

Super Clone "88 " *Melaleuca alternifolia* - what is its value ?

by Tony Burfield and Sylla Sheppard-Hanger

General Introduction

The term "Tea Tree" includes species of the genus *Leptospermum* and *Melaleuca*, the latter covering more than 200 species (Byres 1986) of the family *Myrtaceae*. The most common and economically important member of this group is the Australian Tea Tree, *Melaleuca alternifolia* (Maid. and Bet.) Cheel (syn. *M. linariifolia* var. *alternifolia* Maid. and Bet.). Its native habitat is restricted to the swamps and water-courses of the subtropical coastal region of New South Wales in Australia between 32° and 27°S, growing best in high-rainfall areas, or low rain-fall areas with irrigation. It is a small tree 5-7m, naturally forming impenetrable thickets, and being coppiced easily. As one travels from South to North of its natural habitat, so the leaf oil composition changes, being 1,8-cineole rich in the South, and high terpinen-4-ol, low 1,8-cineole in the North. Leaf oil content can range from 0.5 to 3.0%, but yield from traditional design water-distillation is 1%, unless more modern still design is used. In the last few years the tree has also been successfully cultured in more diverse locations such as Zimbabwe, Kenya, Vietnam, India, Guatemala and China, among others.

In this paper we will focus on the attempts to develop the potential of a specific cloned tea tree plant, a project undertaken by the Oil Fields Ltd. with financial help from Australian Tea Tree Management Limited. The aims for this project were:

- to produce fast growing tea tree plants, which could also grow in colder climates (were frost resistant and therefore increase universal saleability potential),
- to increase oil yields to at least 200 Kg/hectare/annum with a target plant density of 25,000 trees/hectare, and
- to produce high terpineol-4-ol and low 1,8-cineole oils (see below).

These particular oils would go beyond the 1996 ISO 4730 specification requirements (which defines compositional criteria in terms of minimum and maximum limits for fourteen oil components) , eventually leading to a situation where buyers would determine targets of 39.5% minimum terpinen-4-ol and 1,8-cineole of less than 3%, with a low para-cymene content. Much Oilfields clonal material analyzed at 1,8-cineole levels below 1% and terpinen-4-ol percentages in the high 40's. Eventually however, Oilfields would go into receivership (late 1999), and tea tree oil prices would (temporarily?) fall, which is the current situation as at the time of writing. Following the history of this project therefore has involved some detective work to try and elucidate the exact facts. Hopefully, however, the commercial market for tea tree oil will stabilize, and Australian tea-tree growers will be able to promote and commercialize other native-Australian plant essential oils, to add to their portfolios.

The Tea Tree Industry

The Australian tea tree oil industry provided perhaps some 150 metric tons of product per annum in 1995 (anonymous (1997): 300 metric tons) to an estimated 350 metric tons in 1999, reflecting output which has developed progressively in the past decade. The development trend of existing plantations to provide larger supply volumes of oil (which are predicted to extend to 1000 metric tons or beyond within a few years) has caused prices to fall, as sellers to attempt to provide themselves a guarantee of stability by securing sales contracts at reduced selling prices. This problem had been foreseen many years earlier as the potential and popularity of tea tree escalated, but the perceived problems of increased volume adversely affecting prices were predicted to be offset, by envisaging and planning for a demand for a tea tree product, targeted at a specific market. Therefore commercial promotional imaging was developed to sell tea tree oil with a high and broad spectrum antimicrobial activity, and further, to persuade the market into extending its range to include products containing the oil as an active ingredient for therapeutics. This step necessarily required certified plantation practices. Some further selective targeting has subsequently established a market niche for organic tea tree oil, and some smaller companies still can lay claim to the old ways of wild-harvested tea tree oil, the latter promoting the bush-cutter (farming rather than exporting) image. Since tea tree oil has few adverse safety reactions (except as a moderate irritant when applied to the skin at levels over 75%: see Reidl R. 1996), it was felt that in the marketplace these products will be formulated to provide effective levels of treatment for various maladies, without the deleterious side effects associated with some synthetic activities.

