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Improved Tea Tree Varieties for a Competitive Market

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Improved tea tree varieties for a competitive market

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Foreword

The Australian tea tree oil industry has become an important regional industry in Australia with a farm-gate value in excess of $21M. Around 90% of the product is exported each year. Fluctuating prices and growing international competition are drivers for producers to use higher yielding varieties in order to remain economically viable in the long term.

The tea tree breeding project commenced in 1993 and has released progressively improved seed to the industry that has increased oil yield from 148kg/ha prior to the start of breeding to about 250kg/ha. Seed releases since 2005 are expected to outperform this 69% gain as the genetic quality of all seed orchards have been progressively improved.

Tree breeding is a long term investment. Continued support from all project clients and stakeholders will ensure that growers can maximise oil production and profit through use of improved seed and clones and thus enhance the long-term viability of the Australian tea tree oil industry.

This project was funded from industry revenue that was matched by funds provided by the Australian Government.

This report, an addition to RIRDC's diverse range of over 2,000 research publications, forms part of our Tea Tree Oil R&D program, which aims to establish production systems that are both ecologically sustainable and profitable.

Most of RIRDC's publications are available for viewing, free downloading or purchasing online at www.rirdc.gov.au. Purchases can also be made by phoning 1300 634 313.

Craig Burns
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Rural Industries Research and Development Corporation

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Abbreviations

ANU	Australian National University
ATTIA	Australian Tea Tree Industry Association
ATTIA 1	best provenance seed
ATTIA 2A	SSO1 seed
ATTIA 2B	CSO1 seed
ATTIA 3A	SSO2p seed
ATTIA 3B	SSO2 seed
CC	controlled cross
CC1	bulked controlled crossed progeny plots est. 2000
CC2	bulked controlled crossed progeny plots est. 2002
CSO1	first clonal seed orchard
CSO2	second clonal seed orchard
CSOlc	clonal seed orchard low cineole
CST1	clonal spacing trial est. 2004
CST2	clonal spacing trial est. 2006
CYT1	clonal yield trial est. 2004
CYT2	clonal yield trial est. 2006
GxE	genotype by environment
Hedge	bank of clones at WPII est. 1995
Hedge Lc	bank of low cineole clones at WPII est. 1999
IRR	Internal Rate of Return
IS	industry standard
ISEL	industry select
ISTD	industry standard
LSD	least significant difference
NPV	Net Present Value
NSW DII	New South Wales Department of Industry and Investment
OP	open pollination
PT1	progeny trial est. 2000
PT2	progeny trial est. 2002
RIRDC	Rural Industries Research and Development Corporation
T-4-ol	terpinen-4-ol
SCU	Southern Cross University
SSO1	first generation seedling seed orchard
SSO2	second generation seedling seed orchard
SSO2p	partial second generation seedling seed orchard
WPII	Wollongbar Primary Industries Institute
YT1	yield trial est. 1999
YT2	yield trial est. 2002
YT3	yield trial est. 2008

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What the report is about

This report details the fourth phase (2006 - 2009) of the tea tree breeding project. During this phase the project has sold 2.3kg of improved seed, enough to plant over 300ha of plantation. Improved seed has increased yields by 69% compared with unimproved seed (seed available prior the first breeding project of 1993). Newly developed orchards (SSO2 and CSO2) will further increase these already impressive gains.

Project seed has increased grower profitability and market access and will continue to secure the long-term viability of the Australian tea tree oil industry.

Who is the report targeted at?

This report provides the opportunity to disseminate research findings to clients and stakeholders (RIRDC, ATTIA and NSW DII). The project's client base is the Australian tea tree oil producer or potential producer who requires seed or clones to establish, enlarge or replace a plantation.

As the project is focussed on breeding *Melaleuca alternifolia* (an essential oil bearing Australian native), interest in the project and its findings extends to those involved in other essential oil industries both in Australia and overseas.

Background

The Australian tea tree oil industry has become an important regional industry in Australia since the demand and price for the antimicrobial oil increased during the 1990s. Seed used to establish the first plantations were sourced from natural stands. Plantations were, as a consequence highly variable in oil yield and oil quality.

The potential of a breeding project to provide progressive gains in productivity and quality was recognised when industry and RIRDC supported the first phase of the breeding project in May 1993. The project has progressively increased oil yield from 148kg/ha prior to the start of breeding to c.250kg/ha. Further improvements in yield are expected from the on-going development of seed orchards.

Objectives

Objectives for this fourth phase were:

- To release improved seed with the capacity to increase yields by more than 60%
- To have elite clones available for release
- Progressively develop seedling and clonal orchards
- Assess progeny and yield trials
- Undertake controlled crosses
- Monitor genotypes for potential allergens (limonene and linalool)
- Monitor genotypes for insect tolerance

Methods

Progressive genetic improvement is achieved through the on-going development of established seed orchards (CSO1, SSO2, SSO2p) using a recurrent breeding strategy together with the establishment of new orchards (e.g. CSO2) to ensure the long-term advancement of genetic gain.

In association with the establishment of new orchards, new progeny and yield trials are established to provide data for orchard culling and selections for cloning and to quantify yield gains from orchard progeny and elite clones.

Results

Seed from the project has been made available to industry since 1997 and sales now total 9.6kg enough to plant 1300ha. Grower uptake of project seed has been excellent. Once the best seedlot (ATTIA 2B) was identified from the 2002 yield trial, sales only comprised this seedlot. From 2007, seed from the second-generation seed orchards has also been released. It is expected that the progeny from SSO2 (ATTIA 3B) will out perform (ATTIA 2B) so SSO2 is now the preferred source. During 2008/2009, higher oil prices increased new plantings and hence the demand for seed. Sales in 2008 were 1.4kg and higher sales are expected in 2009.

The development of clones in the project is a complementary strategy to the seed orchard program and offers the means to capture further improvements in oil production. From the 20 clones tested, four clones from the 2006 clonal trials have been identified as very high yielding (>500kg/ha at first harvest), while four clones from the 2004 clonal trials have been identified with high oil concentrations (>75mg/g averaged over three harvests).

The systematic improvement of the genetic quality of seed has included the on-going development of the second generation seedling seed orchard (SSO2), with thinnings in 2007 and 2008, and the establishment of a new clonal orchard (CSO2) in February 2007. The development of these two orchards will give substantial genetic gain, and provide improved seed for future releases and seedlots for the establishment of a third generation seedling seed orchard.

Two yield trials were established (2002 and 2008) to estimate gains in yield from use of breeding project seedlots and another two yield trials were established (2004 and 2006) to estimate gains in yield from use of breeding project clones. Yield gains from project seed were outstanding: ATTIA 1 (selected wild seed of best natural provenance), since first released in 1997, had a 39% yield increase (average of five harvests) over industry standards; ATTIA 2A (from a 45% flowering in SSO1), released in 2000, had a 33% yield increase; and ATTIA 2B (CSO1), also first released in 2000, had a 69% increase in oil yield over industry standards. All five seed releases (including ATTIA 3A and 3B) will be assessed in 2009 from the 2008 yield trial.

Oil yields for clones at a first harvest were 518kg/ha while for seedlings only 168kg/ha. These clonal yields are exceptional; however the 300% higher relative yield over seedlings will reduce as the oil concentration for seedlings increases as they mature. The implication for growers is that clones as physiologically mature plants would return higher yields earlier than seedlings.

The successful control crosses undertaken in 2007 will provide 8 seedlots for the third-generation seedling seed orchard.

All breeding trees in the project have been measured for the potential allergens, limonene and linalool. This has been undertaken in the event that higher range limonene and linalool trees could be removed thereby reducing the potential for higher limonene and linalool values in the progeny of that orchard.

As part of the growth assessment of trees, insect damage has been monitored. Damage has been minimal as yield trials are located in commercial plantations and receive regular insect control. The selection for insect tolerant plants is mainly indirect through selection for a higher leafiness score.

Implications

Industry adoption of the project's improved seed would increase average yields by 69%, and at $45/kg this could mean an annual $14M windfall to Australian growers.

Since 1997, the project has sold 6.7kg of selected wild seed of best natural provenance and 2.9kg of improved seed. Increases in average yields for selected and improved seed are 39% and 69% respectively. This resultant yield increase means an annual income increase of $2.6M and $1.9M to growers of selected and improved seed respectively. On the basis of seed sales, current plantings and applying a wholesale value output multiplier of 4, the benefits to the Australian economy are in excess of $18M annually.

A proposal for a fifth phase (2009 - 2014) of breeding has been approved by RIRDC and ATTIA. This project will build on the success of the four previous phases where oil yields from project seed have been progressively increased. The new project aims to further increase yields through improved seed from the project's seed orchards (SSO2 and CSO2) program and through the use of high yielding clones. Releasing highly improved germplasm will greatly assist with the long-term economic viability of the Australian tea tree industry.

Recommendations

Recommendations for industry are based on using improved germplasm to maximise profit and market access. The use of higher yielding varieties through genetic improvement is one proven path to higher efficiencies of production. Oil yields from project released seed have progressively increased from 148kg/ha which was the industry average at the start of breeding to c. 250kg/ha. The cost of seed is a minor component to establishing a plantation yet its genetic quality is a major determinant of both oil yield and quality. Use of elite clones will provide growers with higher earlier yield and income than that of seedlings. Further developments with orchards and clones will ensure the progressive genetic improvement of future releases. Uptake of future releases by industry will increase the economic long-term viability and competitiveness of Australian producers.

The recommendation for stakeholders is for continued support for the project. Tea tree improvement is a long-term investment. Any curtailment of stakeholder support would risk current and future releases, corporate knowledge and the substantial past investment in the project. The project has produced outstanding oil gains for the industry. Any threat to this would threaten the future competitiveness of the Australian industry.

Recommendations for the breeding project have come from studies associated with the project, NSW DII, RIRDC, ATTIA and growers. A study of the reproductive biology of *M. alternifolia* has recommended that, if practical, seed orchards be sited where winter temperatures are low, with minima below 5ºC. Earlier and more intense flowering under these conditions will shorten generation times and increase seed yields. A Quality Management System (ISO 9001) for the project has been implemented to meet the needs of clients and stakeholders. To ensure personnel competency and performance within the project it is recommended that the quality management system be reviewed regularly, enhanced and further training provided when necessary. A review to reduce risks to the project from personnel succession is to be submitted to RIRDC by 2012. Grower feedback and recommendations for the project have been largely very positive reflecting, in part, the success of a good communication between the project, stakeholders and clients. As part of the new phase of the breeding project, the ATTIA board has recommended research be undertaken on frost and insect tolerance.

1. Introduction

The medicinal value of Australian tea tree oil, sourced principally by steam distillation of plantation-grown foliage of *Melaleuca alternifolia* (Maiden & Betche) Cheel, as an effective antimicrobial agent (antibacterial and antifungal properties) was first confirmed scientifically by Government Chemist, Dr A. R. Penfold, in the 1920s. Subsequent study has shown that tea tree oil also possesses anti-inflammatory and antiviral properties (Southwell and Lowe 1999). Today tea tree oil is used in many cosmetic and personal care products including acne control face-washes, creams, gels, hand-washes, mouth-washes and other dental care products, shampoos and vaginal pessaries as well as being used as a topical antiseptic in its pure form on cuts, abrasions and insect bites. Tea tree oil is also widely utilised by naturopaths and aromatherapists and has potential in agriculture and animal husbandry. The oil's potential efficacy in controlling MRSA and VRSA resistant strains of Golden Staph (*Staphylococcus aureus*) is one focus of continued research aimed at expanding the market for tea tree oil (ATTIA 2009).

Annual demand for Australian Tea Tree Oil is presently around 500 tonnes (farm-gate value of $21.5M at current prices) and, although there has been a shortfall in production over the last two years, ATTIA (2009) predicts that this could turn into an oversupply in the years ahead. Increases in production are predicted as a result of current substantial new and replacement plantings (>200 ha/annum with ATTIA seed alone) in Australia and increased production in China. The number of growers and producers in the industry in the principal growing regions of NSW and Queensland is presently fewer than 100, down from 300 in earlier times, and the area under plantation is estimated to be in the order of 3,000 ha, down from 4,000 ha which was the estimate in our report of the previous breeding project (Baker *et al*. 2006). On a positive note, the farm-gate price for tea tree oil has improved dramatically over the past three years from about $16.50/kg in July 2006 to about $43/kg in July 2009 with a peak of $57.50 during the summer of 2008/09 (ATTIA 2009). With 90% (Bolster per comm. 2009) of Australia's production for export, tea tree oil is a significant part of Australia's essential oil industry and remains a valuable rural industry particularly in the production centres of northern New South Wales and northern Queensland.

The seed that underpinned at least the first ten years (80s to mid-90s) of tea tree plantation development in Australia was sourced from natural stands with only rudimentary selection of seed trees based on oil characteristics. Much of the earlier plantation resource was, as a consequence, highly variable in productivity. Then in 1993, with the recognition that tree breeding could provide progressively, economically significant gains in oil yield and oil quality in *M. alternifolia*, RIRDC and ATTIA, organizations funding R&D in the industry, supported the first 3-year tea tree breeding project from 1993 to 1996 (Doran *et al*. 1997). This first project has been followed by an uninterrupted sequence of tea tree breeding projects: 1996 to 2001 (Doran *et al*. 2002); 2001 to 2006 (Baker *et al*. 2007); 2006 to 2009 (this project) and 2009 to 2014 (now in progress). A 2008 tea tree industry in-house review of the breeding projects concluded that they had lead to exceptional gains in the yield of tea tree oil plantations over a relatively short period compared with other industries. The benefits to producers were considered numerous: greater profitability per unit land area through higher yields; significant increases in efficiencies of the harvest and distillation processes through increased oil content from similar biomass levels, thus enhancing margins per kilogram of oil produced; and greatly reduced variability in yields and oil qualities (EOPAA 2008).

This, the Final Report for RIRDC/ATTIA DAN-254A 'Improved tea tree varieties for a competitive market' describes progress over the three years of the project, 2006 to 2009, in implementing a comprehensive breeding strategy for the genetic improvement of *M. alternifolia*. It should be read in conjunction with the three earlier Final Reports (Doran *et al*. 1997, Doran *et al*. 2002, Baker et al. 2007) of previous RIRDC tea tree breeding projects (1993 to 1996; 1996 to 2001; 2001 to 2006 respectively) as each is a continuum under a common breeding strategy which has been twice independently reviewed but only slightly revised from project to project.

2. Objectives

To maximise profit and market access for Australian tea tree oil producers by releasing highly improved seed and clones from a recurrent breeding strategy that ensures on-going genetic improvement. This is to be achieved through: developing the SSO2 with selected culling to give greater than 80% gain by completion of the second generation of breeding; establishing a second clonal seed orchard by 2007; assessing existing yield trials (YT2, CYT1, CYT2) and establishing a new yield trial (YT3) to quantify genetic gains from improved seed and identify the best clones for release; undertaking controlled crosses of selected genotypes for use in the third generation seed orchards and for clonal selections; monitoring all breeding trees for potential allergens (limonene and linalool) and instigating removal of higher limonene and linalool trees from the breeding populations if appropriate; and monitoring all genotypes for insect tolerance and selecting trees with higher leafiness scores.

3. Breeding Strategy

3.1 Determinants of the Strategy

The determinants of the breeding strategy adopted for this species have been fully explained elsewhere (Doran 1992, Doran *et al*. 1997, Baker 1999, Doran *et al*. 2002, Baker *et al*. 2007). A brief overview of these determinants follows:

3.1.1 Genetic Diversity and Genetic Parameters

Provenance/progeny trials established at the outset of breeding activities demonstrated very substantial provenance and within and between family variation in all commercial oil traits (Doran *et al*. 1997). Families from the best performing provenances of Devils Pulpit and Candole now dominate the genetic base of the breeding population at conclusion of the second generation of breeding. There remains, however, a very wide range of provenances represented overall and this will be maintained through infusions of seedlots from previously untested provenances into each new generation.

Heritability and genetic and phenotypic correlations for *M. alternifolia* as estimated from previous tea tree breeding projects and related progeny trials are summarised in Table 3.1 (see also Ivković and Zhang in Section 4.5.3 of this report). The key economic traits of oil concentration, oil quality (%1,8-cineole and % terpinen-4-ol) and leaf biomass proved to be moderately to highly heritable. Estimates of correlations between growth traits, such as tree height and leafiness, and oil concentration and quality traits were low indicating that they could be improved simultaneously in a breeding program. Height and leafiness scores were positively correlated with leaf biomass, confirming their suitability as selection traits.

Table 3.1 Heritability and genetic and phenotypic correlations for *Melaleuca alternifolia*. Genetic correlations are presented below the diagonal and phenotypic correlations above the diagonal.

Trait	Heritability	Leaf biomass	Oil conc.	Height	Leafiness	1,8-cineole	Terpinen-4-ol
Leaf biomass	0.250[a]	1	0.068[c]	0.742[c]	0.776[c]	-0.03[a]	0.1
Oil conc.	0.917[b]	0.1	1	-0.1[c]	-0.1[c]	0.03[a]	-0.03
Height	0.115[b]	0.6[a]	-0.1	1	0.619[c]	-0.05	0.05
Leafiness	0.113[b]	0.9[a]	-0.1	0.5[a]	1	0	0
1,8-cineole	0.513[b]	-0.1	0.15[b]	-0.05	0	1	-0.5
Terpinen-4-ol	0.431[b]	0.1[a]	-0.14[b]	0.05	0	-0.66[b]	1

[a] Butcher *et al*. (1996); [b] Doran *et al*. (2002); [c] Williams (pers comm. 2009)

The significance of Genotype × Environment (G×E) interaction has still to be studied systematically but indications from limited, but geographically widespread trials, established at the commencement of breeding were that oil characteristics are very stable across sites but growth traits are less stable (Doran *et al*. 1997). Reports of increased oil yields from project seed from all tea tree growing areas suggest that G×E in growth traits is of far less importance than selecting for superior oil traits and that the one breeding program centred on northern NSW will provide benefits to all growers.

