coppice of clones and seedlings at 18 months from second harvest in the 2004 CST.

Trees in both the 2006 and the 2004 CST gave similar behaviour in responding to plant spacing. Trees at a wider spacing typically have a higher leafiness score as they are given more space and there is less competition for light. However, these trees had lower oil concentration than those at the narrower spacing. The higher leaf yields coincide with higher oil concentration of trees planted in a narrower spacing i.e. plant stocking of 33,333 plants/ha indicates that the maximum oil yield can be obtained from this plantation rather than from those at wider spacings. To support these findings, financial analyses to evaluate financial viability of establishing tea tree plantations using clones are discussed in Chapter 5.

Chapter 5 Financial analysis of commercial clonal tea tree plantations

5.1 Introduction

The assumptions used in all previous financial analyses of tea tree plantations outlined in Chapter 2 are now dated as there have been significant changes in costs and oil price over the years. It is clear however, that growing tea tree as a commercial plantation crop can give large profits resulting from good internal rates of returns despite substantial costs. The profitability of this venture is very sensitive to changes in oil yield and oil price (Colton *et al.*, 2000; Reilly, 1991). Growers have been unable to control oil price to maximize their profits. The oil price is largely determined by the market which is influenced by supply and demand. Oil yields of tea tree plantations are very dependant on the management of the plantation itself. The optimum oil yield of tea tree plantations can be achieved by maximizing leaf oil concentration and leaf yield of the plantation (Murtagh, 1999). Most of the earlier tea tree plantations were established using seedlots of a very narrow and often sub-optimal genetic base (Baker, 1999). The consequence of this unsophisticated genetic sourcing is substantial variability of oil yield and oil quality (Baker *et al.*, 2007).

To improve plantation productivity, improved seedlots resulting from the RIRDC/ATTIA tea tree breeding programme can be used for establishing new plantings or replacing existing plantations. Doran *et al.* (2006) found that the average improvements in yield of improved seed sources (CSO1 (ATTIA 2B), SSO1 (ATTIA 2A) and a selected provenance (ATTIA 1) over industry standards were 83%, 55% and 43% respectively. Superiority of improved seedlots over industry standards in oil quality was also demonstrated through higher levels of terpinen-4-ol (higher by 4%) and lower levels of 1,8-cineole (Doran *et al.*, 2006). These improved seeds have been released to industry since 2001 (Baker *et al.*, 2007). The enhancement in plantation productivity as result of using breeding programme seedlots will directly affect the profitability of planting tea tree as a commercial venture.

The development of a clonal seed orchard (CSO1) and selection of elite clones have been key features of the breeding strategy to give substantial genetic gain through release to industry of highly improved and selected clones. These releases are designed to give a progressive improvement in financial profitability of tea tree plantations. The elite clones included in the 2006 CST are looking promising in terms of oil yield compared with seedlings grown from seed from CSO1, as described in Chapter 4. Estimated oil yield of the first harvest of clones included in the 2006 CST is about 2.4 times greater than oil yield of industry standard and about 0.4 times greater than oil yield of ATTIA 2B (511.7 kg/ha cf. 148.5 kg/ha and 369.6 kg/ha) for plant stocking of 33,333 plants/ha. When the best three clones, C64, C66 and C70[*)], are selected for deployment, they will boost the estimated oil yield of the clones to 522.6 kg/ha. Clones will likely also show a superior oil yielding capacity throughout the life of the plantation, particularly in the first two harvests as they exhibit quantities of oil production only reached by seedlings at the third harvest (Baker *et al.*, 2007).

There are several impediments to tea tree industry adoption of clonal plantations of tea trees as discussed in Chapter 1. One of the major impediments is growers' concern that the benefit/cost ratio does not favour clones due to clones being too costly compared to seedlings in the establishment phase (50 cents per propagule as opposed to 12 cents for seedlings).

5.2 Objectives

The objectives of this study are:

1. to develop a spreadsheet-based financial model to compare profitability of commercial *M. alternifolia* plantations established from selected clones against those established using improved seed from a breeding programme,

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*) C64 and C66 are clones of family 128 in the yield trial est. 2002
C70 is a clone of family 12 in the controlled-cross progeny plots est. 2002

2. to compare Net Present Value (NPV) and Internal Rate Return (IRR) of *M. alternifolia* plantations established using seedlings grown from improved seed from a breeding programme and three best selected clones planted at two different plant spacings. These comparisons use yield data from the experiments described in Chapter 4.

5.3 Materials and methods

5.3.1 Approach and assumptions

Approach

This component of the study involves development of a spreadsheet-based financial model of commercial tea tree plantations established from selected clones against those established using seedlings grown from improved seed from a breeding programme. Two different within-row spacings are compared. Baseline scenarios of commercial tea tree plantations for each option were initially modelled utilising the baseline production parameters presented in Table 5.1. The model interface is included as Appendix 1 to Appendix 4. Subsequent simulation of amendments based on a number of alternative production parameters were carried out to evaluate sensitivity to these key production variables.

To determine which plantation option is best in terms of financial profitability, the NPV and IRR of each option is compared. Generally, a project with NPV more than or equal to zero and IRR more than or equal to a real discount rate is acceptable in financial terms. The situation is different when selecting the most profitable option to be accepted among mutually exclusive projects. An option is preferable when its NPV and IRR are higher than other options. In some cases, however, the NPV and IRR decision-rules can end up giving a conflicting result because of the ‘switching’ phenomenon which allows changes in the ranking of the options. Because of the possibility of switching, Perkins (1994) and Campbell and Brown (2003) suggested that the decision-rule for mutually exclusive projects such as this is to accept the option with the highest NPV.

Assumptions

The objective of the financial analyses of commercial *M. alternifolia* plantations in this study is to evaluate the financial viability of replacing 20 ha of established commercial tea tree plantations with either selected clones or seedlings grown from improved seed from a breeding programme. Four plantation options were modelled i.e. (1) plantations established using seedlings grown from improved seed (i.e. ATTIA 2B) planted at a 30 cm within-row spacing and 1 m between-row spacing (33,333 plants/ha), (2) plantations established using seedlings grown from improved seed (ATTIA 2B) planted at a 60 cm within-row spacing and 1 m between-row spacing (16,667 plants/ha), (3) plantations established using the three best selected clones (C64, C66 and C70), planted at a 30 cm within-row spacing and 1 m between-row spacing (33,333 plants/ha), and (4) plantations established using the three best selected clones (C64, C66 and C70), planted at a 60 cm within-row spacing and 1 m between-row spacing (16,667 plants/ha).

It was assumed that no capital costs e.g. purchase of land and machinery, are required, as all these options involve replacement plantations. These costs were therefore excluded from the analysis. Plantation establishment costs cover only costs needed for replanting using the two types of propagule (seedlings or clones). Production parameters used for modelling commercial tea tree plantations are shown in Table 5.1. The establishment and operating costs employed in this study are determined from an average of growers' best practices in establishing tea tree plantations using seedlings. It was assumed that operating costs of plantations at a plant stocking of 16,667 plants/ha is 90% of those at a plant stocking of 33,333 plants/ha as there would be some differences in the amount of fertilizers, insecticides and herbicides required but not in other cost components. Details of establishment and operating costs are shown in Appendix 5.

There were confounding factors that influenced oil yield of seedlings over time in both the 2004 and 2006 CSTs as discussed in Chapter 4. To avoid using these atypical results, the oil yields over time of seedlings (ATTIA 2B) employed in this financial analysis were taken from the 2002 yield trial (Baker *et al.*, 2007) while oil yields of clones were derived from the average yields of three best clones i.e. C64, C66 and C70 in the 2006 CST as reported in Chapter 4. It was assumed that the

survival rate of seedlings is the same as of clones in the 2006 CST i.e. 99% throughout the life of the plantations. Proportions of mature yield of seedlings in year 1 and year 2 (i.e. at first and second harvests) were taken from the 2002 yield trial (Baker *et al.*, 2007). In contrast, it was assumed that clones have the same levels of oil yield throughout the life of the plantation. Oil yield of plantations using seedlings at a plant stocking of 16,667 plants/ha was assumed to be 68% lower than that of at a plant stocking of 33,333 plants/ha which was the same as of clones in the 2006 CST. Replanting the whole 20 ha of plantation was assumed to be carried out in the first year. Oil price during September 2008 ($45/kg of oil) was used in the baseline model. Income from marketing of spent leaf after oil as mulch production was determined from information provided by growers.

Table 5.1 Parameters used for modelling commercial tea tree plantations established using selected clones and seedlings grown from improved seed from a breeding programme at two different plant spacings

Parameters	Plantation options			
	Seedlings @30 cm	Seedlings @60 cm	Clone s @30 cm	Clones @60 cm
Plant price ($/unit)	0.12	0.12	0.50	0.50
Number of plants/ha	33,333	16,667	33,333	16,667
Cost of plants/ha ($/ha)	4,000	2,000	16,667	8,334
Other plantation establishment cost ($/ha)*	1,847	1,662	1,847	1,662
Total plantation establishment cost ($/ha)	5,847	3,663	18,514	9,996
Operating costs in year 1 ($/ha)*	1,439	1,295	1,439	1,295
Operating costs in year 2 onward ($/ha)*	5,178	4,660	5,178	4,660
Proportion of mature yield in year 1	0.77	0.77	1.00	1.00
Proportion of mature yield in year 2	0.88	0.88	1.00	1.00
Oil yield in year 1 (kg/ha)	283.8	193.0	522.6	356.6
Oil yield in year 2 (kg/ha)	326.7	222.2	522.6	356.6
Mature yield in year 3 onward (kg/ha)	369.6	251.3	522.6	356.6
Spent leaf production/ha (m^3)	67.5	45.9	67.5	45.9
Farmgate price ($/kg)	45	45	45	45
Spent leaf price ($/$m^3$)	21	21	21	21
Life of tea tree plantation (yrs)	15	15	15	15
Area of plantation (ha)	20	20	20	20
Discount rate (%)	7%	7%	7%	7%

* see Appendix 5 for details of establishment and operating costs

A real discount rate must be used to discount the net cash flow of the project (Perkins, 1994). The choice of discount rate used in calculating net present value (NPV) is of critical importance because a change in the rate will change the NPV. Use of an incorrect rate will give an unrealistic value for the NPV (Campbell and Brown, 2003; Sinden and Thampapillai, 1995). The real discount rate is calculated by deflating the market interest rate by the expected rate of inflation in the economy. The real rate of return of the government bond rate was recommended by Campbell and Brown (2003) as closest to the real market interest rate. However, due to the variability of interest rates and the wide range of factors which impact on interest rates, deriving the appropriate discount rate from the rate of return of government bonds can give an erroneous value (NSW Treasury, 2007). Because there is no universally accepted discount rate, the NSW Treasury (2007) suggested that one should test real discount rates of 4%, 7% and 10% to see if outcomes are sensitive to such variations. In this study, therefore, the discount rate of 7% was used as a central real discount rate with sensitivity tests on 4% and 10%.

5.3.2 Sensitivity analysis

A number of alternative production parameters were modelled, each resulting in a discounted accumulative cashflow, NPV and IRR for the particular scenario. This enables financial comparisons to be made with the base scenario for each plantation option. A sensitivity analysis was carried out by examining the effects of the modification in discount rate, farmgate oil price, fluctuation in oil yield and decrease/increase in plantation costs on profitability of tea tree plantations for each option. The implication of the Goods and Services Tax (GST) and other forms of taxation were not accounted for in these simulations.

To determine the sensitivity of the baseline scenario for each plantation option to the discount rate, the model was run using discount rates of 4%, 7% (central value) and 10%. To determine sensitivity to farmgate oil price, the model was initially run assuming that a market was available for spent leaf mulch at a steady level. Subsequently the model was run in various farmgate oil prices starting from $15 to $55 with an increment of $10.

There are several factors that can affect productivity of tea tree plantations; therefore fluctuation in oil yields is inevitable. To evaluate these fluctuations, a sensitivity analysis on variations in oil yield was carried out. The model was run utilising various oil yields. Values reflecting of up to a 50% decrease and increase in oil yields were chosen. The model was also run utilising various costs in establishing and operating commercial tea tree plantations. The situations where plantation costs are halved and doubled from the baseline scenario were also tested.

5.4 Results

5.4.1 Baseline scenarios

There are differences in NPV and IRR of the four tea tree plantation options (see Table 5.2) generated using production parameters presented in Table 5.1.

Table 5.2 Summary of the financial analysis results for a 20-hectare commercial plantation using the production parameters given in Table 5.1

Criteria	Plantation options			
	Seedlings @30 cm	Seedlings @60 cm	**Clones @30 cm**	Clones @60 cm
PVB ($)	2,308,614	1,377,725	3,668,695	2,312,679
PVC ($)	116,944	73,251	370,274	199,920
NPV ($)	2,191,671	1,304,474	**3,298,420**	2,112,759
IRR (%)	211.5	213.8	**117.6**	143.4

PVB is present value of benefit; PVC is present value of cost

It is clear that replanting a tea tree plantation using selected clones at 30 cm within-row spacing (33,333 plants/ha) is the most profitable option compared with other plantation options under the assumptions applied. The NPV of this 20 ha plantation over 15-year time frame was $3,298,420 compared to $2,191,671 for a plantation using improved seedlings at the same plant stocking. Clones planted at the wider spacing (plant stocking of 16,667 plants/ha) give an NPV that is nearly as high as seedlings at plant stocking a 33,333 plants/ha, whereas seedlings planted at plant stocking of 16,667 plants/ha resulted in an NPV far below the other options.