3.1.2 Biological Information and Available Genetic Resources

Breeding system – flowers are bisexual, protandrous, largely outcrossing and insect pollinated (see Baskorowati in Section 4.5.1 of this report);

Flowering and seeding – time to first major flowering of orchard trees varies markedly between sites (e.g. 14 months at West Wyalong cf 3.75 years at Wyrallah), also there is substantial year to year variation in abundance of spring-summer (late October to mid-November) flowering. Time from flowering to seed maturity is 16 to 18 months (see Baskorowati in Section 4.5.1 of this report);

Seed (rather than clones) to be the main means of deployment of improved varieties from the breeding program;

Controlled crossing is successful using conventional techniques first developed for eucalypts (see Baskorowati in Section 4.5.1 of this report). However, the method is time consuming and costly and mainly of use in developing an elite nucleus population for selection of clonal candidates and as a research tool;

Vegetative propagation/clonal plantations – *M. alternifolia* is relatively easy to propagate vegetatively by stem cuttings and clones can be selected that root readily in the nursery, grow vigorously and give superior oil traits for deployment in clonal plantations (see Prastyono in Section 4.5.2 of this report);

Genetic resources for the second and subsequent generations of breeding – Genetic resources available for the second generation Seedling Seed Orchard (SSO2) were substantial with a total of 99 seedlots and one clone from SSO1 (originally 199 seedlots from 23 provenances) and a wide range of other natural and manipulated sources (see Doran *et al*. 2002). This project will provide the genetic resources for the third generation Seedling Seed Orchard (SSO3) to be established during the following project (2009-2014).

3.1.3 Breeding Objective

M. alternifolia is grown commercially to produce essential oil which is extracted from the leaves. Thus the overall goal of breeding this species must be to increase the yield of high quality oil per unit area by improving yield of leaves and concentration of oil in those leaves while ensuring that the oil produced contains high levels of terpinen-4-ol, the main therapeutic agent in the oil.

The objectives of the recurrent breeding strategy through 4 projects since 1993 were-

- To increase oil yields in new seedling plantations by 30% in the first project (1993-1996), by 60% in the second project (1996-2001), by 90% in the third project (2001-2006), and by >100% at conclusion of this the fourth tea tree breeding project;
- To ensure that oil produced is of consistent, desirable quality;
- To establish a broadly based breeding population to enable flexibility and on-going improvements;
- To identify and make available superior oil-producing clones suitable for mass vegetative propagation.

3.1.4 Selection Criteria

Plantations of *M. alternifolia* are managed as coppice crops to produce the highest possible yields of terpinen-4-ol–rich tea tree oil. Oil yield is a function of leaf biomass and oil concentration of that leaf. To maximise gains from breeding, selection should aim to achieve improvements in both these traits. An ideal plant for use in commercial plantations would have the following features-

1. A large leaf biomass as a seedling and when coppiced.

2. High leaf oil concentration

3. An oil quality that meets the current Australian Standard AS 2009 (Standards Association of Australia 2009) for this oil type and, more importantly for market acceptance, is compliant with industry needs (1,8-cineole <4% of total oil, terpinen-4-ol c. 40% or more of total oil and relatively free of the potential allergens limonene and linalool).

4. Broad adaptability to different growing conditions

5. Resistance to pests and diseases

A multi-trait selection index (predictive model) is used to improve efficiency of individual tree selection in breeding project trials and is described in detail by Baker *et al.* (2007).

The model is:

$$\mathit{Index} = (h*0.115/26.27) + (s*0.113/0.45) + (o*0.917/6.89) - (c*0.2*0.513/3.15) + (t*0.2*0.431/2.41)$$

Where: h = plant height

s = score for canopy density

o = oil concentration

c = cineole

t = terpinen-4-ol

And each variable is multiplied by heritability (eg. 0.115 for height) and divided by the variance (eg. 26.27 for height), and an economic weight of 0.2 is applied to cineole and terpinen-4-ol.

Economic weights based on an economic model of the industry, from which the effect of changes in the two key breeding traits of leaf biomass and oil concentration on industry profitability can be derived, have been estimated during this project (see Ivković and Zhang in Section 4.5.3 of this report).

Application of these economic weights will enhance the estimation of breeding values and will be applied in selecting trees for the third generation breeding population.

The breeding populations established as part of this project, contain plants with a wide range of oil composition. Project progeny trials provide a means of archiving the complete range of possible oil types for possible future use in breeding, while the main emphasis in the seed orchards is on selecting only the low 1,8-cineole oils.

3.1.5 Implementation of the Strategy

This project has followed the successful implementation strategy of the previous tea tree breeding projects. NSW Department of Industry and Investment (formerly NSW Department of Primary Industry) at its Wollongbar Primary Industries Institute (formerly Wollongbar Agricultural Institute) was the project's lead organization providing 1) the Project Leader (Dr Trevor Olesen), 2) the Project Manager (Gary Baker) responsible for implementation of the breeding strategy and financial and human resources management. Gary has the only full-time position funded by this project, 3) technical support in oil chemistry, entomology and pathology, 4) most orchard planting sites and 5) organization of the biennial tea tree industry workshop. Dr John Doran, previously through CSIRO but now as a private contractor, is responsible for breeding strategy and planning and participating in research to enhance genetic gains from the project, and similarly Dr Emlyn Williams, formerly of CSIRO, provides biometric support as a private contractor.

In implementing this and previous tea tree breeding projects, plantation owners have played an important role in providing sites for orchards, progeny and genetic gain trials and maintenance of project plantings. Australian Plantations, Craig Chapman, Geoff & Richard Davis, Rob Dyason, David Martin, John Murtagh, Peter Rose, and John Seccombe are to be acknowledged for their support which has contributed greatly to project outcomes.

A Breeding Committee, chaired by an industry representative (Richard Davis), and also comprising representatives of RIRDC (Roslyn Prinsley), ATTIA (Craig Chapman and others) and NSW DII (Deb Hailstones) in addition to members of the project team (see above) meets annually. It is the role of this committee to review progress in implementing the breeding strategy, ratify plans for each new stage of the project and develop strategies for commercialisation of project outputs of seed and cuttings. The industry members of this committee report on progress to the Research Committee of ATTIA and played a vital role in negotiating the necessary industry financial support in the lead up to this project which coincided with low oil prices unprecedented in the industry.

Funding support for tea tree breeding comes from RIRDC, ATTIA, the research providers and industry participants. In brief, RIRDC and ATTIA provided funding support of $402,700 for the three years ending 30 June 2009. An additional supplement of $3,200 was provided by RIRDC in 2009 to support the additional oil analyses required for the DNA studies described in Section 4.5.4 of this report. This was complemented by additional direct and in-kind support from the research providers and industry participants of $726,984.

3.2 Outline of the Breeding Strategy

At the outset of designing a practical breeding strategy compatible with the biological features of the species and the limited resources available to the Australian tea tree oil industry, it was obvious that the breeding strategy would need to be relatively simple. This meant application of a minimum of labour intensive and costly tasks such as controlled pollinations or vegetative propagation, and restriction to one geographic region (i.e. northern NSW). The main strategy adopted from the start and applied now through a continuum of four projects (1993 to 1996, 1996 to 2001, 2001 to 2005 and 2006 to 2009) was based on establishing breeding populations in progeny trials, which, after they have supplied data on variation in growth and estimates of genetic parameters, are converted to seedling seed orchards to supply genetically-improved seed for plantation establishment by open pollination. Similar strategies have been successfully applied in other hardwood breeding programs in Australia and abroad (Eldridge *et al*. 1993, Harwood *et al*. 2001). Complementing the main strategy were interim measures (wild seed from selected provenances and a clonal seed orchard) to deliver seed of some genetic improvement to the industry quickly, and a limited controlled crossing and clonal program. The principal components of the main recurrent breeding strategy for *M. alternifolia* are given diagrammatically in Figure 3.1.

Important elements of the strategy, some already implemented in earlier projects and now redundant, with respect to the supply of improved germplasm to industry are:

Development of a seed stand

Conversion in 1994 of a natural stand near Coombell into a seed stand by selective thinning to meet short-term demand for slightly improved seed was undertaken. Seed of this slow growing provenance was not released to industry. Some seedlots were included in SSO2, but most have now been removed because of their low family ranking.

Release of seed from best-performing natural stands

Seed from selected trees within two natural populations that had performed best in project breeding populations was made available during 1997 to 2000. Genetic gain trials have confirmed an average 39% improvement in paddock yields from use of ‘best provenance’ seed. 6.7 kg of this seed, enough for about 1000 ha of plantation, was taken up by industry generating about $300K for tea tree research.

Development of first generation seedling seed orchard (SSO1)

Establishment of the first generation seedling seed orchard (SSO1) and provenance/progeny trial including 199 seedlots from 23 provenances and 5 industry standards took place in 1994 at Wyrallah. First seed from the orchard was available in 2000 with an estimated genetic gain of 33%. As gain was below that of CSO1 (see below) uptake by industry has been limited. The main contribution of this orchard has been as a source of 49 superior seedlots, both open pollinated and controlled cross, for SSO2.

Development of the first clonal seed orchard (CSO1)

A clonal seed orchard (CSO1) from selected trees in a 60-family progeny trial established at Wyrallah in 1992 was planted at NSW DII Wollongbar in 1995. Seed from this source has been available to industry since 2000 and uptake has been substantial, 1.1kg. A genetic gain trial has shown a 69% improvement in paddock yields compared with industry standards (Doran *et al*. 2006 and this report). It will be replaced by SSO2 seed due for release in 2009.

Development of two second generation seedling seed orchards (SSO2 and SSO2p)

A second generation seedling seed orchard (SSO2) comprising 99 families and one clone and a partial second generation seedling seed orchard (SSO2p) comprising 36 families were establishment in 2001 at NSW DII WPII and West Wyalong respectively. SSO2 has been culled of the worst families based on breeding values and now comprises 70 families. Seed from this orchard will be released in 2009 and it is confidently predicted that it will be superior in genetic gains to CSO1, although the extent of this improvement has still to be quantified (refer Section 4.1.2 of this report). Gains from SSO2p seed are similarly awaiting quantification. Seed from SSO2p has been available since its 2007 release.

Development of the second clonal seed orchard (CSO2)

Ramets of 28 clones selected for their oil yielding potential in various project progeny and yield trials were used to establish a second clonal seed orchard (CSO2) at WPII in 2007. Growth and oil characteristics were assessed in 2009 and culling will follow. The first yield testing of this seed might be possible in 2011 if there is good flowering in spring of 2009.

Genetic gain trials of 2002 and 2008

A genetic gain trial established in 2002 has now seen five harvests (see Section 4.1.1 of this report). The first harvest of the 2008 genetic gain trial is scheduled for later in 2009.

Clonal field trials of 2004 and 2006

Intensive screening for elite individuals in PT1 in 2001 and in PT2 in 2003 followed by nursery rooting trials resulted in 2 sets of 10 clones being further multiplied for clonal field trials. The first set of 10 clones was established in field trials (yield and spacing trials) at Bungawalbin in 2004 and the second batch of 10 clones was planted at Bungawalbin in 2006. The results of these trials are given in Section 4.4 of this report. Eight superior clones have been identified from the two trials for further testing.

Controlled crossing program

Controlled cross seedlots from the three half diallels, comprising 5 unrelated parents in each diallel, undertaken at NSW DII WPII during the 2001, 2002 and 2003 flowering seasons were sown (see Baker *et al*. 2007). These were undertaken to estimate the importance of Specific Combining Ability in relation to General Combining Ability in breeding for economic traits in *M. alternifolia*. The loss of all germinants in a nursery watering failure means that this work will need to be repeated in the next project.

Further controlled pollinations were undertaken in SSO2 and SSO2p during the course of this project. Most crosses were part of a postgraduate study of the reproductive biology of *M. alternifolia* (see Baskorowati in Section 4.5.1 of this report) but some additional best × best crosses were undertaken in SSO2 in 2007.

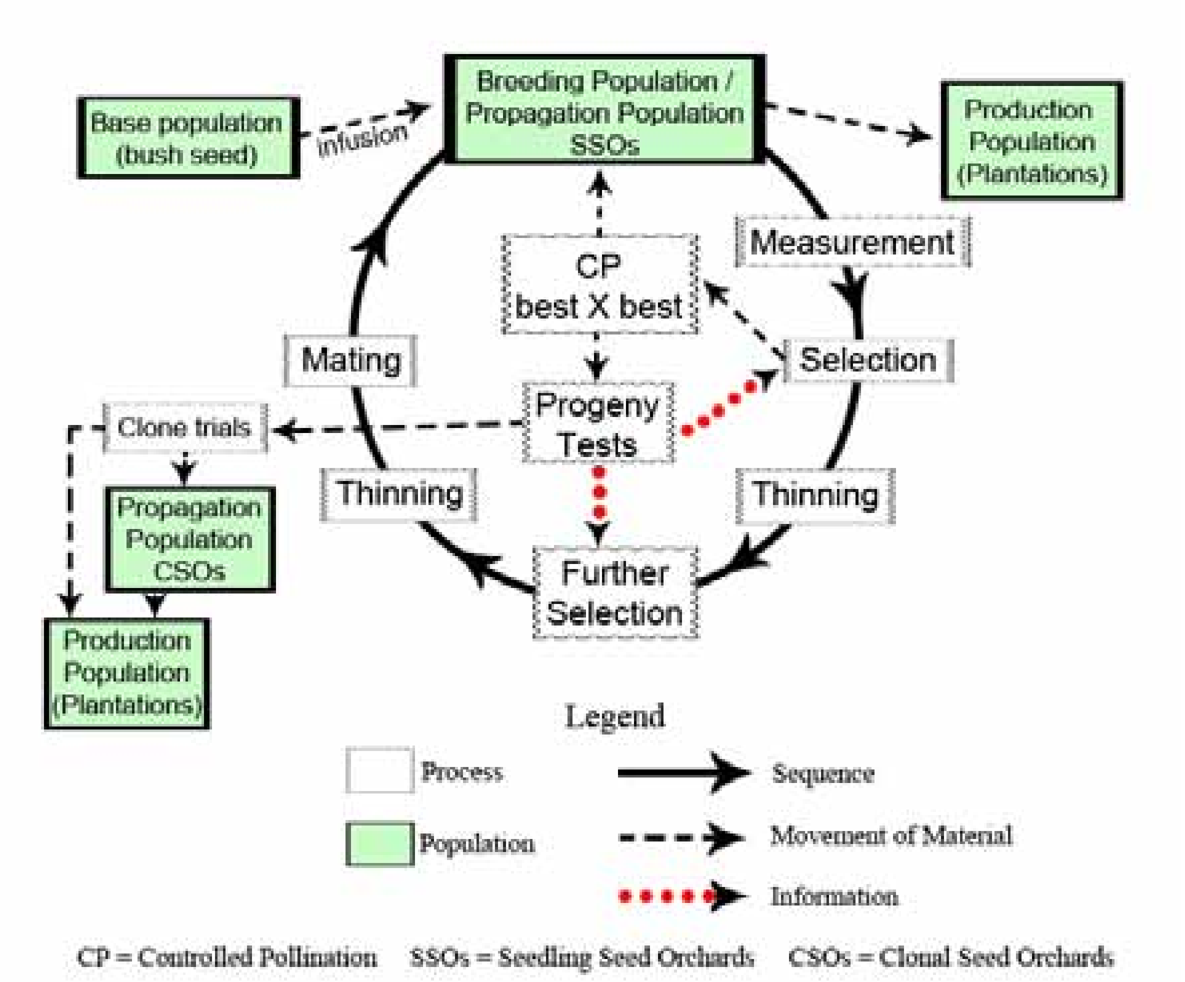

Figure 3.1 Diagrammatic representation of the main recurrent breeding strategy to supply progressively improved seed and cuttings of *M. alternifolia* for plantation production

4. Main Activities

4.1 Yield Trials

The justification for the tea tree breeding project is the genetic gain realised in improved plantations and the financial return on the investment in breeding. Yield trials with larger plots than progeny trials and a good statistical design, where the commercial scale products of the breeding program are compared with well defined commercial alternatives, are usually employed to give realistic estimates of genetic gain.

Predicted gains from implementation of the breeding strategy for tea tree were: 30% in the first project (1993-1996); by 60% in the second project (1996-2001); by 90% in the third project (2001-2006) and by >100% at conclusion of this fourth (2006-2009) tea tree breeding project (Doran *et al*. 1996, Doran *et al*. 2002, Baker *et al*. 2007). The trials described below were undertaken to test these predictions in practice.

4.1.1 Yield trial 2002 (YT2)

4.1.1.1 Introduction

It was important to the project and those using project seed to obtain an unequivocal estimate of the gain achieved through breeding activities. This section reports the results of a yield trial established in 2002 and designed to compare the oil yield and quality of seedlots produced by the first two breeding projects against themselves and a bulk of four commercially available seedlots as control.

4.1.1.2 Materials and Methods

Four seedlots were compared in the 2002 yield trial: a bulked seedlot from the clonal seed orchard (CSO1); early seed from the first generation seedling seed orchard (SSO1); a specially bulked seedlot from a selected natural stand (SP); and, a bulk of four seedlots representing the germplasm available to growers from commercial seed suppliers and nurseries servicing the industry in 2001 (IS or industry standard).