A 20 ha clonal plantation at 30 cm within-row spacing, however, resulted in the lowest IRR of 117.6% while IRR of 211.5%, 213.8% and 143.4% were estimated for 20 ha plantations established using seedling at 30 cm within-row spacing, seedlings at 60 cm within-row spacing and clones at 60 cm within-row spacing. The IRRs of all plantation options were very high, i.e. more than 100%. This indicates that all of the plantation options tested would be highly profitable, as long as oil prices remain high.

As previously discussed, where, as here, the NPV and IRR values give a decision-rules conflicting result, the ranking based on NPV should be given priority in comparing options (Campbell and Brown, 2003). Therefore, at this stage, replacing existing tea tree plantations using selected clones at a plant stocking of 33,333 plants/ha is preferred. Figure 5.1 illustrates the discounted accumulative cashflow of the four plantation scenarios. From the graph it can be seen that the cashflow break-even point occurred at first harvest, 1 year from planting.

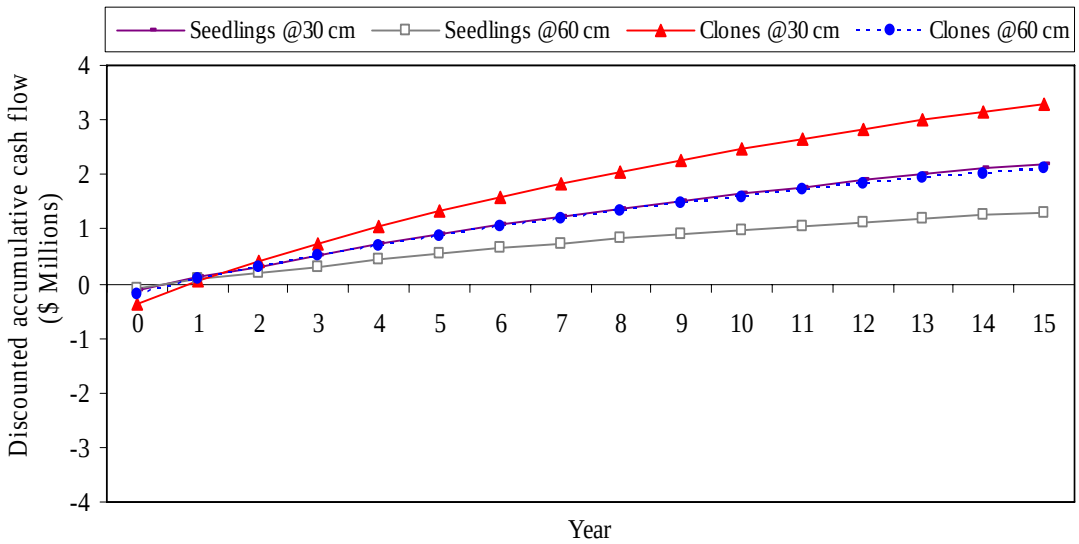

Figure 5.1 Discounted accumulative cashflow of the baseline scenario for replanting tea tree plantations using selected clones and seedlings grown from improved seed planted at two different within-row spacings

5.4.2 Sensitivity analysis

Sensitivity to discount rate

Table 5.3 presents the result of the impact of discount rate on the NPV of the ventures. There was no significant change in the NPV ranking of the ventures when discount rate was modified from 7% to 4% and 10%. The high value of IRR will, certainly, guarantee that all the plantation options are highly profitable as here all discount rates are far below the IRR values.

Table 5.3 Sensitivity of the NPV of the plantation options to discount rate

Criteria	Discount rate (%)	Plantation options			
		Seedlings @30 cm	Seedlings @60 cm	Clones @30 cm	Clones @60 cm
NPV ($)	4	2,707,181	1,609,659	4,094,835	2,611,188
	7	**2,191,671**	**1,304,474**	**3,298,420**	**2,112,759**
	10	1,806,935	1,076,651	2,703,095	1,740,069

Sensitivity to farmgate oil price

Table 5.4 Sensitivity of NPV and IRR of the plantation options to farmgate tea tree oil price at 7% of discount rate

Criteria	Price ($/kg)	Plantation options			
		Seedling @30 cm	Seedling @60 cm	Clone @30 cm	Clone @60 cm
NPV ($)	***12***	***47,598***	***-153,495***	***156,955***	***-30,844***
	15	242,514	- 20,953	442,543	164,029
	25	892,233	420,856	1,394,502	813,606
	35	1,541,952	862,665	2,346,461	1,463,182
	45	**2,191,671**	**1,304,474**	**3,298,420**	**2,112,759**
	55	2,841,390	1,746,283	4,250,380	2,762,335
IRR (%)	***12***	***15.3***	**-**	***14.2***	***3.4***
	15	40.1	-3.9	25.4	21.7
	25	103.0	90.4	57.6	65.5
	35	158.5	154.5	87.9	105.1
	45	**211.5**	**213.8**	**117.6**	**143.4**
	55	263.1	271.0	147.0	180.9

Profitability of tea tree plantations is very sensitive to oil price as demonstrated in Table 5.4. When the oil price is declining the NPV and IRR decrease concomitantly and *vice versa*, provided other production parameters are constant. A clonal

plantation at a plant stocking of 33,333 plants/ha is predicted to give the greatest profit at any of the oil prices tested, followed by plantations using improved seedlings at a plant stocking of 33,333 plants/ha, plantations using clones at a plant stocking of 16,667 plants/ha, and plantations using seedlings at a plant stocking of 16,667 plants/ha. A plantation using improved seedlings at 16,667 plants/ha will not be financially viable when oil price has fallen to $15/kg or less while the other options are still feasible. At the lowest oil price so far experienced in the industry of $12/kg, as occurred during 2004-2005, both clones and seedlings at a plant stocking of 16,667 plants/ha are financially viable. The break-even prices for tea tree oil production, using the production parameters in this model were $11.3/kg, $15.5/kg, $10.4/kg and $12.5/kg for plantation options 1, 2, 3 and 4 respectively. At these levels of oil price, the NPV was $0 and the IRR was 7% for each plantation option.

Sensitivity to oil yield

Table 5.5 Sensitivity of the NPV and IRR of the plantation options to oil yield at 7% of discount rate

Criteria	Increase/ decrease in oil yield (%)	Plantation options			
		Seedlings @30 cm	Seedlings @60 cm	Clones @30 cm	Clones @60 cm
NPV ($)	50	3,653,538	2,298,544	5,440,329	3,574,306
	40	3,361,165	2,099,730	5,011,947	3,281,996
	30	3,068,791	1,900,916	4,583,565	2,989,687
	20	2,776,418	1,702,102	4,155,184	2,697,377
	10	2,484,044	1,503,288	3,726,802	2,405,068
	Control	**2,191,671**	**1,304,474**	**3,298,420**	**2,112,759**
	-10	1,899,297	1,105,660	2,870,039	1,820,449
	-20	1,606,923	906,846	2,441,657	1,528,140
	-30	1,314,550	708,032	2,013,276	1,235,830
	-40	1,022,176	509,218	1,584,894	943,521
	-50	729,803	310,404	1,156,512	651,212
IRR (%)	50	326.6	340.7	183.3	227.2
	40	303.9	315.8	170.2	210.6
	30	281.0	290.7	157.2	193.9
	20	258.0	265.3	144.0	177.2
	10	234.9	239.8	130.9	160.3
	Control	**211.51**	**213.85**	**117.63**	**143.39**
	-10	187.9	187.5	104.3	126.3
	-20	163.9	160.6	90.9	109.0
	-30	139.5	132.9	77.4	91.5
	-40	114.4	103.9	63.7	73.6
	-50	88.3	72.7	49.8	55.2

As previously outlined, the profitability of tea tree plantations is very dependent on oil yields and oil price. Table 5.5 shows that the NPV and IRR of the plantations increase concurrently with an increase in oil yield, and *vice versa*. In the extreme condition, when oil yields of the plantations decrease up to 50% from their optimum levels i.e. 184.8 kg/ha, 125.7 kg/ha, 261.3 kg/ha and 178.3 kg/ha for plantations using options 1, 2, 3 and 4 respectively, the ventures are still financially viable.

Sensitivity to tea tree plantation costs

Table 5.6 gives values for NPV and IRR for the four plantation options where plantation costs are halved or doubled from standard costs used in the baseline scenario. It is more likely that costs will increase rather than decrease. Increases in production costs will reduce the profitability of the venture. However, as shown in the Table 5.6, all plantation options are still profitable when the production costs are doubled from the baseline scenario.

Table 5.6 Sensitivity of the NPV and IRR of the plantation options to plantation costs at 7% of discount rate

Criteria	Increase/ decrease in costs (%)	Plantation options			
		Seedlings @30 cm	Seedlings @60 cm	Clones @30 cm	Clones @60 cm
NPV ($)	100	1,201,396	445,226	2,054,815	1,126,841
	Control	**2,191,671**	**1,304,474**	**3,298,420**	**2,112,759**
	-50	2,686,808	1,734,098	3,920,223	2,605,717
IRR (%)	100	75.0	56.3	45.6	49.5
	Control	**211.5**	**213.8**	**117.6**	**143.4**
	-50	463.1	490.1	255.9	319.4

5.5 Discussion

All plantation options modelled in the baseline scenario of this study are financially viable as indicated by the very high values of IRR and NPV. The absence of capital costs in this financial analysis and the high yielding capacity of the plant material studied coinciding with a high farmgate oil prices, accounted for the high values of IRR and NPV of the baseline models. Selling spent leaf as mulch in conjunction with the sales of oil also contributed to those values. A previous financial analysis by

Agtrans Research (2001) *cited in* Doran *et al.* (2002) also indicated that tea tree plantations using seedlings grown from improved seed and selected clones from the tea tree breeding programme are financially viable although with lower oil yields and oil prices than those used in this study.

The third plantation option which is replanting using the three best clones form the 2006 CST at a plant stocking of 33,333 plants/ha, was shown to be the most financially viable using the NPV decision-rule. The next most profitable option was replanting using seedlings of ATTIA 2B at a plant stocking of 33,333 plants/ha followed by clones at a plant stocking of 16,667 plants/ha, and ATTIA 2B seedlings at a plant stocking of 16,667 plants/ha. Establishing the entire 20 ha of plantation in the first year of operation incurs a greater initial cashflow burden, particularly for clonal plantations where their establishment costs are about four times higher than for seedlings. However, the higher yielding capacity of clones throughout the life of the plantations, which was assumed to hold firm at the same levels from the first harvest onward, can justify their additional costs in the first year. Figure 5.1 shows that establishment and operating costs incurred during year 1 of the plantations were recovered by returns from the venture at first harvest (12 month from planting). This result, therefore, confirmed the earlier hypothesis by Doran *et al.* (2000).

Due to higher establishment costs in the first year, clonal plantations at a plant stocking of 33,333 plants/ha had the lowest discounted accumulative cashflow. This situation, however, changed from year two onwards where this plantation option showed its superiority over the other options. The implication is that replanting tea tree plantations using clones at this plant stocking will be more attractive as compared with seedling plantations from the best seed currently available from the breeding programme at the same stocking, provided there is no natural catastrophe before their second harvest. Replanting tea tree plantations using clones at any plant stocking will have higher risks of losing capital in the worst case scenario of loss of the entire plantation before first harvest (e.g. loss to flooding).

The NPVs of all plantation options remained positive until the discount rate reaches a value that is higher than the IRR for that plantation option (Campbell and Brown, 2003). With IRR values of more than 100%, all plantation options will remain in a

financially viable although the magnitude of the profit will vary among plantation options and according to the discount rate applied.

At a current farmgate tea tree oil price of $45/kg, which is far above their break-even price, all plantation options provided high returns on capital invested. The break-even prices for tea tree oil production, using the production parameters in this model were $11.3/kg, $15.5/kg, $10.4/kg and $12.5/kg for plantation options 1, 2, 3 and 4 respectively. Hence, assuming an oil price of $12/kg which is the lowest price so far experienced (ATTIA, 2006; Davis, 2003), clonal plantations at a plant stocking of 33,333 plants/ha will be more attractive because they provide the highest NPV of $156,955 as oppose to only $47,598 from seedling plantations at the same plant stocking whereas other plantation options are not financially viable. It is impossible for the small producers to be able to set the market price received for bulk oils. It is, however, realistic to improve their production efficiency. If the oil price drops, efficiency must be improved in order to reduce production costs to maintain the viability of the plantation. With the reasonably low break-even price of all plantation options in the baseline scenario, it appears that currently production efficiency is good and urgent action to improve efficiencies is not required.