Seed released from the CSO1, SSO1 and SP are ATTIA 2B, ATTIA 2A and ATTIA 1 respectively.

The yield trial was laid out as a 4×4 Latin Square. Plots comprised 96 trees using 6 rows 1 m apart each of 16 trees at 0.3 m spacing. It was planted in March 2002 amongst a new commercial planting of tea tree at Codrington (Lat. 28º56.959'S; Long. 153º15.529'E; Alt. 29 m) near Coraki, New South Wales.

Five growth assessments were undertaken just prior to the first, second, third, fourth and fifth commercial harvest in 2003, 2004, 2005, 2006 and 2008 respectively. Survival, plant height and crown leafiness score [1 (sparse canopy) to 5 (bushy with no gaps and good leaf/twig retention)] were recorded as measures of growth.

As it was impractical to sample every tree in the trial for oil yield, a sampling scheme was used to estimate leaf biomass, oil concentration and oil quality for each seedlot. Frequency distributions were constructed for height (five classes) and leafiness score (six classes). Trees were randomly chosen from the matrix of height / leafiness score classes, proportional to class numbers and such that seven trees were sampled from each 96-tree plot. In this way a total of 112 trees were harvested and their components (stem, twig and leaf) determined.

On leaf subsamples oil concentration and composition was determined by ethanol extraction and then gas chromatography (Baker *et al.* 2000). Leaf biomass and oil concentration values were then combined to give an estimate of oil yield for each seedlot.

4.1.1.3 Results

Survival in the trial was very high (mean of 95% at fifth harvest) and there were no significant differences between seedlots in this attribute. Seedlot means from the first (2003), second (2004), third (2005) fourth (2006) and fifth (2008) harvests for height (cm), leafiness score (1-5), predicted leaf mass (g) per plant on an oven-dry weight basis, oil concentration in the leaves (mg/g, DW) and the oil quality traits, 1,8-cineole % (of total oil) and terpinen-4-ol % (of total oil), are given in Table 4.1. There were significant differences (not shown) between seedlots in most traits. The industry standard was significantly poorer in performance than seedlots from the breeding program for the key variates affecting yield, leaf mass per plant for harvests 1 and 2 and oil concentration for all five harvests. Breeding program seedlots did not differ significantly amongst themselves for leafiness score or leaf mass per plant but oil concentration of clonal seed orchard progeny was significantly better than the other two lots at all harvests.

Overall, the seedlots provided by the breeding program gave on average 10% greater predicted leaf mass per plant and 33% greater oil concentration in those leaves than the industry standard. Stand out performers were progeny from the clonal seed orchard seedlot which gave on average 10% greater leaf mass per plant and 50% greater oil concentration than the industry standard.

Oil yield per plant (g) by seedlot and harvest, estimated from the means for leaf mass per plant and oil concentration, is the variate of most interest for comparing performance of the various seedlots and for estimating realised genetic gains because of its direct link to the economics of production (Table 4.1). Seedlots from the breeding program gave on average 70%, 50%, 29%, 45% and 42% (mean 47%) greater oil yield than industry standard from the first, second, third, fourth and fifth harvests respectively. Progeny from the clonal seed orchard were 91%, 75%, 43%, 79% and 56% (mean 69%) superior to industry standard, from the seedling seed orchard 56%, 28%, 24%,29% and 30% (mean 33%) superior, and from selected provenance 62%, 47%, 19%, 28% and 41% (mean 39%) superior over the five harvests. Extrapolation to oil yield per hectare, assuming a per hectare plant stocking of 30,000 plants, gives 258kg, 189kg, 357kg, 312kg and 504kg for first, second, third, fourth and fifth harvests of progeny of the clonal seed orchard, 210kg, 138kg, 309kg, 225kg and 420kg for the seedling seed orchard, 219kg, 159kg, 297kg, 222kg and 456kg for the selected provenance and 135kg, 108kg, 249kg 174kg and 324kg for the industry standard.

Oil quality, as directed by the present medicinal market for tea tree oil, is assessed on oil composition. The two compounds in the oil of most interest are terpinen-4-ol, the main indicator of antimicrobial activity where levels approaching 40% (of total oil) or greater are deemed desirable, and 1,8-cineole that is erroneously regarded as a skin irritant where low levels (c. 3% or lower) are required by the market (Southwell and Lowe 1999). The results show all seedlots tested to be of desirable oil quality with the breeding project seedlots overall (average of the five harvests) superior to the industry standard with higher levels of terpinen-4-ol (higher by 4%) and lower levels of 1,8-cineole (lower by 27%).

Table 4.1 Growth and oil characteristics for the 4 seedlots in the 2002 yield trial over 5 harvests

Variate	Harvest	Seed Sources*				Least significant difference
		CSO1	SSO1	SP	IS	
Height (cm)	1st	172	183	174	168	8.0
	2nd	122	127	126	124	3.5
	3rd	158	168	163	167	7.1
	4th	169	170	171	167	6.0
	5th	190	197	193	190	9.3
Leafiness score (0.5–5)	1st	2.96	2.90	2.97	2.79	0.07
	2nd	3.55	3.43	3.49	3.28	0.18
	3rd	3.67	3.53	3.58	3.58	0.04
	4th	3.83	3.73	3.77	3.72	0.13
	5th	3.58	3.41	3.50	3.41	0.13
Pred. Leaf mass/ plant (g, DW)	1st	140.1	140.8	140.3	116.3	9.4
	2nd	88.6	82.2	87.8	75.5	7.1
	3rd	165.0	171.4	168.2	172.8	10.7
	4th	131.9	125.9	132.4	121.5	8.7
	5th	193.6	193.0	197.2	179.2	21.9
Oil conc. (mg/g, DW)	1st	61.6	50.0	51.7	38.9	7.8
	2nd	71.2	56.3	60.0	47.2	5.4
	3rd	72.3	60.4	59.0	48.1	5.3
	4th	72.3	58.6	59.4	49.1	5.4
	5th	86.9	72.3	76.9	60.3	7.2
Est. oil yield/ plant (g)	1st	8.6	7.0	7.3	4.5	1.8
	2nd	6.3	4.6	5.3	3.6	1.2
	3rd	11.9	10.3	9.9	8.3	3.0
	4th	10.4	7.5	7.4	5.8	1.5
	5th	16.8	14.0	15.2	10.8	2.7
1,8-cineole % (of total oil)	1st	2.0	1.9	2.2	3.1	0.8
	2nd	2.8	3.0	3.0	3.7	1.0
	3rd	2.3	2.3	3.0	2.9	0.8
	4th	2.2	2.6	2.2	3.6	1.0
	5th	1.5	2.1	1.7	2.6	0.5
Terpinen-4-ol % (of total oil)	1st	36.9	36.0	35.5	34.8	1.6
	2nd	38.7	37.5	37.9	36.7	0.5
	3rd	38.5	37.4	37.1	36.5	1.4
	4th	40.9	40.5	40.6	39.4	1.0
	5th	39.0	37.7	37.8	36.6	1.2

* CSO1 is clonal seed orchard; SSO1 is 1st generation seedling seed orchard; SP is selected provenance seed from the best performing natural population in progeny trials with seed trees chosen for superior oil characteristics; IS is industry standard, a bulk of seed supplied to the project by 4 commercial suppliers of seed and seedlings to the industry in 2001.

4.1.1.4 Discussion

Oil yields on a per hectare basis (kg/ha) at first harvest for breeding program seedlots (258 – CSO1; 210 – SSO1; 219 - SP) were excellent while the industry standard at 135kg/ha was typical of the first harvest yields experienced throughout the industry (Southwell and Lowe 1999). Oil yields per ha at second harvest (189kg – CSO1; 138kg – SSO1; 159kg - SP; 108kg - IS) were substantially below those at first harvest. This is atypical for tea tree plantations in NSW where a steady increase in oil yields is usually experienced up to the third harvest. Although oil concentration of each seedlot did increase by an average of 16% between harvests, this was well below what would normally be expected (25–50%, Colton et al. 2000). This was accompanied by a dramatic and atypical decrease (38%) in leaf biomass between first and second harvests. This is thought to be a combination of a very dry year during 2004 causing premature leaf drop and a reduction in the increase in oil concentrations. While there is no clear evidence of an association between water stress and oil concentration in tea tree (Murtagh1999), a reduction in oil concentration due to drought has been demonstrated in young eucalypt leaves (Doran and Bell 1994). Significant insect attack causing leaf loss leading up to second harvest is thought to have also contributed. Nevertheless a substantial improvement in yield of breeding program seedlots was evident.

The third harvest oil yields per ha (357kg – CSO1; 309kg – SSO1; 297kg – SP; 249kg – IS) were excellent. All seedlots performed well, with seedlots adding roughly 100 kg/ha to their first harvest yields. Oil concentrations at harvest 3 for all seedlots were similar to those at harvest 2, so that the increase in oil yield at harvest 3 was a function of more leaf and this is probably due to more rain during 2005 and better insect control.

Oil yields per ha at fourth harvest (312kg – CSO1; 225kg – SSO1; 222kg – SP) were good while the industry standard (IS) at 174kg/ha was typical of yields in the industry. Oil concentrations at harvest 4 for all seedlots were similar to those at harvest 3, so that the decrease in oil yield at harvest 4 was a function of less leaf and probably due to drier growing conditions during 2006.

The fifth harvest oil yields per ha (504kg – CSO1; 420kg – SSO1; 456kg – SP; 324kg – IS) were substantially higher than any previous harvest. All seedlots performed excellently, with seedlots adding roughly 200 kg/ha to their fourth harvest yields. The very high oil yields were a function of both high leaf production (the highest of any harvest) and high oil concentrations (also the highest of any harvest). Favourable rain events during 2007/08 would have contributed significantly to the high leaf production and to some extent the increase in oil concentrations. In addition to the favourable growing conditions the increasing age of the trees contributes to higher oil concentration.

The first release of seed from the breeding program was selected provenance(SP). During 1997 to 1999, 6.5kg (enough for about 1000ha of plantation) of seed of this grade was purchased by growers. This trial gave a realised genetic gain in yield from selected provenance over industry standard of 43% which compares well with anecdotal evidence from growers of at least a 30% gain in production from use of this seed source (e.g. J. Petrusa pers. comm. 2001). Once the yield results were released and the quantified gains (69%) of the clonal seed orchard were known only seed from this orchard was taken up by growers. During 2008/09 however, seed supply from the orchard was poor and so it was supplemented from seed from SSO2p and SSO2. Realised gains from these orchards (and all past releases) are to be quantified in YT3.

The relative gain in yield (33%) of the seedlot from the first generation seedling seed orchard, although substantial, was somewhat disappointing. The seedlot used to represent the orchard came from its first major flowering in 1997 at less than 3 years-of-age and when only 45% of seed orchard trees flowered. Some self-pollination due to the less-than-optimum numbers of pollen parents is perhaps why this seedlot did not perform better. This orchard met one of its primary goals of providing seed for the second generation seedling seed orchard. It was being maintained as an archive of breeding materials and low priority seed source, but it is now unavailable due to its removal by the land owner.

4.1.2 Yield trial 2008 (YT3)

4.1.2.1 Introduction

The aim of the tea tree breeding program is to increase the yield of high quality oil per unit area by improving yield of leaves and concentration of oil in those leaves while ensuring that the oil produced is of specified and consistent quality to maximise marketability. This section reports on the yield trial established in 2008 that is designed to compare the oil yield and quality of seedlots produced by the breeding program up to 2006 (first 3 projects) against themselves and a bulk of four commercially available seedlots as control.

4.1.2.2 Materials and Methods

Eight treatments are included in a big-plot yield trial established in March 2008 at Bungawalbin and these are described in Table 4.2. Treatments 2 to 7 comprise project seedlots and the five industry seed releases. Treatment 1, a contemporary industry standard of 2001, comprised seedlots obtained from 4 commercial seed suppliers. Treatment 8 was the industry select from one commercial seed supplier.

Table 4.2 Description of the origins of treatments included in the yield trial established at Bungawalbin in March 2008

Treatment	Origin	Release	Description
1	Unimproved	Control	A bulked collection of seed from 4 commercial seed suppliers
2	Best Provenance	ATTIA 1	A bulked collection of seed from 17 Devils Pulpit trees selected on oil characteristics
3	SSO1	ATTIA 2A	A bulked collection of seed from 25 selected SSO1 trees
4	CSO1	ATTIA 2B	A bulked collection of seed from 14 selected CSO1 trees
5	SSO2	ATTIA 3B	A bulked collection of seed from 30 selected SSO2 trees
6	SSO2p		A bulked collection of seed from all SSO2p trees
7	SSO2p	ATTIA 3A	A bulked collection of seed from 12 selected SSO2p trees
8	Industry Select		Seed from a commercial seed supplier with a privately developed seed orchard

The Bungawalbin yield trial was designed to be commercially harvested. There are four replicates for treatments 1 to 7 and only 2 replicates of treatment 8 because of poor nursery performance. For Treatments 1 to 7, each replicate consists of 14 x 200m rows. Plant spacing within a row 1m apart is 40cm (stocking of 25,000 plants per ha), so the total number of plants in the trial is 30,000 including 2000 Treatment 8 trees.

The first harvest will occur in spring 2009. Calculations indicate that each plot (2 rows/treatment/replicate) will provide approximately 25% of the biomass needed to fill a bin. The harvester will cut a plot, weigh the bin, move to the next plot containing the same treatment, harvest and weigh the bin again and repeat for the remaining two plots of that treatment. Differentials in bin weight at each weighing will provide estimates of the total biomass from each plot. Results from the distillation plant will be a composite of all replicates of each treatment and will provide broad-acre evidence of treatment performance. Replication will be obtained by sub-sampling the bin after each harvest run and determining oil concentration (Baker *et al*. 2000).

4.2 Seed Orchards

4.2.1 Clonal Seed Orchards

4.2.1.1 Introduction

Vegetative propagation (cloning) as part of a breeding strategy offers a method to access and maintain genetic gain from selected trees. Superior trees identified in provenance/progeny trials have been selected, propagated and then used to establish clonal seed orchards.

4.2.1.2 Materials and Methods

Coppice shoots from the 50 top ranked plants (by combined index) in the 1992 ANU provenance/progeny trial (see Doran *et al.* 1997) were collected in November 1993. Approximately 10 cuttings per ortet (clone) were set under mist sprays in a glasshouse. Thirteen of the clones that developed roots were used to establish the first clonal seed orchard (CSO1) at WPII in November 1995.

All 61 trees in the orchard were sampled in August 1998 and again in 2000 and 2002 to assess leaf oil characteristics (see Doran *et al.* 2002). This data was used to cull 25 trees from the orchard in May 2003. Trees were culled if their leaf oil contained less than 37% terpinen-4-ol or greater than 4% 1,8-cineole or if oil concentrations were less than 70 mg/g. Removal of inferior trees, further increases the genetic quality of clones in the orchard that will cross breed to produce highly improved seed. Summary details of the clones represented in CSO1 can be found in earlier reports (see Doran *et al*. 2002, Baker *et al*. 2007).

Since 1994, 6 provenance/progeny/yield trials have been established from which elite trees have been identified. These trees were first identified for superior growth and then sampled for oil characteristics with the best selected for propagation by stem cuttings. Cuttings from 28 of these trees selected after nursery screening for rooting ability (Table 4.3) were used to establish the second clonal seed orchard (CSO2) at WPII in February 2007.

In March 2009, all trees in the CSO2 were measured for height and scored (0.5 - 10) for leafiness and then sampled for oil characteristics.

Table 4.3 Details of the origin and number of clones included in CSO2 established at WPII in February 2007

Treatment	Source	Clone number	Number of ramets
1	PT2	3-73-4	3
2	PT2	2 84 3	3
3	PT2	2 44 1	3
4	PT2	2 46 4	4
5	PT2	3 36 5	6
6	PT2	3 91 3	4
7	PT2	4 88 4	6
8	PT2	2 108 1	8
9	PT2	2 110 5	7
10	PT2	2 106 5	4
11	YT2	2 128 56	1
12	YT2	3 128 41	4
13	YT2	3 128 65	4
14	CC2	15 12 50	5
15	CC2	3 117 53	6
16	PT1	3 30 3	2
17	PT1	2 31 3	2
18	PT1	2-24-5	1
19	PT1	1 92 3	2
20	CC1	93 42	4
21	Hedge	9-2	1
22	Hedge	5 3	4
23	Hedge	18 3	4
24	Hedge Lc	44-2	2
25	Hedge Lc	51-3	2
26	Hedge Lc	59-1	1
27	Hedge Lc	71-1	2
28	Hedge Lc	131-1	1
Total			96

4.2.1.3 Results and Discussion

Flowering in CSO1, during the October-November period since 1998 has varied between 6% and 75% (Table 4.4). One of the factors influencing flowering is the genetics/origin of the trees. During 1997, only 5 trees flowered, and these represented 3 clones all from the same local provenance, while the only clones that did not flower during 1998 and 1999 were all from another provenance. By 1998, 11 of the clones had flowered, and by 2001 all 13 clones had flowered.

Table 4.4 Flowering in the clonal seed orchard (CSO1) established at WPII in 1995

Year	Number of clones	Number of clones flowering	Number of trees flowering	Number of trees	Flowering (%)
1997	13	3	5	55	9
1998	13	11	34	55	62
1999	13	11	25	55	45
2000	13	12	15	55	27
2001	13	13	17	53	32
2002	13	12	34	53	64
2003	10	7	14	36	39
2004	10	7	18	36	50
2005	10	9	27	36	75
2006	10	4	5	36	14
2007	10	9	24	36	67
2008	10	2	2	36	6

The pattern of flowering differs between trees, with some flowering each year and others not. The fact that capsules remain on tea tree for many seasons means that a tree does not have to flower each year to provide a source of viable seed.

The first release of seed from CSO1 was from the flowering in 1998 that had matured by autumn 2000. Seed capsules were collected from 31 trees representing 11 clones and this netted 648g of improved seed which was release to the industry (ATTIA 2B). Eight of the best seedlots collected were included in the establishment of the SSO2. Seed capsule is now collected in May each year for release to industry.

Average clonal growth after 25 months in the CSO2 varied from 175cm to 315cm, while all clones scored a 7 or more for leafiness (Table 4.5). The mean leaf oil concentration for the orchard is excellent at 92.4mg/g, with 5 clones averaging over 100mg/g. Cineole levels for the orchard were low at 1.1% while terpinen-4-ol (with precursors) levels were high at 48.2%.

Flowering of the CSO2 in 2008 was a low 12% (21-month old trees) with only 9 of the 28 clones flowering. A better flowering is expected in 2009. Mature seed from this flowering will be available for yield testing in 2011.

Table 4.5 Mean growth, leafiness and oil characteristics for clones in CSO2 at 25 months

Treatment	Number of clones	Height (cm)	Leafiness (Score 0.5-10)	Oil conc. (mg/g)	1,8-cineole (%)	Terpinen-4-ol (%)
1	3	237	9	101.7	2.1	45.7
2	3	287	8	95.4	1.7	49.6
3	3	250	9	99.3	1.7	49.4
4	4	243	8	90.3	0.4	48.8
5	6	283	7	89.2	1.4	48.0
6	4	280	8	104.6	1.2	50.6
7	6	230	9	99.9	0.4	50.1
8	8	224	8	97.5	2.5	48.2
9	7	214	9	89.0	0.5	49.7
10	4	230	9	83.3	1.5	46.2
11	1	200	8	106.9	1.2	53.3
12	4	248	8	92.1	0.5	50.0
13	4	195	7	93.0	2.3	48.1
14	5	224	9	95.7	0.5	48.6
15	6	222	8	82.4	0.5	45.5
16	2	315	9	99.8	0.5	46.4
17	2	245	10	97.4	0.4	46.4
18	1	240	8	75.0	3.0	48.7
19	2	215	10	98.1	0.4	45.6
20	4	210	9	89.6	0.5	47.4
21	1	230	7	81.2	0.4	44.8
22	2	200	7	74.9	3.5	48.3
23	4	223	9	111.2	0.4	53.0
24	2	175	8	77.3	0.5	47.6
25	2	270	8	90.8	0.4	46.5
26	1	200	5	100.5	0.5	46.4
27	2	255	8	90.9	0.5	51.0
28	1	240	10	78.9	2.5	44.3
Mean		235	8	92.4	1.1	48.2

4.2.2 Second Generation Seedling Seed Orchards

4.2.2.1 Introduction

The main second generation seedling seed orchard (SSO2) was established at WPII in April 2001. The orchard was designed to give substantial genetic gain, provide highly improved seed for release to the industry and provide improved seedlots for the establishment of a third generation seedling seed orchard.

A partial second generation seedling seed orchard (SSO2p) was established at West Wyalong in September 2001. A second orchard site increases project security as well as enabling the opportunity to compare flowering between sites.

4.2.2.2 Materials and Methods

The second generation seedling seed orchard (SSO2) was used to assess the growth potential and oil quality of 100 largely unrelated families collected from the first generation seedling seed orchard, the clonal seed orchard (CSO1), best provenance and the infusion population. The orchard was established at WPII in April 2001 with 4 replicates of 100, 5-tree plots.

The partial SSO2 (SSO2p) was established at West Wyalong (G.R. Davis Pty Ltd) in September 2001. The design has 4 replicates of 5-tree line plots with a single row buffer at establishment. The orchard is a sub-set of 35 seedlots from the SSO2.

In May 2002, all trees in the SSO2 were assessed for growth (height and a leafiness score). Growth was then used to select the best 3 trees per 5-tree plot. The 2 non-selected trees in each plot were then removed. In July 2002, twig samples were then taken from all remaining 1200 trees to determine oil concentration and quality. A selection index (combining growth and oil characteristics, see Appendix 1 in Doran *et al*. 2002) was used in May 2003 to reduce the 3 trees per plot to one. All trees remaining in the orchard were again assessed in 2004 for growth and oil characteristics. A family selection index (combining growth from the orchard and progeny trials and oil characteristics from the orchard, see section 4.2.2.2 in Doran *et al*. 2002) was used to cull 18 families from the orchard before flowering in 2005. In 2007 another culling was undertaken when 124 trees were removed and families were reduced to 77. A further culling of 43 trees in 2008 reduced the orchard to 154 trees and 70 families.

Using both growth and oil characteristics, the SSO2p plots were thinned to one tree in September 2003. The trial was again sampled in April 2004 for oil characteristics. The trial underwent another overall thinning in early 2005. A further sampling occurred in December 2008, with a thinning to be undertaken prior to flowering in 2009.

4.2.2.3 Results and Discussion

Flowering in the SSO2 has ranged between 6% (20-month old trees) and 99% in 2007 (Table 4.6). While flowering in the SSO2p has varied between 2.5% (2006 – a drought year) and 98% in 2005.

Site differences appear to have a pronounced effect on a family's ability to flower at a young age. As the trees at WPII matured however, this effect was reduced.

In March 2007, over 2.2kg of SSO2p seed was collected for release (ATTIA 3A). Seed was collected from the best (oil concentration >65mg oil/g leaf DW) 32 trees in the 181 tree orchard.

Table 4.6 Percent flowering by year in the second generation seedling seed orchards

YEAR	SSO2	SSO2p
2001	Planted	Planted
2002	6	48
2003	37	50
2004	64	82
2005	46	98
2006	74	2.5
2007	99	24
2008	42	78

From March to May 2009, over 1.7kg of SSO2 seed was collected for release (ATTIA 3B). Seed was collected from the best (oil concentration >100mg oil/g leaf DW) 49 trees in the orchard of 154 trees.

4.3.3 Future Orchard Activities

Ongoing development of the main second generation seedling seed orchard (SSO2) and the second clonal seed orchard (CSO2) was established at WPII in 2007 from a selection of elite clones will give substantial genetic gain, provide progressively more productive seed for release to industry and seedlots for the establishment of a third generation seedling seed orchard (SSO3) scheduled for establishment in 2009 under the next tea tree breeding project.

4.3 Seed Releases

4.3.1 Early Releases 1997 – 2006

4.3.1.1 Introduction

The main activity of the breeding project is to increase oil yield and oil quality by releasing improved seed to the industry from a recurrent breeding strategy. As an interim measure to provide some improvement in seed quality while the new seed orchards matured, the project collected seed of selected best provenances and released this to the industry.

4.3.1.2 Objectives

To increase oil yields by at least 17% through the release of seed from best provenances and progressively increase yield by 60% and more through the release of seed from seed orchards.

4.3.1.3 Materials and Methods

The results of three field trials (SSO1 and two coppicing trials) planted in January 1994 and including 199 seedlots resulting from single tree collections from throughout the natural distribution (23 provenances representing 13 regions) of *M. alternifolia* and five commercially available seedlots as Industry Standards were used to identify the best performing natural provenances. These trials were assessed in 1995 for leaf biomass and oil characteristics to gain a preliminary estimate of oil yield per hectare by provenance (Doran *et al.* 1997).

The relative gains from the early natural stand and seed orchard seed releases by the project were quantified in the second project yield trial (YT2) established in 2002 and comparing the performance of the 3 project released seedlots (ATTIA 1, ATTIA 2A and ATTIA 2B) and a bulked Industry

Standard. This trial was assessed in 2003, 2004, 2005, 2006 and 2008 for leaf biomass and oil characteristics to give an estimate of oil yield by seedlot (see Section 4.1.1 of this report).

4.3.1.4 Results and Discussion

In the 1994 provenance/progeny trial, many substantially out-performed the industry benchmarks. Devils Pulpit was outstanding (see Doran *et al*. 1997 and section 4.1.1 of Doran *et al*. 2002 [the RIRDC final report, 1996–2001]). Other provenances giving relative higher yields included Barcoongere and Candole. Having identified the best provenances, collections were undertaken in 1996, 1998 1999 and 2000. Only trees that had tested well for oil characteristics were included in each seed release.

Yield gains as quantified in YT2 from project released seed were outstanding with 70%, 50%, 29%, 45% and 42% (mean 47%) greater oil yield than industry standard from the first, second, third, fourth and fifth harvest respectively. Progeny from the clonal seed orchard (ATTIA 2B – the best performing release) were 91%, 75% 43%, 79% and 56% (mean 69%) superior to industry standard. Seed release to the industry commenced in 1997 (Table 4.7).

The first three releases (1997 - 1999) were bush seed from best provenance. For the fourth release (2000), the project increased the choice of seed available. Seed offered for sale was again from best provenance (ATTIA 1) and in addition to this and for the first time included improved seed from the seedling seed orchard (SSO1 - ATTIA 2A) and the clonal seed orchard (CSO1 - ATTIA 2B).

Table 4.7 Seed sales (g) and revenue from 1997 to 2006

	Best Provenance	**SSO1**	**CSO1**	**TOTAL**	**REVENUE**
YEAR	**ATTIA 1 (g)**	**ATTIA 2A (g)**	**ATTIA 2B (g)**	**(g)**	**($)**
1997	1500			1500.0	52500
1998	2700			2700.0	94500
1999	2485			2485.0	86975
2000		12.5	12.5	25.0	1000
2001		10.0	20.0	30.0	1200
2002	50	35.3		85.3	4745
2003			40.5	40.5	2180
2004			325.5	325.5	26040
2005			81.0	81.0	6480
2006			364.0	364.0	29120
TOTAL	6735	57.8	843.5	7636.3	304,740

The realised gains of the 3 seedlots were not available until results from the 2002 yield trial were assessed. Once known (2003/04), only ATTIA 2B was released.

During 1999/2000, the downward pressure on oil prices restricted new plantings and hence the demand for seed. As a result of the lower oil prices, seed sales were slow. Oil prices and hence seed sales remained depressed during 2000 - 2006.

4.3.2 Releases 2006 – 2009

4.3.2.1 Introduction

This project, as a continuum of the earlier projects, shares the same primary objective: to release improved seed to the industry to maximise profit and market access from a breeding strategy that ensures on-going improvement of seed quality.

4.3.2.2 Objectives

The stated aim during this project was to release highly improved seed from the seed orchards with the aim of increasing oil yields by more than 80% in 2006/07 and 100% by 2007/08 once orchard culling is completed.

4.3.2.3 Materials and Methods

A third yield trial (YT3) was established in 2008 to compare the performance of progeny from three seedling seed orchards (SSO1, SSO2p and SSO2), the first clonal seed orchard (CSO1), an industry select and a bulked industry standard.

The trial is to be assessed for leaf biomass and for oil characteristics in spring 2009. The two measures of leaf biomass and oil concentration will be combined to give an estimate of oil yield by seedlot.

4.3.2.4 Results and Discussion

Recent poor flowering in the CSO1 has meant low seed production. In 2007 and 2008, CSO1 seed was supplemented with SSO2p seed (Table 4.8).

Table 4.8 Seed sales and revenue from 2007 to 2009

	CSO1	**SSO2p**	**SSO2**	**TOTAL**	**REVENUE**
YEAR	**ATTIA 2B (g)**	**ATTIA 3A (g)**	**ATTIA 3B (g)**	**(g)**	**($)**
2007	210.55	68.0		278.55	22284
2008	49.0	1339.0		1388	111052
2009			275.1	275.1	36015
TOTAL	259.55	1407.0	275.1	1941.65	169,351

It is expected (YT3 – to confirm) that the progeny from SSO2p will perform similar to CSO1 seed. However, progeny from SSO2 is expected to out perform both SSO2p and CSO1 so now only SSO2 seed (ATTIA 3B) is released.

During 2008/2009, higher oil prices increased new plantings and hence the demand for seed. Sales in 2009 of mainly SSO2 seed are expected to exceed 1000g.

4.3.3 Future Releases

The seed available for future releases will be progressively improved. Progressive improvement is possible as the second generation seedling seed orchard (SSO2) begins to produce even more highly improved seed. A second clonal seed orchard established from elite clones is expected to produce seed by 2011. A third generation seedling seed orchard is planned for establishment in 2009/10 to enable continued improvement. Use of improved seed for new plantings of tea tree will increase industry productivity and profitability.

The future success of seed releases will depend on a demand for new plantings. New plantings will occur with high oil prices or producers begin to replace paddocks of non-profitable tea tree with improved seedlings to increase productivity and profitability.

Current high oil prices should ensure a continued high demand for seed.

4.4 Clonal Work

Cloning as a propagation technique, offers the quick capture of genetic gains from selection and breeding and production of a uniform material for processing. Deployment of clones from selection and breeding programs in the rubber and oil palm industries, for example, has resulted in very substantial increases in latex and oil yields.

This project and other work have shown *M. alternifolia* to be a species amenable to mass vegetative propagation by stem cuttings (Doran *et al*. 2002). However, rooted cuttings of *M. alternifolia* are three to four times more expensive to produce than seedlings thus significantly increasing establishment costs. It was an objective of this project to demonstrate to tea tree growers that planting clones is an economically viable proposition (i.e. the enhanced income generated through the increased yields and uniform oil qualities of clones more than compensates for the increased establishment costs). This was to be achieved by selection and testing of a suite (20 genotypes) of elite clones from the breeding program and the eventual release to industry of several reliable and IP-protected clones suitable for broad-acre planting. The tests, undertaken on two batches of clones selected from PT1/CC1 and PT2/CC2/YT2 respectively, were to include nursery rooting abilities, trials to determine optimum planting densities for clones and yield comparisons with highly improved seedlots from the breeding program.

4.4.1 Clonal Spacing Trials

4.4.1.1 Introduction

Improved tea tree growth from ongoing selection and breeding may enable growers the opportunity to reduce planting density and thus reduce establishment costs (particularly for clones) without reducing total oil production. To evaluate this, two clonal spacing trials (CST1 and CST2) were established during September 2004 and October 2006 respectively at Bungawalbin.

4.4.1.2 Materials and Methods

The first spacing trial (CST1) compared the performance of 3 selected (superior growth and rooting ability in PT1 and at WPII respectively) clones (see Table 4.9) and 2 seedlots (ATTIA 2A and 2B) over 3 different spacings.

The trial, a split-plot design consisting of 3 main-plot treatments (spacing) and 5 sub-plot treatments (varieties) with 4 replicates. The 3 spacings of 30cm, 45cm, and 60cm within a 1m row spacing are equivalent to 33,333, 22,222 and 16,667 plants per ha respectively. Individual plots comprised 2 rows each of 10 plants.

Table 4.9 Details of the origin of clones and seedlots included in the 2004 clonal spacing trial (CST1) at Bungawalbin

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

The second spacing trial (CST2) compared the performance of 10 selected (superior growth and rooting ability in PT2, YT2 and CC2) clones (see Table 4.10) and a seedlot (ATTIA 2B) over 3 different spacings.

Table 4.10 Details of the origin of clones and seedlots included in the 2006 clonal spacing trial (CST2) at Bungawalbin

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	ATTIA 2B	CSO1	Bulked
12	Seedling	ATTIA 2B	CSO1	Bulked

The trial, a split-plot design consisting of 3 main-plot treatments (spacing) and 12 sub-plot treatments (varieties) with 4 replicates. The 3 spacings of 30cm, 45cm and 60cm within a 1m row spacing are equivalent to 33,333, 22,222 and 16,667 plants per ha respectively. Individual plots comprised 2 rows each of 10 plants.

Seedlings and cuttings supplied by the project were raised in the Toolara Nursery of Queensland Primary Industries and Fisheries, near Gympie and transported by road to the trial sites.

Weed and insect control was undertaken by the co-operator as part of normal plantation practice. As the trials were part of a commercial plantation they are harvested annually.

Measurements in both trials include survival, height, leafiness score, biomass production and oil characteristics (Prastyono 2009, Prastyono *et al.* 2009). Unfortunately the flood in January 2008 killed many trees in the CST2 and it has since been ploughed out. The CST1 was also compromised in this flood and is to be ploughed out in 2009.

4.4.1.3 Results and Discussion

Measurements of survival, height and leafiness in the CST1 were undertaken in 2005, 2006 and 2008. The analysis of variance (not shown) of this data showed no significant difference between spacing for survival and height however significant differences were indicated for leafiness. Also clones and seedlings were not significantly different for height and leafiness, however leafiness differences between clones were significant.

Survival in the trial was 96% in 2005. It was noted however that many of the cuttings had varying degrees of 'J' roots, a problem caused by repotting cuttings in a holding nursery before planting. This problem reduces a trees ability to develop a normal root system, which will reduce survivability over time. By 2008, survival in the trial for seedlings had reduced to 83% and for the clones to 60% (Table 4.11). The marked difference in survival between clones and seedlings is considered to be associated with the clones having the 'J' root problem.