Fluctuations in oil yield of tea tree plantations is inevitable due to multiple factors, as outlined in Chapter 2. This study shows that fluctuations in oil yields will directly affect the profitability of the four options studied here. A decrease of NPV occurs concurrently with a decrease in oil yield and *vice versa*. At the current farmgate oil price, however, the ventures remain profitable even when oil yields drop up to 50% from the baseline parameter. In the case of higher production costs, as shown in the Table 5.6, the ventures are still viable. The current high oil price is the main reason for this.

5.6 Conclusions

The financial analysis developed in this study clearly showed that replanting tea tree plantations using selected clones and seedlings of CSO1, planted either at 33,333 plants/ha or 16,667 plants/ha were financially viable. Sensitivity analyses showed that discount rate, oil price, oil yield and production costs all affected the amount of

profitability of the venture without affecting the ranking of plantation options. Plantations using selected clones at a plant stocking of 33,333 plants/ha appeared to be the best choice as it gave the highest profit over a 15-year time frame. This scenario, however, has the highest risk of capital loss if an unexpected catastrophe occurs before first harvest. The profitability of plantations using improved seedlings at a plant stocking of 33,333 plants/ha was slightly higher than that of plantations using selected clones at a plant stocking of 16,667 plants/ha. Meanwhile, plantations using improved seedlings at a plant stocking of 16,667 plants/ha gave the lowest profit compared with other options. It is also important to note that the data of oil yields of selected clones used in this study were early, first-harvest estimates and these clones have not yet proven themselves to be adapted to other plantation areas.

Chapter 6 Summary of key findings and recommendations

6.1 Introduction

The main aim of this study was to evaluate whether the use of clones in tea tree (*Melaleuca alternifolia*) plantation establishment is a financially viable proposition. The study was based on clonal spacing trials (CST) established in 2006 and 2004 at Bungawalbin, NSW. Three stockings were evaluated in these trials: 33,333 plants/ha (1 m between-rows x 30 cm within-rows), 22,222 plants/ha (1 m x 45 cm) and 16,667 plants/ha (1 m x 60 cm). The clonal spacing trials were established to evaluate if improved off-paddock oil yield from using selected clones enabled growers to reduce planting density and associated establishment costs while still delivering increased oil production. In order to gain an understanding of these objectives, the following aspects were investigated:

1. Effect of plant spacing on commercial traits for oil production,
2. The difference between clones and seedlings in commercial traits for oil production within each spacing,
3. The difference among clones in commercial traits for oil production within each spacing, and
4. Financial viability of tea tree plantations established using selected clones and improved seedlings at different plant spacings.

Plant materials studied: The 2006 CST comprised 10 clones and two improved seedling controls and the trees were 12 months from planting when assessed. The 2004 CST comprised of three clones and two improved seedling controls. Trees in this trial had been twice harvested before this study. Coppice growth at age 18 months after second harvest and just prior to third harvest was assessed.

6.2 Summary of key findings and recommendations

6.2.1 Effects of plant spacing on commercial traits for oil production

Effects of plant spacing on growth traits

Survival and leafiness scores in response to the different spacings showed similar trends in both trials (Table 4.4 and Table 4.15). There was no significant difference in survival of trees due to differences in plant spacing. Trees at wider spacing typically have a higher leafiness score as they are given more space and there is less competition for light. The response of tree height to the different plant spacings was variable. Trees in 2006 CST planted at narrower spacing were typically taller than those at wider spacings. Coppice in 2004 CST, however, had relatively similar height over all plant spacings. These results suggest that on an individual tree level, trees at a wider spacing will have higher leaf yield compared to those at narrower plant spacings.

Effects of plant spacing on oil traits

Generally, trees at a narrow spacing had higher oil concentration than those at wider spacings (Table 4.7 and Table 4.17). The most likely explanation for this is that the plants given the additional space are placing more resources towards growth than developing secondary products such as essential oils. While there was no significant difference in percentage of total oil comprised of 1,8-cineole and terpinen-4-ol in 2006 CST (Table 4.7) due to plant spacing there was a response in 2004 CST (Table 4. 17). This inconsistent result, however, is of little relevance as the quality of oils from the two clonal spacing trials was excellent and above the levels specified by the International Standard and current market requirement for tea tree oil. Oil quality variation due to plant spacing treatments, therefore, appears to be of little concern.

Effects of plant spacing on predicted dry weights of tree components and estimated oil yields

Dry weights on a per plot basis of all tree components with potential to influence oil yields increased concomitant with increase of plant within-row spacing from 30 cm (33,333 plants/ha) to 60 cm (16,667 plants/ha). Consequently, estimated oil production per plot was also higher at the wider spacing (Figure 4.4 and Figure 4.5). As the number of plants per hectare increased with decrease in plant within-row spacing, predicted stem, fine stem, and leaf mass per unit area increased more than 45% (Figure 4.4 and Figure 4.5-C). In addition, it was found that trees at the narrower spacing (33,333 plants/ha) had higher oil concentration than those at the widest spacing, (16,667 plants/ha). The combination of these factors indicates that larger oil yields per unit area can be obtained from tea tree plantations established at higher stockings, such as are currently typical within the industry, e.g. plant stockings > 30,000 plants/ha.

6.2.2 The difference between clones and seedlings in commercial traits for oil production within each spacing

The difference in growth traits between clones and seedlings

Seedlings were significantly poorer in performance than clones for survival, height, leafiness and frost damage at all spacings in the 2006 CST. Conversely, the clones in 2004 CST were inferior in performance to the seedling controls for survival, height and leafiness score at all spacings. Most of the clones in the 2006 CST appeared to be more tolerant to frost damage compared to seedlings. However, the extent of the differences in growth traits between clones and seedlings in the 2006 CST was most likely associated with the fact that clones were older and bigger than the seedlings at planting and the seedlings had not caught up with the clones when assessed at 12 months from planting. Later comparisons of growth traits of coppice after harvest would probably be more reasonable as the clones and seedlings start from relatively similar circumstances.

There were problems too with the comparison in the 2004 CST, as the growth of the three clones was quite obviously affected by external influences, namely J-rooting in the clones, edge effects and flood effects. It is also possible that these particular clones are not suitable for this site. Hence, the comparison between clones and seedlings in these two trials was considered to be unrepresentative. Further research to evaluate differences in growth traits between clones and seedlings over several harvesting periods is required.

The difference in oil traits between clones and seedlings

In the 2006 CST, eight clones from the second suite of elite clones provided by the tea tree breeding programme gave more than 44% greater foliar oil concentration than improved seedlings at 12 months-of-age from planting both at spacing 1 and 3 (Table 4.7). This is most likely related to their physiological maturity, where clones exhibit mature oil levels from planting that will only be reached by seedlings at the third harvest. Surprisingly, oil concentration of the three clones in the 2004 CST at age 18 months after second harvest and representing the first suite of elite clones from the breeding programme was generally less than those of the improved seedling controls (Table 4.17). It is important to note, however, that a number of extraneous sources of variation are operating in the 2004 CST and these may contribute to this inconsistency. As expected, clones were very consistent in their 1,8-cineole content and substantially less variable in this regard than seedling treatments. Further research to evaluate consistency of oil concentration and composition of clones over several harvesting periods is warranted, as the trials in this study are either young (2006 CST) or compromised (2004 CST).

The differences in predicted dry weights of tree components and estimated oil yields of clones and seedlings

Clones gave superior performance to improved seedlings in predicted dry weights of tree components (stem, fine stem and leaf) and estimated oil yield at both plant stockings of 16,667 plants/ha and 33,333 plants/ha in the 2006 CST (Figure 4.4). The extent of the differences, however, is likely to be exaggerated because the clones were fitter on planting compared to the seedlings. Clones in the 2004 CST, in

contrast, showed inferiority in these traits compared to improved seedlings (Figure 4.5). This comparison is also compromised by the fitness of the planting materials with the clones disadvantaged by extraneous factors such as J-rooting. Oil yields of the seedling controls (ATTIA 2A and ATTIA 2B) in the 2004 CST were greater than the oil yields of identical seedlots in the well established 2002 yield trial. This result is unexpected and contributes to the extent of the differences between clones and seedlings in this trial.

6.2.3 The differences among clones in commercial traits for oil production within each spacing

The difference in growth traits among clones

Survival of all clones was excellent in the 2006 CST averaging more than 99% at all spacings. There were significant differences in plant height, leafiness score, frost damage score and flowering score among the 10 clones from the second suite of elite clones at age 12 months from planting at all spacings. Taller plants tended to have lower leafiness scores and *vice versa*. Most clones in the 2006 CST appeared to be more tolerant to frost damage compared to seedlings. The hypothesis that flowering, as was common amongst the clones in the 2006 CST at 12 months from planting, might lead to reduced oil yield due to lowered oil concentration during flowering was not proved in this study. There was no significant difference in oil concentration between flowering and non-flowering trees. Two clones (Clone 4 and Clone 10) were not included in the evaluation of oil traits because they grew poorly compared to the other clones.

The three clones in the 2004 CST did not differ significantly in survival and height growth but they did differ in leafiness score. A J-rooting problem occurred in these clones and other extraneous sources of variation operating in this trial were considered to account for the poor growth traits of these clones. Clone-by-environment interactions might also be contributing to the poor growth of clones in this trial.

The difference in oil traits among clones

Oil concentrations of the eight clones assessed in the 2006 CST ranged from 82.14 mg/g ODW to 95.78 mg/g ODW and from 78 mg/g ODW to 92.47 mg/g ODW from stockings of 33,333 plants/ha and 16,667 plants/ha respectively. These were considered to be excellent. It is expected that the oil concentration of clones will be held at this level throughout the life of the plantation. Consistency in 1,8-cineole content was also a feature of each clone compared to greater variability amongst seedling stock. A lower than average oil concentration was demonstrated by Clone 1 at both stockings, whereas the highest oil concentrations were given by Clone 3 at 33,333 plants/ha and Clone 6 at 16,667 plants/ha. Oil traits among the three clones in the 2004 CST did not differ significantly. Average oil concentration of these clones was 75 mg/g ODW which was considered to be below their potential based on the far superior oil yields of their ortets. It should be emphasised that the external factors influencing the results from this trial, as discussed under growth comparisons above, were also suspected of influencing oil concentration. Oil compositions, however, seemed not to be influenced by these external factors.

The differences in predicted dry weights of tree components and estimated oil yield among clones

The eight clones assessed for both growth and oil traits in the 2006 CST differed significantly in predicted dry weights of key tree components and estimated oil yields (Figure 4.4 and Table 4.13). The two best performing clones in terms of oil yield in the 2006 CST were clone 5 (C64) and clone 6 (C66) that performed consistently at both narrow and wide spacings. Clone 3 (C56) showed better performance at the narrow spacing and clone 9 (C70) performed better at the wider spacing. There were also oil quality differences between clones e.g. clone 3 contained 2.69% of 1,8-cineole with 42% of terpinen-4-ol as opposed to clone 9 which contained 0.5% of cineole and 44% of terpinen-4-ol which is preferred by market. These results indicate that gains in biomass, oil yield and oil quality of clonal plantations can be best achieved by the careful selection of clones with the best combination of these traits irrespective of the plant stocking that might be employed by a particular grower.

Because of the limited number of clones included in the 2004 CST and the unrepresentative estimation of oil yield derived from these clones under confounding factors, representative conclusions and recommendations cannot be drawn from this trial. Selection of best clones from the first suite of elite clones to be deployed might be based on the 2004 clonal yield trial which was planted adjacent to the spacing trial but not assessed in this study because of time constraints.

6.2.4 Financial viability of tea tree plantations established using selected clones and improved seedlings at different plant spacings.

The financial analysis developed in this study showed clearly that replanting tea tree plantations using the three best selected clones from the second suite of elite clones and improved seedlings from CSO1, planted either at stockings of 33,333 plants/ha or 16,667 plants/ha, were financially viable. Sensitivity analyses showed that discount rate, oil price, oil yield and production costs all affected the amount of profitability of the venture without affecting the ranking of plantation options. Plantations using clones at a stocking of 33,333 plants/ha appeared to be the best choice as it gave the highest profit over a 15-year time frame. This scenario, however, has the highest risk of capital loss if an unexpected catastrophe occurs before first harvest. It is also important to note that the data of oil yields of selected clones used in this study were early, first-harvest estimates and these clones have not yet proven themselves to be adapted to other plantation areas. Establishment of small-scale clonal plantations on a range of sites where tea tree is grown commercially is highly recommended before launching into large scale clonal plantations. This is because of the level of additional investment required in adopting clones and the uncertainty created by the unavailability of data from longer term trials of tea tree clones on a range of sites.

6.3 Synthesis

This study has shown that plant spacing influences *Melaleuca alternifolia* growth and oil traits. Trees at wider spacing typically have a higher leafiness score as they are given more space and there is less competition for light but have a lower oil

concentration than those at a narrower spacing. Dry weights of key tree components and oil yields of tea tree plantations on a per hectare basis were influenced by plant spacing, being larger at the narrow plant spacing equivalent to a stocking of 33,333 plants/ha. Clones from the second suite of elite clones provided by RIRDC/ATTIA tea tree breeding programme showed their superiority over seedlings grown from improved seed from the same breeding programme in commercial oil traits. Conversely, due to extraneous sources of variation operating in the 2004 CST, clones from the first suite of elite clones gave poorer performance than improved seedlings.