Table 4.11 Mean growth traits in 2008 of the clones and seedlings at three spacings in the 2004 clonal spacing trial (CST1) (Prastyono 2009)

Variety Group	**Treatment 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	58.8	60.0	145.8	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	85.6	142.1	136.7	136.8	3.78	4.15	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*

Spacing are 30cm (1), 45cm (2) and 60cm (3) within a 1m row spacing and are equivalent to 33,333, 22,222 and 16,667 plants per ha respectively

Average height growth for 2008 was similar for both clones (139cm) and seedlings (138cm) (Table 4.11). For leafiness, clone 2 at 4.3 was the highest with clones 1 and 3 the least at 3.8.

After several analyses of this trial, there is evidence to suggest that increasing plant spacing from 30cm to 60 cm (within a 1m row spacing) increases tree leafiness together with a trend (although not significant) to reduce plant height. The implication for growers is that increasing plant spacing from 30cm to 60cm (within a 1m row spacing) will have the potential to reduce yields unless the increase in plant leafiness compensates for the reduction in plant number per hectare.

Oil characteristics for the trial at 30cm and 60cm (within a 1m row spacing) for 2008 are presented in Table 4.12. Although there was some variation in oil concentration and oil composition between varieties, there was no consistent trend to indicate that increasing spacing has any effect on oil concentration or oil composition.

Table 4.12 Oil characteristics in 2008 of the clones and seedlings for two spacings in the 2004 clonal spacing trial (CST1) (Prastyono 2009)

Variety Group	**Treatment Number**	**Oil concentration (mg/g DW)**		**1,8-cineole (%)**		**Terpinen-4-ol (%)**	
		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*

Spacing are 30cm (1), 45cm (2) and 60cm (3) within a 1m row spacing and are equivalent to 33,333, 22,222 and 16,667 plants per ha respectively

The biomass data and oil data from 2008 are combined in Table 4.13 to indicate predicted plant components and estimated oil yield.

As a consequence of lower survival, clones had predicted tree components and estimated oil yield lower than seedlings, both on a per plot and a per hectare basis. Analyses of variance of the predicted values (not shown) indicated that there was a significant difference for all variates due to spacing and variety group while variation within each group variety was not significantly different.

Results expressed on a per hectare basis showed a decrease in predicted stem, fine stem and leaf mass by 36%, 33% and 32% respectively for clones and 32%, 28% and 29% for seedlings in response to and increase in plant spacing. Similarly, for estimated oil yields per hectare clones decreased 33% and seedlings 32%. The implication for growers is that increasing plant spacing from 30cm to 60cm (within a 1m row spacing) can reduce yields by 33% on a per hectare basis.

Table 4.13 Estimated per hectare means from the 2008 assessment of predicted tree components and estimated oil yields of the clones and seedling at two spacings in the 2004 clonal spacing trial (CST1) (adapted from Prastyono 2009)

Variety Group	**Treatment Number**	**Predicted stem mass/ha (t DW)**		**Predicted fine stem mass/ha (t DW)**		**Predicted leaf mass/ha (t DW)**		**Predicted total mass/ha (t DW)**		**Estimated oil yield/ha (kg)**	
		Spac.1	**Spac.3**	**Spac.1**	**Spac .3**	**Spac.1**	**Spac.3**	**Spac.1**	**Spac.3**	**Spac.1**	**Spac.3**
Clone	1	2.58	2.04	1.63	1.34	2.65	2.34	6.87	5.74	199	183
	2	3.40	2.79	2.29	1.89	3.62	2.96	9.31	7.66	286	234
	3	4.31	1.74	2.76	1.21	4.46	1.98	11.6	4.94	325	139
	mean	3.43	2.19	2.22	1.48	3.57	2.43	9.24	6.11	272	184
Seedling	4	4.69	3.17	2.99	2.12	4.80	3.36	12.5	8.67	384	269
	5	4.79	3.27	3.04	2.23	4.90	3.53	12.8	9.06	402	258
	mean	4.74	3.22	3.02	2.18	4.85	3.45	12.6	8.86	393	262

Spacing are 30cm (1) and 60cm (3) within a 1m row spacing and are equivalent to 33,333 and 16,667 plants per ha respectively

Survival, height and leafiness in the CST2 were measured in 2007. The analysis of variance (not shown) for these measurements showed no significant difference between spacing for survival, however significant differences for both height and leafiness. There was also a significant difference between varieties for height and leafiness.

Average height decreased from 126cm to 119cm with increasing plant spacing while leafiness increased from 3.3 to 3.7 (Table 4.14). It appears that a tree with more near neighbour competition grew taller but with less leaf. This result is similar to that found for CST1.

Table 4.14 Mean height and leafiness of the clones and seedlings in 2007 at the three spacings in the 2006 clonal spacing trial (CST2) (adapted from Prastyono 2009)

Variety Group	**Treatment Number**	**Height (cm)**			**Leafiness score (0.5-5)**		
		Spac.1	**Spac.2**	**Spac.3**	**Spac.1**	**Spac.2**	**Spac.3**
Clone	1	151	148	147	2.7	3.0	3.2
	2	125	118	115	3.7	3.9	4.1
	3	125	121	116	3.6	3.7	3.5
	4	116	109	110	3.2	3.4	3.3
	5	124	115	114	3.8	4.2	4.5
	6	131	120	118	3.5	3.6	4.1
	7	133	131	132	3.1	3.3	3.4
	8	135	126	130	3.2	3.4	3.5
	9	124	117	120	3.5	4.0	4.1
	10	125	119	116	3.0	3.2	3.5
	mean	129	122	122	3.3	3.6	3.7
Seedling	11	113	113	103	3.2	3.4	3.5
	12	112	107	109	3.3	3.2	3.5
	mean	113	110	106	3.3	3.3	3.5
l.s.d (P=0.05)		*9.3*	*9.3*	*9.3*	*0.38*	*0.38*	*0.38*

Spacing are 30cm (1), 45cm (2) and 60cm (3) within a 1m row spacing and are equivalent to 33,333, 22,222 and 16,667 plants per ha respectively

Oil concentration and oil quality for 30cm and 60cm (within a 1m row spacing) plant spacings as assessed in 2007 are presented in Table 4.15. Although plant spacing had no effect on oil quality, there was a 5% decrease in oil concentration from 91.6mg/g to 86.7mg/g for the clones and a 10% reduction from 61.8 to 55.8mg/g for the seedlings when plant spacing was increased from 30cm to 60cm (within a 1m row spacing).

Clones as physiologically mature plants had a mean oil concentration of 89.2mg/g while the first year seedlings had a mean oil concentration 34% less at 58.8mg/g. All clones had excellent oil concentrations with clone 6 having the highest at 94.3mg/g and clone 1 the lowest at 80.1mg/g.

Table 4.15 2007 data for oil characteristics of the clones and seedlings for two spacings in the 2006 clonal spacing trial (CST2) (Prastyono 2009)

Variety Group	**Treatment Number**	**Oil concentration (mg/g DW)**		**1,8-cineole (%)**		**Terpinen-4-ol (%)**	
		Spac.1	**Spac.3**	**Spac.1**	**Spac.3**	**Spac.1**	**Spac.3**
Clones	1	82.1	78.0	1.6	1.6	43.3	42.9
	2	93.6	87.2	0.5	0.5	42.2	42.7
	3	95.8	88.9	2.7	2.7	42.0	42.1
	5	94.7	91.9	1.6	1.6	43.0	43.9
	6	96.0	92.5	0.8	0.5	43.5	44.0
	7	89.3	82.3	2.4	2.5	42.1	41.6
	8	92.4	87.0	3.2	2.8	41.8	41.6
	9	88.9	85.7	0.5	0.5	44.3	43.8
	mean	91.6	86.7	1.7	1.6	42.8	42.8
Seedling	11	68.0	58.3	1.8	2.8	42.5	41.8
	12	57.6	54.1	2.9	1.8	41.6	42.0
	mean	62.8	55.8	2.3	2.2	42.2	41.9
l.s.d (P=0.05)		*7.40*	*7.40*	*0.97*	*0.97*	*1.43*	*1.43*

Spacing are 30cm (1) and 60cm (3) within a 1m row spacing and are equivalent to 33,333 and 16,667 plants per ha respectively.

Treatment 4 and 10 not sampled.

The biomass data and oil concentration data from 2007 are combined in Table 4.16 to indicate predicted plant components and estimated oil yield for the 30cm and 60cm (within a 1m row spacing) plant spacings.

Results expressed on a per hectare basis showed a decrease in predicted stem, fine stem and leaf mass of 37%, 35% and 34% respectively for clones and 48%, 36% and 37% for seedlings in response to the increase in plant spacing. For estimated oil yields per hectare clones decreased by 38% overall and seedlings by 43%. The implication for growers is that increasing plant spacing from 30cm to 60cm (within a 1m row spacing) can reduce yields in the first harvest by 38 - 43% on a per hectare basis.

Oil yield for clones at 421kg/ha was 219% greater than that of seedlings at 132kg/ha. The greater yield from clones was a function of an 111% greater leaf production and a 52% greater oil concentration when compared with the seedlings.

The highest yielding treatment at 580kg/ha was clone 8 at the 30cm spacing. It had the highest leaf production at this spacing and a high oil concentration of 92.4mg/g.

Although all the clones at first harvest have very substantially out-performed the seedlings in per hectare yield of oil the relative difference in both leaf production and oil concentration will not be maintained. As a seedling matures, its oil concentration increases whereas the clone already expresses a mature age oil concentration. This has been demonstrated in the CYT1 (see Table 4 18). Also any differences in the size of the plants at establishment are pronounced in the first year of growth and decrease with time (CST1 and CYT1). The economic implication of these outstanding clonal yields has been studied by Prastyono (2009) and is presented in section 4.5.2.

Table 4.16 Estimated per hectare means from the 2007 assessment of predicted tree components and estimated oil yield of the clones and seedling at two spacings in the 2006 clonal spacing trial (CST2) (adapted from Prastyono 2009)

Variety Group	**TreatmentNumber**	**Predicted stem mass/ha (t DW)**		**Predicted fine stem mass/ha (t DW)**		**Predicted leaf mass/ha (t DW)**		**Predicted total mass/ha (t DW)**		**Estimated oil yield/ha (kg)**	
		Spac.1	**Spac.3**	**Spac.1**	**Spac.3**	**Spac.1**	**Spac.3**	**Spac.1**	**Spac.3**	**Spac.1**	**Spac.3**
Clone	1	6.1	3.1	2.1	1.5	5.3	3.0	13.7	7.7	441	234
	2	4.4	3.1	2.1	1.4	5.7	4.1	12.2	8.7	538	361
	3	4.7	2.9	2.3	1.3	5.0	2.9	12.0	7.1	479	256
	5	3.9	2.2	1.7	1.2	5.9	3.8	11.5	7.2	560	352
	6	5.2	3.3	1.7	1.3	5.4	4.0	12.4	8.5	522	371
	7	5.9	3.4	2.2	1.2	5.6	3.3	13.9	8.0	500	268
	8	4.9	4.2	1.9	1.5	6.2	4.9	13.0	10.6	580	426
	9	4.4	2.9	2.1	1.2	5.8	3.6	12.3	7.6	521	312
	mean	4.9	3.1	2.0	1.3	5.6	3.7	12.6	8.2	518	323
Seedling	11	2.5	1.0	1.1	0.6	2.7	1.5	6.3	3.1	178	92
	12	2.9	1.7	1.1	0.8	2.7	1.8	6.7	4.3	153	99
	mean	2.7	1.4	1.1	0.7	2.7	1.7	6.5	3.9	168	96

Spacing are 30cm (1) and 60cm (3) within a 1m row spacing and are equivalent to 33,333 and 16,667 plants per ha respectively.

Treatment 4 and 10 not sampled.

4.4.2 Clonal Yield Trials

4.4.2.1 Introduction

To evaluate the performance of clones in a commercial plantation two clonal vs seedling yield trials (CYT1 and CYT2) were established during September 2004 and October 2006 respectively at Bungawalbin.

4.4.2.2 Materials and Methods

The first yield trial (CYT1) compared the performance of 10 selected (superior growth and rooting ability in PT1 and at WPII respectively) clones and 3 seedlots (ATTIA 2A, 2B and Industry Select). Details of the origin of these clones and seedlots are given in Table 4.21 of Doran *et al.* (1997).

The trial had 4 replicates with each replicate having 12 rows x 66 plants = 792 plants and each treatment represented by 66 plants (3 rows x 22 plants) in each replicate. The total number of plants in the trial was 3192 including 24 buffers. It was planted in 2004.

The second yield trial (CYT2) compared the performance of 10 selected (superior growth and rooting ability in PT2, YT2 and CC2) clones (see Table 4.10 above) and a seedlot (ATTIA 2B).

The trial had 4 replicates with each replicate having 12 rows x 60 plants = 720 plants and each treatment represented by 60 plants (3 rows x 20 plants) in each replicate. The total number of plants in the trial was 3384 including 504 buffers. It was established in 2006.

Seedlings and cuttings supplied by the project were raised in the Toolara Nursery of Queensland Primary Industries and Fisheries near Gympie and transported by road to the trial sites.

Weed and insect control was undertaken by the co-operator as part of normal plantation practice. As the trials are were of a commercial plantation they are harvested annually.

Measurements in both trials include survival, height, leafiness score and oil characteristics. Unfortunately the flood in January 2008 killed many trees in the CYT2 and it has since been ploughed out. The CYT1 was also compromised in this flood and is to be ploughed out in 2009.

4.4.2.3 Results and Discussion

Survival in the CYT1 was 96% in 2005. As in the clonal spacing trial (CST1), ‘J’ rooting was also observed. By 2008, survival in the trial of seedlings had reduced to 81% and for the clones to 49% (Table 4.17). The marked difference in survival between clones and seedlings is considered to be associated with the clones having the ‘J’ root problem.

Analysis of variance (not shown) for 2005, 2006 and 2008 indicated significant differences between treatments for both height and leafiness score. For 2005, average height growth was 114cm, clones at 118cm out performed the seedlings at 101cm. It was noted that the clones were a larger plant (both top and root mass) at establishment. This initial advantage would assist growth in the first year. By 2008 (Table 4.17), seedlings at 130cm out performed clones at 97cm. Only one clone (C9) at 131cm had a similar height to the seedlings.

Leafiness for clones in 2008 varied greatly from 2.77 (C62) to 3.80 (C4). C4 was the only clone with a similar leafiness to the seedlings at 3.70.

Clone survival, growth and leafiness was significantly lower than the seedlings. The problem with ‘J’ rooting was thought to be the major cause in the clones poor performance over time.

Table 4.17 Mean growth traits in 2008 of the clones and seedlings in the 2004 clonal yield trial (CYT1) (adapted from Prastyono 2009)

Variety Group	**Treatment Number**	**Source Number**	**Survival (%)**	**Height (cm)**	**Leafiness score (0.5-5)**
Clone	1	C2	64.8	116.9	3.43
	2	C4	62.1	86.3	3.80
	3	C6	27.3	68.5	2.96
	4	C9	51.1	131.3	3.28
	5	C11	55.7	114.3	3.54
	6	C30	53.0	121.0	3.23
	7	C57	61.0	77.2	3.24
	8	C62	24.6	62.7	2.77
	10	C53	42.8	97.2	3.03
	mean		49.2	97.3	3.25
Seedling	11	ATTIA 2A	77.3	131.8	3.70
	12	ATTIA 2B	84.1	125.6	3.70
	13	IS	81.1	131.9	3.65
	mean		80.8	129.8	3.68
l.s.d (P=0.05)			*21.39*	*21.68*	*0.38*

Treatment 9 (C41) not sampled.

Analysis of variance (not shown) for oil characteristics (concentration, 1,8-cineole and terpinene-4-ol) for 2005, 2006 and 2008 indicated some significant differences between treatments. Oil characteristics for the clones and seedlings for these 3 years are presented in Table 4.18. The mean oil concentration for the seedlots ATTIA 2A and ATTIA 2B was 46.6mg/g, 61.2mg/g and 84.9mg/g for 2005, 2006 and 2008 respectively and this was 10%, 11% and 19% higher than that for the Industry Select for those three years. Seedling oil concentrations at 45.2mg/g at the first harvest (2005) were 35% lower than clones, however by 2006 concentrations increased to 59.1mg/g and only 9% less than clones and by 2008 concentrations were 80.4mg/g which were similar to clones.

As physiologically mature plants, the clones demonstrated a 54%, 36% and 0% higher oil concentration than the seedlings for 2005, 2006 and 2008 respectively. It was not until the third harvest that the oil concentrations of seedlings reached mature levels and were comparable with that of the clones. The implication for growers is that clones return higher yields earlier than seedlings.

For the clones, oil concentration was similar for 2005 and 2006, however with wet conditions during 2008 oil concentrations increased. Of the clones C2, C4, C57 and C62 have performed the best for oil concentration. Further assessment is needed to confirm the ranking of the clones in terms of survival and leaf production (unfortunately both these variates were compromised with clonal 'J' rooting in this trial) before any release to the industry is undertaken.

Oil quality was excellent for all 13 treatments. It should be noted however that the Industry Select had one of the lowest terpinen-4-ol values and one of highest 1,8-cineole values.

Table 4.18 Mean oil characteristics for the 10 clones, 2 seedlots and IS for 2005, 2006 and 2008 in the 2004 clonal yield trial (CYT1) (adapted from Prastyono 2009)

Variety Group	Treatment Number	Source Number	Oil concentration (mg/g)			1,8-cineole (%)			Terpinen-4-ol (%)		
			2005	2006	2008	2005	2006	2008	2005	2006	2008
Clone	1	C2	75.8	75.3	92.0	0.5	0.4	0.4	39.7	42.4	41.9
	2	C4	72.0	63.3	91.0	0.5	0.5	0.4	39.5	40.9	40.8
	3	C6	67.6	67.6	79.0	2.7	2.9	2.6	38.8	39.2	41.3
	4	C9	64.9	58.0	73.3	2.4	2.4	2.1	39.8	41.7	41.8
	5	C11	71.8	57.0	78.2	0.5	0.5	0.5	40.9	41.7	42.5
	6	C30	61.7	54.6	72.7	1.8	1.9	1.5	40.6	41.5	42.3
	7	C57	75.6	70.3	84.1	0.5	0.4	0.4	40.4	42.3	43.9
	8	C62	83.9	77.6	80.8	0.5	0.4	0.4	39.7	41.4	42.1
	9	C41	60.2	59.5	71.4	1.2	1.1	1.3	39.7	40.3	40.4
	10	C53	63.0	63.5	73.1	1.0	0.5	0.9	39.8	40.9	40.5
	mean		69.7	64.7	79.6	1.2	1.1	1.1	39.8	41.2	41.8
Seedling	11	ATTIA 2A	47.5	59.4	81.3	1.6	2.0	1.7	39.0	41.0	40.0
	12	ATTIA 2B	45.7	63.0	88.5	1.9	2.3	1.5	39.0	40.6	40.6
	13	IS	42.5	55.0	71.4	2.8	2.0	1.6	37.4	40.2	39.1
	mean		45.2	59.1	80.4	2.1	2.1	1.6	38.5	40.6	39.9
l.s.d (P=0.05)			*10.6*		*11.1*	*0.6*		*0.2*	*1.4*		*3.4*

Survival, height and leafiness in the CYT2 were measured in 2007. The analysis of variance (not shown) for these variates showed no significant differences between treatments for survival, however significant differences for both height and leafiness.

Survival in the CYT2 was excellent at 98%. The problem of ‘J’ rooting in the 2004 clones was not apparent in these the 2006 clones.

Average height growth was 134cm. Clones at 137 cm out performed the seedlings at 117cm (Table 4.19). Clones were a larger plant at establishment and this could account for the superior growth in the first year. Clones demonstrated large differences in both height and leafiness with C39 and C67 being the tallest at 170cm and 148cm respectively yet with the lowest leafiness score at 3.1 and 3.3 respectively.

Table 4.19 Mean height and leafiness in 2007 of the clones and seedlings in the 2006 clonal yield trial (CYT2)

Variety Group	**Treatment Number**	**Source Number**	**Height (cm)**	**Leafiness score (0.5-5)**
Clone	1	C39	170	3.1
	2	C52	130	4.1
	3	C56	136	4.0
	4	C57	118	3.8
	5	C64	139	4.1
	6	C66	133	3.9
	7	C67	148	3.3
	8	C68	135	3.5
	9	C70	132	3.9
	10	C71	133	3.5
	mean		137	3.7
Seedling	11 and 12	ATTIA 2B	117	3.6

Oil characteristics for the trial are presented in Table 4.20. There were large differences in oil concentration and oil composition between varieties. Clones as physiologically mature plants had a mean oil concentration of 94.4mg/g while, in this the first year of growth, seedlings had a mean oil concentration 23% less at 72.5mg/g. All clones had excellent oil concentrations with C52 having the highest at 100.9mg/g and C71 the lowest at 84.2mg/g. Oil quality was excellent for all 12 treatments.

Table 4.20 2007 data for oil characteristics of the clones and seedlings in the 2006 clonal yield trial (CYT2)

Variety Group	**Treatment Number**	**Source Number**	**Oil concentration (mg/g)**	**1,8-cineole (%)**	**Terpinen-4-ol (%)**
Clone	1	C39	91.4	1.5	40.1
	2	C52	100.9	0.5	40.0
	3	C56	94.6	2.7	39.9
	4	C57	90.2	1.0	39.8
	5	C64	100.1	1.6	41.7
	6	C66	97.9	0.5	41.1
	7	C67	94.9	2.1	39.6
	8	C68	98.2	2.4	38.8
	9	C70	91.4	0.5	41.0
	10	C71	84.2	0.5	39.2
	mean		94.4	1.3	40.1
Seedling	11	ATTIA 2B	72.1	2.3	40.1
	12	ATTIA 2B	72.9	2.4	39.5
	mean		72.5	2.4	39.8

4.4.3 Future Work

The clonal trials (CST1 and CYT1) established in 2004 provide information on the oil characteristics of the first suite of selected clones. Unfortunately, these clones suffered from a nursery induced problem of ‘J’ roots which disrupts root growth and this in turn inhibits top growth and reduces survival thus compromising the growth results from these trials. A second suite of 10 elite clones were selected and another spacing trial (CST2) and yield trial (CYT2) were established in spring 2006. The 2006 clones did not suffer from the ‘J’ root problem, however these two trials were lost to the flood in January 2008.

From these trials, four (C2, C4, C57 and C62) from the CYT1 (oil concentration considered) and four (C52, C64, C57 and C62) from the CYT2 (oil yield considered) have been identified as high yielding and should again be field trialled to compare their performances with those of the progeny from the new seed releases (ATTIA 3A and 3B).

4.5 Associated Activities

During the course of the current project, breeding project staff have encouraged and participated in three University studies and one CSIRO-based project that have delivered research findings of significance to tea tree breeding. These projects and a NSW DII initiative concerning adoption of a quality management system inclusive of the tea tree breeding project are summarised below.

4.5.1 Reproductive Biology of *Melaleuca alternifolia* (Maiden and Betche) Cheel

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The efficient and successful conduct of a tree improvement strategy employing sexual reproduction requires species-specific information about flowering and fruiting phenology, floral development characteristics, pollen vectors and pollen dispersal, the sexual system, the mating system and its genetic consequences in terms of inbreeding, and interspecific hybridisation and paternity relationships. This study was conducted to gain a better understanding of the reproductive biology of *M. alternifolia* and to assist the ATTIA/RIRDC tea tree breeding program in its seed orchard management and controlled pollination activities. Components of the study were conducted in a managed natural population of *M. alternifolia* at Coombell, NSW, in two seedling seed orchard populations in NSW at Wollongbar and West Wyalong and in glasshouses in Canberra, ACT. The majority of the work was conducted from 2004 to 2007, although the study also drew on some prior observations.

Readers are directed to Baskorowati (2009), Baskorowati *et al*. (2009a) and Baskorowati *et al.* (2009b) for full details of the study.

Implications of results for the RIRDC/ATTIA Breeding Program

Siting of seed orchards and breeding populations

- Earlier and more intense flowering will result from siting of seed orchards and breeding populations where winter temperatures are low, with minima below 5ºC. This has the potential to shorten generation times and increase seed yields.

- Flowering intensity and success in producing capsules appeared to be associated with total spring rainfall. Drought during flowering causes a heavy abortion of flowers indicating a need for irrigation of breeding sites with unreliable rainfall.

Is selfing an important issue in M .alternifolia breeding?

- A high level of self incompatibility is present in *M. alternifolia,* although some families are capable of producing a few selfed capsules.

- A self-incompatibility system operates in the stigma or the style, although a few self-pollen tubes are capable of germinating and producing a pollen tube. It also appears that late acting self-incompatibility mechanisms discriminate against self pollen tubes when they descend to the ovary.

- Circumstantial evidence suggests that selfed progeny of *M. alternifolia* exhibit suppressed growth (in-breeding depression) and would be culled from a breeding population at an early age.

Making controlled crosses

Controlled pollination as a tool to produce a nucleus of superior trees as a basis for the clonal component of the tea tree breeding program can now be conducted more efficiently. Key findings were:

- The stigma was most receptive during days 3 to 5 after anthesis. Pollen needs only to be applied once during this period to achieve good results, rather than twice which is current practice.

- Pollen can be stored successfully in the deep freezer (-18ºC) from one pollination season to the next. This finding will allow greater flexibility in undertaking controlled pollinations as stored pollen can be substituted for fresh pollen when insufficient quantities are available from new-season flowers.

- Emasculated flowers must be carefully inspected and cleaned of adult and larval thrips (*Thysanoptera* species) before bagging to ensure isolation from unwanted pollen. Barely visible small insects identified as mainly thrips were recorded in large numbers on tea tree flowers and data from the exclusion experiments indicated that they are important pollinators. Varieties of large flying insects, such as different types of flies and bees, also visit tea tree flowers and play a role in pollination.

Other useful findings

- Flowers of *M. alternifolia* are hermaphroditic and protandrous. They are carried in a compound inflorescence and open acropetally over 6 days. Flowering of an entire inflorescence lasts 12 days. No strong separation of male and female phases was found in any individual flower; pollen was shed by 1.4 days after anthesis and the stigma was, by then, partially receptive but well below the peak receptivity reached during days 3 to 6 from anthesis.

- Stamens per flower average 250 while ovules per flower average 78, being high and low for the genus respectively and indicating a preferential outcrossing system.

- The period from flowering to capsule maturation takes 16 to 18 months in *M. alternifolia*. Viable seeds per capsule range from 26 to 57.

- Flowering was synchronous across the three study sites. It occurred over 3 to 4 weeks with peak flowering occurring in November. A low percentage of trees appear able to flower outside of the regular flowering season with by fewer than 10% of the trees studied in the natural population at Coombell flowering sporadically over 6 months.

- An artificial interspecific hybrid between *M. alternifolia* and *M. dissitiflora* was produced for what is believed to be the first time. The cross succeeded only when *M. alternifolia* was used as female parent and then only in low proportions indicating that both physical and physiological barriers may be operating against this species combination. *M. alternifolia* x *M. linariifolia* hybrids were readily manipulated with no barrier to hybridisation evident.

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4.5.2 Potential of Clones to Boost Yields in Tea Tree Plantations

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Clonal forestry is widely practiced today and has achieved notable success in the tropics and subtropics the deployment of a suite of well adapted eucalypt species and their hybrids that lend themselves to vegetative propagation. There are compelling reasons for this including maximum capture of genetic gains from tree breeding, uniformity of trees in growth and form and increased yields from plantations and consequent lower production costs. As *M. alternifolia* can be relatively easily vegetatively propagated as rooted cuttings, the RIRDC/ATTIA tea tree breeding project has conducted several trials since its inception aimed at demonstrating to the tea tree industry the potential gains in oil yield that might be achieved by deploying selected clones in place of seedling stock.

This study, which was based on two clonal spacing trials (CSTs) established at Bungawalbin in 2004 and 2006, had the following objectives: (a) to compare the commercial oil traits of selected *M. alternifolia* clones with improved seedling controls at three plantation stockings (33,333, 22,222 and 16,667 plants/ha), and (b) to develop a financial model comparing the financial aspects of planting clones vs. seedlings at two stockings (33,333 and 16,667 plants/ha). The primary purpose of the work was to investigate 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 program.

The work was undertaken during 2007 and 2008 while the author was studying for an MSc at SCU. Readers are directed to Prastyono (2009), Prastyono et al. (2009a) and Prastyono et al. (2009b) for full details of the investigation.

Summary of Key Findings

- Plant stocking had a significant effect on the growth and oil traits of tea tree in these trials. Trees at lower stocking (wider spacing) typically gave 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 were superior in commercial oil traits to seedlings grown from improved seed (ATTIA 2B) from the breeding program. Oil concentration of clones in this trial averaged 91.6mg/g on an oven dry weight (ODW) basis and 86.7mg/g compared with seedlings that averaged 63.6mg/g ODW and 55.8mg/g 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. The performance of clones in this trial, however, was deemed to be unrepresentative because of extraneous factors such as the poor survival and growth of clones due to J-rooting.

- Consistency in 1,8-cineole content was a feature of each clone compared with greater variability amongst seedling stock.

- 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 oil yields in excess of 500kg/ha might be obtained from plantations established using three best clones (8 were compared) at a stocking of 33,333 plants/ha. This is substantially greater than the mature oil yields recorded for improved seedlings (ATTIA 2B) in a breeding program yield trial (357kg/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 while this is not achieved until the third year when deploying seedlings.

- Financial analyses to evaluate the viability of replanting 20-ha tea tree plantations using elite clones and improved seedlings over 15 years 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 these analyses, as all plantation options involve replacement plantations.

- The financial analyses showed that, at an oil price of $45/kg which was current at the time of the study, 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 were $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 $11.30/kg, $15.50/kg, $10.40/kg and $12.50/kg for plantation options 1, 2, 3 and 4 respectively.

Conclusions

This investigation indicated that clonal plantations established at a stocking of 33,333 plants/ha had the potential to give a much higher profit over 15 years compared with plantations established with the best improved seed (ATTIA 2B) available at the time of the study . 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, long-term clonal plantations on a range of sites where tea tree is grown commercially is highly recommended before launching into large scale clonal plantations.

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4.5.3 Progress in Development of an Economic Selection Index for Australian Tea Tree

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Introduction

Growing tea tree in commercial plantations for oil production is costly but can give high profits and good internal rates of return. The main drivers of profitability of the production system are leaf biomass, leaf oil concentration and oil quality (Baker *et al.*, 2007, Prastyono 2009). Therefore, significant gains in profitability could be made using combined index selection for biomass and leaf oil concentration, with a restriction to maintain oil quality at acceptable levels. Such an index should be based on trait economic weights derived through formal bio-economic modelling and genetic parameters obtained from previous studies.

The objectives of this study were to: (1) modify recently developed bio-economic model of tea tree oil production system in Australia so it can be used for evaluating economic importance of breeding objective traits, (2) estimate economic weights for breeding objective traits based on the bio-economic modelling, (3) compile the estimates of genetic parameters including heritability and genetic and phenotypic correlations for tea tree, and (4) to derive an economic selection index so as to maximise the profitability of the tea tree oil production system.

Materials and Methods

Restricted Selection Index

A selection index (I) is a linear function of phenotypic (measured) values for n tree traits (p_i), each of which is weighted by a coefficient (b_i), such that the index value relates the phenotype to the genotypic worth of that tree. The genotypic worth (H) is composed of m breeding values (g_j) weighted by their relative economic values (w_j) per unit change:

$$I = b_1 p_1 + b_2 p_2 \ldots b_n p_n$$

$$H = w_1 g_1 + w_2 g_2 + w_m g_m$$

The vector of index weights (*b*) is obtained as partial regression coefficients of genotypic worth on phenotypic values (Cotterill and Dean 1990):

$$\boldsymbol{b} = \boldsymbol{P}^{-1}\boldsymbol{G}\boldsymbol{w}$$

where ***P*** is the matrix of phenotypic variances and co-variances among selection traits; ***G*** is the genetic variance-covariance matrix between selection and objective traits; and ***w*** is the vector of economic weights for the objective traits.

While the above index results in the largest response in a linear combination of characters, often we wish the largest possible response in some linear combinations of characters while ensuring that another set of characters remains unchanged. This is done by constructing a restricted (or constrained) index. A general analytical solution to this problem was first developed by Kempthorne and Nordskog (1959). Restriction of response in oil quality traits (i.e. 1,8-cineole% and terpinen-4-ol%) while maximising response in objective traits (i.e. leaf biomass and leaf oil concentration was achieved using the “Generalized Reduced Gradient” nonlinear optimization (Fylstra *et al.* 1998) implemented in the Microsoft Excel Solver®.

Expected genetic gains or mean response in breeding objective traits expressed per standardised selection differential (*i*) can be calculated as:

$$\Delta G = i \frac{Gb}{\sqrt{b' Pb}}$$

Genetic Parameters

Heritability and genetic and phenotypic correlations for *M. alternifolia* as estimated from previous projects and related progeny trials are summarised in Table 1. Leaf biomass was moderately heritable (0.250) as estimated by Butcher *et al.* (1996). Heritability estimates for oil content (0.917), oil quality (0.513 for 1,8-cineole%, 0.431 for terpinen-4-ol%), showed strong, while height (0.115) and leafiness (0.113) showed weak heritability (Doran *et al*., 2002). Estimates of correlations between growth traits, such as tree height and leafiness, and oil concentration and quality traits were low. Furthermore, oil concentration and growth traits were negatively correlated, indicating that selection for growth traits may adversely (but weakly) affect oil concentration. Correlations of leaf biomass with oil quality were weak but favourable, suggesting that they could be improved simultaneously in a breeding program. Height and leafiness scores were positively correlated with leaf biomass, suggesting potential for their use as selection traits.

Table 1. Heritability and genetic and phenotypic correlations for *Melaleuca alternifolia*. Genetic correlations are presented below the diagonal and phenotypic correlations above the diagonal

Trait	Heritability	Leaf biomass	Oil conc.	Height	Leafiness	1,8-cineole	Terpinen-4-ol
Leaf biomass	0.250[a]	1	0.068[c]	0.742[c]	0.776[c]	-0.03[a]	0.1
Oil conc.	0.917[b]	0.1	1	-0.1[c]	-0.1[c]	0.03[a]	-0.03
Height	0.115[b]	0.6[a]	-0.1	1	0.619[c]	-0.05	0.05
Leafiness	0.113[b]	0.9[a]	-0.1	0.5[a]	1	0	0
1,8-cineole	0.513[b]	-0.1	0.15[b]	-0.05	0	1	-0.5
Terpinen-4-ol	0.431[b]	0.1[a]	-0.14[b]	0.05	0	-0.66[b]	1

[a] Butcher *et al*. (1996); [b] Doran *et al*. (2002); [c] Williams (pers comm. 2009).