The variation in growth and oil traits of clones in the 2006 CST indicates that gains can be optimised by selecting the best performing and stable clones for tea tree clonal plantation establishment. Average oil yields of a clonal plantation established from three best clones, clone 5 (C64), clone 6 (C66) and clone 9 (C70), were estimated to be 522.6 kg/ha and 356 kg/ha at stockings of 33,333 plants/ha and 16,667 plants/ha respectively. It was assumed for the financial analysis that these yields will be maintained throughout the 15-year life of the average tea tree plantation, however, this needs to be confirmed by further research.

The oil yields of the select clones were substantially more than the mature yields of a plantation of CSO1 (ATTIA 2B) seedlings derived from the 2002 yield trial which were estimated to reach 369.6 kg/ha and 251.3 kg/ha from same plant stockings.

The financial analysis developed in this study showed that replanting tea tree plantations using the three best selected clones from the second suite of elite clones and CSO1 (ATTIA 2B) seedlings at stockings of either at 33,333 plants/ha or 16,667 plants/ha were financially viable. The greatest profit can be realised from a clonal plantation at a stocking of 33,333 plants/ha.

The establishment of long term clonal trials testing all clones from the first and second suite of elite clones across a range of sites is recommended to address the limitations of the trials included in this study. These new trials will deliver information on clone-by-environment interactions and should provide reliable, longer-term data on clonal performance which is missing currently. This knowledge is necessary to efficiently deploy clonal material.

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Appendices

Appendix 1 Discounted cashflow for a 20 ha tea tree plantation at 7% of discount rate – plantation option 1 (seedlings at a stocking of 33,333 plants/ha)

Year	Oil yield (kg/yr)	Spent leaf production (m3/ha)	Gross Receipts ($/yr)	Establishment cost ($)	Operating cost ($)	Annual cashflow ($)	Annual Discounted cashflow ($)	Accumulated Discounted cashflow ($)
0	-	-	-	116,943.53	-	-116,943.53	- 116,943.53	-116,943.53
1	5,675.94	1,350.00	283,767.45	-	28,783.33	254,984.11	238,302.91	121,359.38
2	6,533.93	1,350.00	322,377.06	-	103,560.00	218,817.06	191,123.29	312,482.67
3	7,391.93	1,350.00	360,986.67	-	103,560.00	257,426.67	210,136.85	522,619.52
4	7,391.93	1,350.00	360,986.67	-	103,560.00	257,426.67	196,389.58	719,009.09
5	7,391.93	1,350.00	360,986.67	-	103,560.00	257,426.67	183,541.66	902,550.75
6	7,391.93	1,350.00	360,986.67	-	103,560.00	257,426.67	171,534.26	1,074,085.02
7	7,391.93	1,350.00	360,986.67	-	103,560.00	257,426.67	160,312.39	1,234,397.41
8	7,391.93	1,350.00	360,986.67	-	103,560.00	257,426.67	149,824.67	1,384,222.08
9	7,391.93	1,350.00	360,986.67	-	103,560.00	257,426.67	140,023.05	1,524,245.13
10	7,391.93	1,350.00	360,986.67	-	103,560.00	257,426.67	130,862.67	1,655,107.80
11	7,391.93	1,350.00	360,986.67	-	103,560.00	257,426.67	122,301.56	1,777,409.36
12	7,391.93	1,350.00	360,986.67	-	103,560.00	257,426.67	114,300.52	1,891,709.88
13	7,391.93	1,350.00	360,986.67	-	103,560.00	257,426.67	106,822.92	1,998,532.80
14	7,391.93	1,350.00	360,986.67	-	103,560.00	257,426.67	99,834.50	2,098,367.30
15	7,391.93	1,350.00	360,986.67	-	103,560.00	257,426.67	93,303.27	2,191,670.57

Criteria	Value
PVB ($)	2,308,614.11
PVC ($)	116,943.53
NPV ($)	2,191,670.57
IRR (%)	211.5

Appendix 2 Discounted cashflow for a 20 ha tea tree plantation at 7% of discount rate – plantation option 2 (seedlings at a stocking of 16,667 plants/ha)

Year	Oil yield (kg/yr)	Spent leaf production (m3/ha)	Gross Receipts ($/yr)	Establishment cost ($)	Operating cost ($)	Annual cashflow ($)	Annual Discounted cashflow ($)	Accumulated Discounted cashflow ($)
0	-	-	-	73,250.70	-	- 73,250.70	- 73,250.70	- 73,250.70
1	3,859.64	918.00	192,961.86	-	25,905.00	167,056.86	156,127.91	82,877.21
2	4,443.08	918.00	219,216.40	-	93,204.00	126,012.40	110,064.11	192,941.32
3	5,026.51	918.00	245,470.94	-	93,204.00	152,266.94	124,295.18	317,236.50
4	5,026.51	918.00	245,470.94	-	93,204.00	152,266.94	116,163.72	433,400.22
5	5,026.51	918.00	245,470.94	-	93,204.00	152,266.94	108,564.22	541,964.44
6	5,026.51	918.00	245,470.94	-	93,204.00	152,266.94	101,461.89	643,426.33
7	5,026.51	918.00	245,470.94	-	93,204.00	152,266.94	94,824.20	738,250.53
8	5,026.51	918.00	245,470.94	-	93,204.00	152,266.94	88,620.74	826,871.27
9	5,026.51	918.00	245,470.94	-	93,204.00	152,266.94	82,823.13	909,694.40
10	5,026.51	918.00	245,470.94	-	93,204.00	152,266.94	77,404.79	987,099.19
11	5,026.51	918.00	245,470.94	-	93,204.00	152,266.94	72,340.93	1,059,440.11
12	5,026.51	918.00	245,470.94	-	93,204.00	152,266.94	67,608.34	1,127,048.45
13	5,026.51	918.00	245,470.94	-	93,204.00	152,266.94	63,185.37	1,190,233.82
14	5,026.51	918.00	245,470.94	-	93,204.00	152,266.94	59,051.74	1,249,285.56
15	5,026.51	918.00	245,470.94	-	93,204.00	152,266.94	55,188.55	1,304,474.11

Criteria	Value
PVB ($)	1,377,724.81
PVC ($)	73,250.70
NPV ($)	1,304,474.11
IRR (%)	213.8

Appendix 3 Discounted cashflow for a 20 ha tea tree plantation at 7% of discount rate – plantation option 3 (clones at a stocking of 33,333 plants/ha)