Economic weight derivation

An economic weight is formally defined as the expected change in overall profitability of an enterprise as a result of a unit increase in a given breeding-objective trait (Cotterill and Dean 1990). Economic weights for breeding-objective traits reflect how the improvement in those traits impacts on the overall profitability of an enterprise. In tea tree, economic weights for breeding-objective traits have not been previously formally derived. A bio-economic model that provides a framework for simultaneously considering production management and breeding decisions has been developed recently (Prastyono 2009). The bio-economic model was modified to include effects of leaf biomass and oil yield on production system components and on overall profitability of the tea tree production system and was used for economic weight derivation.

The bio-economic model considered in this study was of a commercial M. alternifolia plantation established using seedlings grown from improved seed from the RIRDC/Australian Tea Tree Industry Association clonal seed orchard - ATTIA 2B (Baker et al. 2007). The seedlings were planted at a 30cm within-row spacing and 1m between-row spacing (33,333 trees/ha), which is a common and widely accepted production system. Production system parameters used for modelling the plantation established using seedlings from a breeding program are shown in Table 2.

Table 2 Parameters used for modelling a commercial tea tree plantation established using selected seedlings grown from improved seed from a breeding program.

Parameter	Plantation options
Plant price ($/unit)	0.12
Number of plants/ha	33,333
Cost of plants/ha ($/ha)	4,000
Other plantation establishment cost ($/ha)	1,847
Total plantation establishment cost ($/ha)	5,847
Operating costs in year 1 ($/ha)	1,439
Operating costs in year 2 onward ($/ha)	4,228
Proportion of mature yield in year 1	0.77
Proportion of mature yield in year 2	0.88
Oil yield in year 1 (kg/ha)	283.8
Oil yield in year 2 (kg/ha)	326.7
Mature yield in year 3 onward (kg/ha)	369.6
Spent leaf production/ha (m^3)	67.5
Farm-gate price ($/kg)	45
Spent leaf price ($/$m^3$)	21
Life of tea tree (yrs)	15
Area of plantation (ha)	20
Discount rate (%)	7%

Plantation establishment costs covered only costs needed for replanting using the seedling planting material. The establishment and operating costs were determined from an average practice in establishing tea tree plantations using seedlings. Oil yield of seedlings (ATTIA 2B) used in this economic analysis was taken from the 2002 yield trial (Baker *et al.*, 2007). Proportions of mature yield in year 1 and year 2 of seedlings were taken from the 2002 yield trial. It was assumed that survival rate of seedlings will be at the 99% level throughout the life of the plantations. Replanting the whole 20ha of plantation was assumed to be carried out in the first year. Oil price during September of 2008 (A$45) was used in the baseline model (Prastyono 2009). Spent leaf production of tea tree plantation was determined from information provided by growers.

Costs and incomes occurred at different times in the production system, and a discounted cash flow analysis was used to adjust differences in the timing of costs and returns. It was assumed that the cash flow occurs at the end of each year. The overall profitability of a plantation grower and an integrated enterprise was measured by two economic indicators: net present value (NPV) and the internal rate of return (IRR). Present value (PV) was calculated as:

$$PV = V \Big/ \left(1 + \frac{d}{100}\right)^{(t_V)}$$

where PV is the value at planting year of a cost or an income V incurred t_V years after planting, and d is the discount rate. Net present value was calculated as the difference between the total present-value income (I_{PV}) and total present value costs (C_{PV}) as

$$NPV = I_{PV} - C_{PV}$$

The IRR was calculated as the break-even discount rate or the discount rate at which the NPV equals zero (Klemperer 1996). In this study, a base discount rate of 7% was used. Because there is no universally accepted discount rate, the NSW Treasury (2007) recommended use of a set of real discount rates of 4%, 7% and 10% to see if the outcomes are sensitive to such variations.

The baseline scenario of commercial tea tree plantation was initially modelled utilising the baseline production parameters presented in Table 1. Subsequently a production system modification (i.e. 10% trait improvement in leaf biomass or oil content) was applied and the effect on production system parameters was evaluated. To compare the effects of trait change on profitability, both NPV and IRR were calculated. Generally, a selection option was preferable when its NPV and IRR are higher than other options. However, in some cases, the NPV and IRR decision-rules can have conflicting results because of the changes in the ranking of the projects. Perkins (1994) and Campbell and Brown (2003) suggested that the decision-rule for mutually exclusive projects, such as this, is to accept the project with the highest NPV.

Results and Discussion

Replanting a tea tree plantation using selected seedlings was, as expected, highly profitable (Table 3). The NPV at 7% discount rate over a 15-year time frame of a 20-hectare plantation was $2,347,029 and IRR was very high at 217%, for a seedling plantation at a stocking of 33,333 plants/ha. The expected increase in NPV after a 10% increase in leaf biomass was $304,348 ($15,217/ha) or 5.7%. The expected increase in NPV after a 10% increase in oil content was $392,374 ($14,619/ha) or 5.6%. The expected change in plantation profitability as a result of a unit increase in leaf biomass and oil concentration were NPV $2.196/kg and $205.9/mg/g, respectively.

Table 3 Baseline economic parameters: present value benefit (PVB), present value cost (PVC) and net present value (NPV) for a 20-hectare commercial tea tree plantation based on seedlings at a stocking of 33,333 plants/ha and economic weights for changes to leaf biomass and oil concentration.

Economic parameters	Base Values	After 10% improvement in Leaf Biomass (kg/ha)	After 10% improvement in Oil Concentration (mg/g)
PVB ($A/ha)	159,097	175,007	173,716
PVC ($A/ha)	41,746	42,438	41,746
NPV Profit ($A/ha)	117,351	132,569	131,970
IRR (%)	217	242	240
Economic Weights (NPV $A/(unit)		2.196	205.9

Using optimal Smith-Hazel selection index at selection intensity of 1.76 standard deviation above current population mean (i.e. approximately 10% best trees), the response in the breeding objective (i.e. leaf biomass and oil concentration) and early selection traits (i.e. oil conc., height, leafiness, 1,8-cineole% and terpinen-4-ol%) is presented in Table 4. Selecting the best 10% trees based on Smith-Hazel's index resulted in positive response in the objective traits of leaf biomass (32.0kg/ha, or 0.6%) and oil concentration (4.7mg/g or 5.5%), and a positive response in all selection traits (i.e. oil conc., height, leafiness, and 1,8-cineole%) except for terpinen-4-ol%. The positive response in 1,8-cineole% and negative response in terpinen-4-ol% where both adverse responses (i.e. there is an established industry threshold of acceptability of <4%% for 1,8-cineole% and >36% for terpinen-4-ol%. The use of a restricted selection index resulted in zero change in either 1,8-cineole% or terpinen-4-ol%, but at an opportunity cost especially in oil content (i.e. 4.7mg/g vs. approximately 3.9mg/g). However, even after this adverse response the two oil characteristics were well below (i.e. 1,8-cineole @ 1.6%) or above (i.e. terpinen-4-ol @ 39.3%) the required threshold. Gibson and Kennedy (1990) cautioned that the use of restricted index comes at such a cost in terms of decreased response in merit relative to a Smith-Hazel index that they should only be used in highly specialized situations.

Table 4 Optimal selection index coefficients and expected genetic responses in breeding objective and early selection traits (percent response is given in brackets).

Trait	Means	Standard Deviation	Opt. Index Coefficients	Genetic Resp. Opt. Index	Genetic Resp. Rest. Cineole	Genetic Resp. Rest. T-4-ol
Leaf Biomass (kg/ha)	5000	250	-	32.0 (0.6)	62.2 (1.2)	61.1 (1.2)
Oil Conc. (mg/g)	71	3.0	-	4.70 (5.5)	3.88 (4.6)	3.90 (5.5)
Juv. Oil Conc. (mg/g)	61	2.6	195.1	0.55 (0.7)	0.36 (0.4)	0.37 (0.4)
Height (cm)	114	5.13	9.72	0.14 (0.1)	0.65 (0.6)	0.65 (0.6)
Leafiness (1-5 score)	3.6	0.67	117.0	0.30 (8.4)	1.20 (33.2)	1.16 (32.2)
Cineole (%)	1.4	1.77	15.53	0.16 (11.1)	0.00 (0.0)	0.01 (0.5)
Terpinen-4-ol (%)	39.5	1.55	-12.23	-0.15 (-0.4)	0.00 (0.0)	0.00 (0.0)

Further work will be done on sensitivity of economic weights and genetic response to varying key production variables such as discount rate and farm-gate oil price. Linearity and independence of economic weights for the two breeding objective traits of leaf biomass and oil concentration will also be examined.

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4.5.4 Using Gene Information to Improve Selection Efficiency for Oils in Breeding Programs of *Melaleuca alternifolia*

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The RIRDC/ATTIA tea tree breeding project has collaborated with ANU in two projects aiming to identify genes governing terpene synthesis in *M. alternifolia*. This information will be used to develop better selection strategies for oil types as well as oil concentration. Brief summaries of the two projects are given below.

Gene diagnostic tests for oil chemotypes

In *M. alternifolia*, three major compounds determine the established chemotypes: terpinen-4-ol, 1,8-cineole and terpinolene. In the chemotypes that are dominated by only one of these three compounds, different monoterpene synthases appear in the foliar RNA pool. In chemotype 5, the major monoterpene synthase is responsible for the biosynthesis of cineole, α-pinene, limonene and α-terpineol. In chemotype 2, the major monoterpene synthase synthesizes terpinolene, while in the desired chemotype 1, we have a sabinene hydrate synthase. This corresponds to measurements of in-vivo non enzymatic rearrangements in chemotype 1 tea tree leaf, whereby the sabinene hydrate of young leaf gradually rearranges into terpinen-4-ol and α- and γ-terpinene. The gene encoding for sabinene hydrate synthase can only be found in the genome of individuals showing significant amounts of terpinen-4-ol in their leaf oil. As few as 17 non-synonymous mutations separate the cineole and sabinene hydrate synthase genes. These findings have allowed us to design diagnostic probes that can distinguish between the different chemotypes reliably.

Genes associated with leaf oil concentration

This work is presently in progress. In the first approach the aim is to test whether variation in upstream genes in oil pathways is associated with variation in oil concentration. If DNA sequence variants (SNPs) are associated with oil concentration then the SNPs can be used in selection screens to increase oil concentration within breeding material.

A baseline field trial consisting of 200 open-pollinated families from natural populations was established at Teven near Ballina in the 1990's within the RIRDC/ATTIA breeding program of *M. alternifolia*. These families consisted predominately of the terpinen-4-ol chemotype. In April 2009 this trial was sampled for leaf material for both oil analysis and for DNA. Within replicate four of this trial two individuals per family were sampled. DNA was extracted from leaf samples with a Qiagen Dneasy 96 plant kit. Oil profiles and oil concentrations are currently being determined at Wollongbar by Gary Baker.

The genes DXR, DXS, IPPI, MVK and GPPS were isolated and sequenced in *M. alternifolia* using the homologues from *Eucalyptus globulus*. SNP genotypes at variable sites in these genes will be assayed for the individuals from the 200 families. Statistical tests will be carried out to test whether any SNPs are associated with variation in oil concentration.

A second approach is to test whether gene expression levels are responsible for differences in oil concentrations between breeding lines within *M. alternifolia*. The aim is to have a set of samples with a wide range in oil concentrations so that variation in gene expression in the five genes can be compared with variation in oil concentrations. To do this, leaves from 79 trees were collected and immediately frozen in liquid nitrogen and then stored at -80^o C. Twenty nine of these were sampled from known high oil concentration lines in seed orchards at Wollongbar. The other 50 trees were from the Teven trial (see above).

RNA will be extracted from the 79 trees and expression levels determined by direct PCR techniques. The expressions levels will be compared with oil concentrations to find if oil concentrations are significantly correlated with enzyme amounts. It is expected that these studies will be completed by October 2009.

4.5.5 A Quality Management System for the Breeding Project

During this current phase of the project, the breeding team has worked towards full implementation of a Quality Management System (ISO 9001) to cover its chemical analysis and research activities. ISO 9001 certification was obtained in January 2009.

The quality objectives of the Breeding Project are:

- To identify clients and stakeholders and to understand and meet their needs.
- To attract external funding for the continuation of research and development of the tea tree industry in accordance with the current NSW DII Corporate Plan.
- To develop, use and document procedures and methods that demonstrate the technical competence of the team.
- To manage equipment inventory, checks, servicing and calibrations.
- Provide appropriate training and development opportunities for staff to ensure high levels of competency and performance.
- To disseminate research findings to clients and stakeholders through scientific publications, written reports and industry seminars.

The breeding project has several clients and stakeholders which include producers, funding bodies (RIRDC, ATTIA), industry associations (ATTIA) and other research institutes (SCU, ANU). Tea tree growers require progressively improved seed to increase yields. The project develops this seed and informs ATTIA of the amounts available. ATTIA tenders the release and the project processes allotments as needed.

The breeding project is funded by industry (ATTIA) and government (RIRDC and NSW DII) funds. RIRDC in conjunction with ATTIA requests research providers for project proposals. The funding application is prepared by the research provider (breeding team), approved by the Research Leader and the External Funding Unit Manager (NSW DII) before submission to the funding bodies (RIRDC). Project proposals are competitively evaluated by the institutions and funds allocated to projects they feel offer maximum benefit to their industry. Contracts detailing expected outputs and funding are signed by RIRDC and the External Funding Unit Manager before the project commences. Records of funding applications and project contracts are held by the External Funding Unit.

Five procedures (Biomass, Oil Extraction, Oil Analysis, Sample and Seed Management) document and describe the process for ensuring samples are identified, processed, preserved or disposed of consistently once arriving at the laboratory and that systematic records and reports are made.

The Wollongbar site has procedures in place to back up and protect records stored electronically and to prevent unauthorised access. These include;

- passwords protection
- ‘read/write’ access to designated staff
- storage facility at G drive on the LAN, specifically G:\res\EO\QA\
- NSW DII Information Technology Section backs up the LAN weekly.

The Equipment List identifies laboratory equipment and highlights items that require calibration and checking. The WPII Technical Manager oversees the servicing and maintenance of general laboratory infrastructure and equipment. Maintenance of all laboratory equipment follows the corporate procedure. Items checked by the team are listed on the QA schedule which is updated each year.

To ensure that personnel are competent in performing specific tasks relating to procedures or equipment an operator competency record has been established. This spreadsheet lists the specific laboratory and other competencies of each team member. Operator competencies are reviewed every 6 to 12 months.

In most cases reports are communicated to industry bodies via the Research Leader (NSW DII) responsible for the project. Face to face meetings also occur with industry, funding bodies, etc. This usually occurs at a local level. Funding Body Research Reports are usually Milestone Reports, Research in Progress Reports or Key Finding Reports. Reporting of results to client organisations usually occurs every 6 months. A final report is submitted when due. Results can also be communicated to stakeholders through public forums, i.e. field days, conferences, reports, Fact Sheets and by scientific publications. Seed orders (letter, email or phone) from ATTIA are recorded and processed by the project in a timely manner. Feedback received is documented and stored electronically in the relevant project file. Communication and feedback is monitored and reviewed annually at the Management Review.

5. Results

A high level of adherence to the project objectives, set at the start of the project in July 2006, has been achieved. A summary of results against objectives follows –

Objective: *Release improved seed to maximise profit and market access for Australian tea tree growers:*

Result: The main objective of the breeding project is to improve oil yield and oil quality by the production and release of improved seed. Improved seed is produced from established seed orchards.

As an interim measure the project released seed to industry from better performing natural seed sources (bush seed), as identified in its trials, to give some increase in yields while the seed orchards matured. Best bush seed, seed from the first generation seedling orchard (SSO1), the first clonal orchard (CSO1) and the second generation orchards (SSO2p and SSO2) are the sources for the five seedlot releases ATTIA 1, ATTIA 2A, ATTIA 2B, ATTIA 3A and ATTIA 3B respectively. Seed from the project has been made available to industry since 1997. Seed sales from 1997 to 2002 totalled 6825g and comprised ATTIA 1, ATTIA 2A and ATTIA 2B seedlots. Once the best seedlot (ATTIA 2B) was identified from the 2002 yield trial, sales only comprised this seedlot. Since 2003 sales of ATTIA 2B have exceeded 1070g. In 2007/08, low seed production in the CSO1 has meant releases also comprise SSO2p seed (ATTIA 3A). It is expected (YT3 – to confirm) that the progeny from SSO2p will perform similar to CSO1 seed. However, progeny from SSO2 are expected to out perform both SSO2p and CSO1 so now SSO2 seed (ATTIA 3B) is preferred.

During 2008/2009, higher oil prices increased new plantings and hence the demand for seed. Sales in 2009 are expected to exceed 1000g.

Objective: *Systematically improve the genetic quality of tea tree seed:*

Result: The development of the second generation seedling seed orchard (SSO2) has been a key feature of the breeding strategy: to give substantial genetic gain; provide highly improved seed for release; and seedlots for the establishment of a third generation seedling seed orchard. The orchard has been thinned in 2002, 2003, 2005, 2007 and 2008 using individual and family selection indices. The remaining (154 selected) trees represent 7.7% of the original 2000 trees. Also the remaining trees are from 70 of the original 100 families. Flowering in the orchard has been good 37% – 99%. The yield trial (YT3) established in 2008 will be assessed at its first harvest in 2009 to compare all 5 releases and thus quantify expected oil gains from SSO2.

In addition to this orchard, a second clonal seed orchard (CSO2) was established at WPII in 2007 from a selection of elite clones. Ongoing development of these two orchards will give substantial genetic gain, provide improved seed for future releases and seedlots for the establishment of a third generation seedling seed orchard.

Objective: *Release elite clones to growers to maximise profit and market access:*

Result: The use of clones in the project is a complementary strategy to the seed orchard program and offers the quick capture of genetic gain and a uniform plant. This project and other work have shown *M. alternifolia* to be a species amenable to mass vegetative propagation by stem cuttings (Doran *et al.* 2002).