Year	Oil yield (kg/yr)	Spent leaf production (m3/ha)	Gross Receipts ($/yr)	Establishment cost ($)	Operating cost ($)	Annual cashflow ($)	Annual Discounted cashflow ($)	Accumulated Discounted cashflow ($)
0	-	-	-	370,274.33	-	- 370,274.33	- 370,274.33	- 370,274.33
1	10,452.00	1,350.00	498,690.00	-	28,783.33	469,906.67	439,165.11	68,890.78
2	10,452.00	1,350.00	498,690.00	-	103,560.00	395,130.00	345,121.84	414,012.62
3	10,452.00	1,350.00	498,690.00	-	103,560.00	395,130.00	322,543.78	736,556.40
4	10,452.00	1,350.00	498,690.00	-	103,560.00	395,130.00	301,442.79	1,037,999.19
5	10,452.00	1,350.00	498,690.00	-	103,560.00	395,130.00	281,722.23	1,319,721.41
6	10,452.00	1,350.00	498,690.00	-	103,560.00	395,130.00	263,291.80	1,583,013.22
7	10,452.00	1,350.00	498,690.00	-	103,560.00	395,130.00	246,067.11	1,829,080.32
8	10,452.00	1,350.00	498,690.00	-	103,560.00	395,130.00	229,969.26	2,059,049.58
9	10,452.00	1,350.00	498,690.00	-	103,560.00	395,130.00	214,924.54	2,273,974.12
10	10,452.00	1,350.00	498,690.00	-	103,560.00	395,130.00	200,864.06	2,474,838.18
11	10,452.00	1,350.00	498,690.00	-	103,560.00	395,130.00	187,723.42	2,662,561.59
12	10,452.00	1,350.00	498,690.00	-	103,560.00	395,130.00	175,442.45	2,838,004.04
13	10,452.00	1,350.00	498,690.00	-	103,560.00	395,130.00	163,964.90	3,001,968.94
14	10,452.00	1,350.00	498,690.00	-	103,560.00	395,130.00	153,238.23	3,155,207.17
15	10,452.00	1,350.00	498,690.00	-	103,560.00	395,130.00	143,213.30	3,298,420.46

Criteria	Value
PVB ($)	3,668,694.80
PVC ($)	370,274.33
NPV ($)	3,298,420.46
IRR (%)	117.6

Appendix 4 Discounted cashflow for a 20 ha tea tree plantation at 7% of discount rate – plantation option 4 (clones at a stocking of 16,667 plants/ha)

Year	Oil yield (kg/yr)	Spent leaf production (m3/ha)	Gross Receipts ($/yr)	Establishment cost ($)	Operating cost ($)	Annual cashflow ($)	Annual Discounted cashflow ($)	Accumulated Discounted cashflow ($)
0	-	-	-	199,919.90	-	- 199,919.90	- 199,919.90	- 199,919.90
1	7,132.00	918.00	340,218.00	-	25,905.00	314,313.00	293,750.47	93,830.57
2	7,132.00	918.00	340,218.00	-	93,204.00	247,014.00	215,751.59	309,582.16
3	7,132.00	918.00	340,218.00	-	93,204.00	247,014.00	201,637.00	511,219.17
4	7,132.00	918.00	340,218.00	-	93,204.00	247,014.00	188,445.80	699,664.96
5	7,132.00	918.00	340,218.00	-	93,204.00	247,014.00	176,117.57	875,782.53
6	7,132.00	918.00	340,218.00	-	93,204.00	247,014.00	164,595.86	1,040,378.39
7	7,132.00	918.00	340,218.00	-	93,204.00	247,014.00	153,827.90	1,194,206.29
8	7,132.00	918.00	340,218.00	-	93,204.00	247,014.00	143,764.40	1,337,970.69
9	7,132.00	918.00	340,218.00	-	93,204.00	247,014.00	134,359.25	1,472,329.94
10	7,132.00	918.00	340,218.00	-	93,204.00	247,014.00	125,569.39	1,597,899.33
11	7,132.00	918.00	340,218.00	-	93,204.00	247,014.00	117,354.57	1,715,253.90
12	7,132.00	918.00	340,218.00	-	93,204.00	247,014.00	109,677.17	1,824,931.07
13	7,132.00	918.00	340,218.00	-	93,204.00	247,014.00	102,502.03	1,927,433.10
14	7,132.00	918.00	340,218.00	-	93,204.00	247,014.00	95,796.29	2,023,229.39
15	7,132.00	918.00	340,218.00	-	93,204.00	247,014.00	89,529.24	2,112,758.63

Criteria	Value
PVB ($)	2,312,678.53
PVC ($)	199,919.90
NPV ($)	2,112,758.63
IRR (%)	143.4

Appendix 5 Tea tree plantation establishment and operating costs

No.	Parameter	Cost ($)	
	Establishment costs		
1.	Seedling cost	0.12	per plant
2.	Cutting cost	0.50	per plant
3.	Land preparation and bed making (include machinery costs, labour and other costs)	567	per hectare
4.	Planting, replanting and irrigation after planting (include machinery costs, labour and other costs)	1,231	per hectare
5.	Sundries	50	per hectare
	Operating costs in year 1		
1.	Land opportunity cost	600	per hectare
2.	Weed control costs (include machinery costs, labour, herbicide and its application costs)	373	per hectare
3.	Insect control costs (include machinery costs, labour, insecticide and its application costs)	150	per hectare
4.	Fertilising costs (include machinery costs, labour, fertiliser and its application costs)	317	per hectare
	Operating costs in year 2 onwards		
1.	Land opportunity cost	600	per hectare
2.	Weed control costs (include machinery costs, labour, herbicide and its application costs)	200	per hectare
3.	Insect control costs (include machinery costs, labour, insecticide and its application costs)	360	per hectare
4.	Fertilizing costs (include machinery costs, labour, fertiliser and its application costs)	550	per hectare
5.	Harvesting and distillation costs (include machinery costs, fuel/gas and oil, labour, distilling costs, electricity and other costs)	1,798	per hectare
6.	Oil marketing costs	35	per hectare
7.	Post-harvest cultivation costs (if any) (include machinery costs, labour, and other costs)	50	per hectare
8.	Lease payments of leased equipment (which is not included in other machinery costs elsewhere)	505	per hectare
9.	Machinery repair and maintenance	158	per hectare
10.	General repair and maintenance of area around the plantation (include road, fence, drains etc.)	65	per hectare
11.	Depreciation of plant and equipment	300	per hectare
12.	Overhead costs (insurance, rates and legal and accounting fees, etc.)	100	per hectare
13.	Management	408	per hectare
14.	Sundries	50	per hectare