An objective of the project was to examine the potential of deploying elite tea tree clones to growers by demonstrating that clones are an economically viable proposition (i.e. the enhanced income generated through the increased yields and uniform oil qualities of clones more than compensates for the increased establishment costs of the clones). This has been achieved by selection and testing a suite (20 genotypes) of elite clones from the breeding program. The selection of two batches of 10 clones from PT1/CC1 and PT2/CC2/YT2 respectively, involved screening for survival, coppice regrowth (height and leafiness) and oil characteristics (oil concentration and oil quality). The tests, undertaken on both batches, included nursery rooting abilities, trials to determine optimum planting densities for clones and yield comparisons with highly improved seedlots from the breeding program.

From the 20 clones tested, four clones from the 2006 clonal trials have been identified as very high yielding (>500kg/ha at first harvest). While four clones from the 2004 clonal trials have been identified with high oil concentrations (>75mg/g averaged over three harvests).

Although elite clones have been identified and recent economic analyses (Prastyono 2009) show an economic advantage by using clones the industry is still reluctant to use them. One of the main reasons why clones are not preferred to seedlings is that the rooted cuttings of *M. alternifolia* are three to four times more expensive to produce than seedlings thus significantly increasing establishment costs. Another constraint to their deployment is the risk of a much larger financial loss if plantation establishment fails (e.g. through floods).

For industry to make an informed decision about the use of clones more evidence on the economic benefits of clones is needed. New trials will help secure this information.

As seed is being progressively improved, so the potential for selecting a better clone improves. The establishment of SSO3 and its associated progeny trials planned for the new project offers the potential for better clones.

Objective: *Assess existing trials and establish new trials to quantify genetic gains from improved seed and clones:*

Result: The success of the tea tree breeding project can be measured by comparing the performance of released project seedlots and selected clones with alternative seed sources in genetic gain trials (yield trials). The estimates of genetic improvement provided by these large-plot trials show the progress of improvement delivered by the project and the increased financial returns possible through using the improved varieties. The breeding strategy predicted a 60% increase in oil yields by conclusion of the first generation of breeding, about year 2003. Greater gains (80 – 100%) are expected by the conclusion of the second generation of breeding.

Two yield trials were established (2002 and 2008) to estimate gains in yield from use of breeding project seedlots and another two yield trials were established (2004 and 2006) to estimate gains in yield from use of breeding project clones. In summary the results from the 2002 yield trial were: ATTIA 1 (bush seed of best provenance), since first released in 1997, has averaged a 39% yield increase (average of five harvests) over industry standards; ATTIA 2A (from a 45% flowering in SSO1), released in 2000, has averaged a 33% yield increase; and ATTIA 2B (CSO1), also first released in 2000, has averaged a 69% increase in oil yield over industry standards. All this was achieved in parallel with modest improvements in oil characteristics affecting marketability (i.e. relative increases in terpinen-4-ol and decreases in 1,8-cineole). Releases of ATTIA 2B after 2005 are expected to outperform that from the yield trial as the genetic quality of the orchard was improved after a thinning in May 2003.

All five seed releases will be assessed in the 2008 yield trial from 2009.

The results of the 2002 yield trial demonstrate that the project has delivered to industry what was promised in project proposals since the 1993 commencement of breeding, if by a slightly different route (i.e. CSO1 the premier source of seed rather than SSO1) than was initially envisaged. Oil yield from project seed has been progressively increased from 148kg/ha (industry average) at the beginning of the breeding program to c. 250kg/ha. Gains of 69% have been achieved in a little more than a decade of selection and breeding.

In summary the results of the two clonal yield trials (2004 and 2006) were: as physiologically mature plants, clones demonstrated a significantly higher oil concentration (54% and 30% for the 2004 and 2006 clonal trials respectively) at the first harvest. By the second harvest, oil concentration for seedlings increased and the relative advantage of the clones was reduced. By the third harvest, the oil concentration for seedlings had again increased and to the level of the clones. The implication for growers is that clones would return higher yields earlier than seedlings. For the 2006 trial, the oil yield for clones at the first harvest was 518kg/ha while for seedlings only 168kg/ha. These clonal yields are exceptional, however the 300% higher yield over seedlings will diminish over time. As both 2004 and 2006 clonal plantings have now been lost to floods, new trials will be needed to track longer-term changes.

Economic analyses (Prastyono 2009) have shown that, at an oil price of $45 per kg, plantations of clones or improved seedlings are both highly profitable with clones predicted to give the greatest profit at any of the oil prices tested. The break-even prices for tea tree oil production, using the production parameters in his model were $11.30/kg for clones and $15.50/kg for improved seedlings.

Objective: *Undertake controlled crosses to enhance rate-of-gain in breeding and cloning:*

Result: The project has undertaken conventional controlled pollination techniques, since 1996. The controlled crosses produced previously (1997, 98 and 99 flowering seasons) are described in detail in Doran *et al*. (2002). The crosses were best × best unrelated individual tree selections in SSO1. The seedlots produced were used in the establishment of SSO2 and in the clonal program with the establishment of CC1and CC2.

The controlled crosses produced in the 2001 to 2005 flowering seasons are described in Baker *et al*. (2007). Their purpose was mainly to produce disconnected half diallels involving the crossing of five parents in each of four years (2001, 2002, 2003 and 2005). Seedlots produced were to be used in the estimation of additive and non-additive genetic variances and co-variances. Unfortunately, the resulting seedlings were lost to a weekend breakdown of the nursery watering system at WPII during June 2006. Seed in excess to this study is still available to the breeding program.

The purpose of the controlled crosses in 2007 was to produce an elite population to be used in the establishment of the third generation seed orchard by crossing eight best × best unrelated individual tree selections in SSO2.

Objective: *Undertake research on allergens – superior genotypes to be assessed for potential allergens (limonene and linalool) to enhance oil marketability:*

Result: As part of the oil assessment for any tree in the breeding project, the two components limonene and linalool have been measured since 2006. Generally, values of limonene range from 0.4 to 1.5% (mean 0.8%) of total oil and linalool from 0.1 to 0.6% (mean 0.2%) of total oil. At these levels the two components represent a very small amount of the total oil.

Both limonene and linalool have been measured for each tree in all project orchards. This has been undertaken so that higher range limonene and linalool trees could be removed, reducing the potential for higher limonene and linalool values in the progeny of that orchard.

Objective: *Undertake research on insect tolerance – clones screened for insect tolerance:*

Result: As part of the growth assessment for any tree in the breeding project, insect damage has been measured both in terms of a direct score for insect leaf damage and indirectly by measuring plant height and scoring for total leafiness. Where a score for insect damage tends to indicate the presence of insects, differences in height and leafiness can indicate the relative yield reduction through the reduction of leaf.

Generally, insect damage in all project progeny and yield trials has been minimal. This is because the trials are located in commercial plantations and co-operators control insects regularly as part of normal plantation practice.

Insect tolerant plants express a large leafiness score which is a selection criteria for both clones and orchard trees. A high level of insect control in field trials reduces the effectiveness to select for insect tolerance. It is proposed as part of the new project to select for quick maturing leaves to increase insect tolerance.

6. Implications

Australian tea tree oil producers, with the cyclical nature of oil prices and growing international competition, need to be innovative with a focus on improving efficiency of production if they are to remain economically viable in the long term. Presently, Australian growers provide 85% of world production of this oil. However, the local industry currently faces the risk of significant overseas competition developing as the oil price improves. Increasing on-farm profitability through the use of higher yielding varieties through genetic improvement is one proven path to higher efficiencies of production and this was recognized in this industry in 1993 when RIRDC and ATTIA funded the first of four (1993/96, 96/01, 01/06 and 06/09) consecutive tea tree breeding projects.

The breeding project has progressively increased oil yields from 148kg/ha prior to the start of breeding to c.250kg/ha. This is equivalent to 70% improvement achieved in less than a decade of selection and breeding. Industry adoption of the project's improved seed would increase average yields by 70% and at $45/kg this could mean an annual $14M windfall to Australian growers.

Since 1997, the project has sold 6.7kg of best bush-selected seed and 2.9kg of improved seed. Increases in average yields for selected and improved seed are 40% and 70% respectively. This resultant yield increase means an annual income increase of $2.6M and $1.9M to growers of selected and improved seed respectively. On the basis of seed sales, current plantings and applying a wholesale value output multiplier of 4, the benefits to the Australian economy are in excess of $18M annually.

The project has established the genetic resources to further increase the level of gains and profits for Australian growers through the development of the second generation seed orchard. Seedlots (ATTIA 3B) already released to industry during 2009 from this orchard are predicted to give much greater than 80% improvement in off-paddock yields compared with typical yields at the start of tree improvement activities.

Even greater gains will be available when the third generation SSOs come into production possibly as early as 2012. Third generation seedling seed orchards with supporting progeny and yield trials will be established and brought into production during the new tea tree breeding project (2009/14).

The project has developed a suite of elite clones for deployment to growers as a complementary strategy to the release of improved seed. Yields from clonal trials in 2007 (first harvest) were shown experimentally to be 300% higher than the best available improved seed (ATTIA 2B) and, although subject to higher risk factors, to be a better option economically than improved seedlings. Prastyono (2009) recently modelled the financial returns of tea tree plantations deploying either (a) improved seed (ATTIA 2B) or (b) elite clones. He found that plantations of elite clones were 51% more profitable than improved seed as represented by CSO1 (ATTIA2B), but cautioned that this was based on early data from one trial. He recommended the geographically wider and longer term testing of clones before large scale adoption.

The major benefit from project germplasm (seed and clones) is to maximize profit and market access for the Australian tea tree oil producer. Increasing productivity of high quality oil through the use of improved seed and clones is a most cost effective way to maximize grower profits and market access. Competition for market share is strong both nationally and internationally and with high oil prices competition will increase.

7. Recommendations

Recommendations for industry

- The use of improved seed

Australian producers of essential oil of *Melaleuca alternifolia* (Australian tea tree oil), in the face of the cyclical nature of their industry and growing international competition, need to be innovative with a focus on improving efficiency of production if they are to remain economically viable in the long term. Use of higher yielding varieties through genetic improvement is one proven path to higher efficiencies of production. Oil yields have been progressively increased from 148kg/ha which was the industry average at the start of breeding to c. 250kg/ha as a result of this project. The cost of seed is a minor component to establishing a plantation yet its genetic quality is a major determinant of both oil yield and quality. Use of improved seed maximizes profits and market access.

Further development of existing orchards and the establishment of SSO3 will ensure the progressive genetic improvement of future seed and its use by industry will increase the economic long-term viability and competitiveness of Australian producers.

- The use of clones

A study comparing the financial performance of clonal vs improved seedling plantations at different stockings was undertaken by SCU MSc student, Prastyono (Prastyono 2009) to assist the tea tree breeding project in an economic evaluation of clonal oil production. In brief, the recommendation (based on a first harvest and with the caveat that clones warrant more widespread and longer term testing) was to use clones at 33,333 plants/ha to give the greatest profit at any of the oil prices tested, followed by use of improved seedlings (ATTIA 2B) at the same stocking rate. The break-even prices for tea tree oil production, using his model were $11.30/kg of oil for clones and $15.50 for improved seedlings.

Recommendations for stakeholders

- Continued support of the project

Tree improvement is invariably a long-term activity and the genetic improvement of *M. alternifolia* for oil production is no exception. Tea tree breeding commenced in 1993 and a series of RIRDC/ATTIA projects has been releasing seed to growers since 1997. To curtail these activities at this point will risk the substantial past investment made in support of breeding activities and will contribute to a declining Australian production as local growers lose orders to countries where labour and costs are cheaper. For this project to continue the progressive improvement of germplasm, adequate levels of financial and human resources are required. The main project team members who commenced breeding activities in 1993 are still involved in this project. This corporate experience and knowledge will continue to contribute to the ongoing success of the project. The project offers tea tree growers' seed that will increase oil yields by 69%. Even greater yield increases will be progressively delivered to growers.

Recommendations for the breeding project

Recommendations on the activities of the breeding project have come from three studies undertaken during the course of the project 2006/09, NSW DII policy, a RIRDC recommendation, grower feedback and an ATTIA board recommendation.

- Climatic site for seed orchards

A study of the reproductive biology of *M. alternifolia* undertaken by ANU PhD student, Liliana Baskorowati (Baskorowati 2008) assisted the tea tree breeding project in its seed orchard management and controlled pollination activities. A key finding was that tea tree seed orchards should be sited where winter temperatures are low, with minima below 5ºC. Earlier and more intense flowering under these conditions will shorten generation times and increase seed yields. Reliable spring rainfall, or applied irrigation, are recommended at such sites to ensure that a high proportion of flowers develop into capsules.

- Developing an economic selection index

A study of an economic selection index for Australian tea tree was undertaken by Milos Ivković and Guojun Zhang (2009) to provide the tea tree breeding project with a selection index, including economic weightings for selected breeding traits, to maximise the profitability of oil production. The study found that replanting a tea tree plantation using improved seedlings was highly profitable. The net present value (NPV) at 7% discount rate over 15 years for a 20ha plantation was $2,347,029 and internal rate of return (IRR) was very high at 217%. The expected increase in NPV after a 10% increase in leaf biomass was $304,348 ($15,217/ha) or 5.7%. The expected increase in NPV after a 10% increase in oil content was $392,374 ($14,619/ha) or 5.6%. The expected change in plantation profitability as a result of a unit increase in leaf biomass (i.e. kg) and oil concentration (i.e. mg/g) were NPV $2.196/kg and $205.9/mg/g, respectively.

This study is on-going and further work on the independence of economic weights for the two breeding objective traits of leaf biomass and oil concentration is recommended by Ivković and Zhang.

- Using gene information to improve selection efficiency

A study of the genes associated with leaf oil concentration was initiated by Gavin Moran of ANU (Section 4.5.4 of this report) aimed at providing the tea tree breeding project with a gene diagnostic test for screening leaf oil concentration.

This study is in progress and will test if DNA sequence variants (SNPs) and/or gene expression levels are markers for differences in leaf oil concentration. These studies are to be completed by October 2009 and if gene information is successful and cost effective it is to be recommended as a selection technique for the breeding project.

- A Quality Management System

A Quality Management System (ISO 9001) for the tea tree breeding project has been implemented to cover its chemical analysis and research activities. The quality objectives of the breeding project are to meet the needs of clients and stakeholders and to develop, use and document procedures that demonstrate technical competence. It is recommended that the quality management system be reviewed regularly and enhanced with on-going training to ensure personnel competency and performance.

- Report on the management of project personnel succession

To reduce the possible impacts of personnel succession within the project, the project team has worked towards and achieved full ISO 9001 certification for its laboratory and research activities. An implication of certification is that all sample and data management procedures are now fully documented. Another proposed initiative to better ensure the long term security of project data is to obtain a tree improvement data base program such as the Oil Mallee Data Base (OMDaT) developed by Richard Mazanec. A review of these initiatives to reduce risks to the project from personnel succession is to be submitted to RIRDC by 2012

- Communication

The project has conducted 14 trials on 7 growers’ properties. Communication with growers during establishment and maintenance of these trials is paramount to the success of the trials. Such trials also demonstrate the success of the project to growers. Maintaining good communication between the project and industry is a major reason for the ongoing success of the project. To facilitate the two way flow of information between and within the project, formal and informal opportunities are organized. Within the project, fortnightly meetings of the tea tree breeding project team are held at WPII while communication with the breeding advisor (John Doran) is on a day-by-day basis via emails and the phone. Annual breeding committee meetings are held between stakeholders to discuss project activities, and the importance of these meetings to the progress of the project cannot be overstated. At the WPII biennial Tea Tree Symposium, project results and other industry research issues are presented to growers. Project aims and achievements are also regularly communicated through local media outlets, field days and formal publications. Grower feedback and recommendations for the project have been largely very positive to date reflecting, in part, the success of a good communication strategy.

- Research on frost and insects

It has been recommended by the ATTIA board that the new project (2009/14) include research on frost and insect tolerance. This has been agreed. In terms of insect tolerance, several tea tree families will be screened for susceptibility to grazing by pyrgo beetle. In terms of frost tolerance, families will be grown at frost prone sites and screened for frost damage.

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Harvesting tea tree plantation. Photo: Pat Bolster

Improved Tea Tree Varieties for a Competitive Market

by G. Baker, J. Doran, E. Williams and T. Olesen

RIRDC Pub. No. 10/188

The Australian tea tree oil industry has become an important regional industry in Australia with a farm-gate value in excess of $21M. Around 90% of the product is exported each year. Fluctuating prices and growing international competition are drivers for producers to use higher yielding varieties in order to remain economically viable in the long term.

The tea tree breeding project commenced in 1993 and has released progressively improved seed to the industry that has increased oil yield from 148kg/ha prior to the start of breeding to about 250kg/ha. Seed releases since 2005 are expected to outperform this 69% gain as the genetic quality of all seed orchards have been progressively improved.

Tree breeding is a long term investment. Continued support from all project clients and stakeholders will ensure that growers can maximise oil production and profit through use of improved seed and clones and thus enhance the long-term viability of the Australian tea tree oil industry.

This project was funded from industry revenue which is matched by funds provided by the Australian Government.

This report, an addition to RIRDC's diverse range of over 2,000 research publications, forms part of our Tea Tree Oil Program, which aims to to support the continued development of an environmentally sustainable and profitable Australian tea tree oil industry that has established international leadership in marketing, in value-adding, and in product reliability and production.

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