History:

With the pharmaceutical market in mind a plant breeding programme was initiated in 1990 to obtain stock of superior trees of *M. alternifolia* and *M. linariifolia* for increased production and oil quality. This was initiated by screening some 250 trees in natural stands and selecting those which might should desirable characteristics such as increased leaf oil yield, vigor of growth (as measured in biomass per unit of time), and superior oil quality (as measured by the index of high of terpinen-4-ol content). Increased productivity in biomass/hectare and comparative growth rates were also carefully monitored. Ease of seedling propagation or clones was also a weighting factor. An initial choice of twenty-five clones were selected for planting, in the area of Port Macquarie district of NSW and in Far North Queensland. It was predicted that the development of these superior plants would realize oil yields in excess of 400 kg/hectare/annum, as well as giving high activity oil with terpinen-4-ol content over 40%, thereby producing an extra valuable oil compared with existing commercial grade tea tree oils.

So the initiating step for the selection and breeding program was to identify trees in natural stands which produced oils with high terpin-4-ol (>40%). These trees

tend to have low 1,8-cineole (<2%). The reasoning behind the commercial propagation of seedlings from the same source (the clonal approach) was the hope that the composition of the oil would be mainly influenced by genetic factors. Thus extrinsic factors such as soil, climate, irrigation, etc may well affect the growth history after the seedlings have been propagated into mature trees, but should have little effect on the composition of the oil (the nature/nurture controversy within an arboreal context!). The productivity of a particular clone was measured by actual growth in trial plots. Factors determined vigor of growth, ratio of leaf to stalk and oil content, and ability to reproduce readily by vegetative means. Since oil yield varies with the time of year, comparisons had to be made with material sampled at identical times. After 5 years of selection and evaluation, one particular clone was chosen for commercial production, which was designated No.88. It was highly productive, had a vigorous rate of growth, a good ratio of leaf to stalk and high percent of oil. This oil contained a terpineol-4-ol level of 42-45% and 1,8-cineole content of 0.5% to 1%. Williams (1995) notes that 200,000 clones per month were to be planted at Masterton Park in Queensland as part of "The Oil Fields Project". (By the time Oilfields went into receivership in 1999, 22 million trees were in the ground.) The site at Atherton Tableland in Far North Queensland was to provide the combination of a mild climate and adequate irrigation in Far North Queensland, which would afford optimal conditions for growth. As expected the first harvest that took place in late 1998 provided commercial quantities of the oil from No 88 so other studies were able to be conducted with this oil.

Had the company not gone into receivership, the long-term sustainability of the clonal crop is less clear, however. Jarmyn (1999) has subsequently remarked that, in his opinion, the clones lacked sustainable root structure, and during an experimental transfer onto Good Oil Group's land 90% of imported plants died back during trials.

Chemical Components

Interestingly, tea tree oil is an example of oil where human intervention has led to a change in marketing policy with respect to chemical make-up of certain commercially offered oils. Lassak in 1991 showed that high 1,8-cineole, moderate terpinen-4-ol Tea tree oils as well as low 1,8-cineole high terpinen-4-ol oils could be produced from endemic tea tree plants. Later Williams *et al.* (1988) reported *M. alternifolia* oils could be found with a range of 0-64%, generally the 1,8-cineole content was in the range 6-15% . However given that the price of tea tree oil was greater than eucalyptus oil (which then was a mere 6\$ US/Kg), the oils was prone to adulteration by eucalyptus oil or other 1,8-cineole sources by unscrupulous dealers and importers further down the selling chain. 1,8-cineole was also portrayed by some workers as an undesirable characteristic of the oil, making it more irritant, and more toxic (this was subsequently challenged and disproven: see below). Some semi-hysterical orchestration lead to a marketing policy of low 1,8-cineole types, which as we have seen elsewhere in this paper, coincidentally could be shown by certain studies to be more efficient against

certain micro-organisms. Southwell *et al.* (1996) eventually argued against the fact that high terpinen-4-ol oils were superior anti-microbiological agents than those with higher levels of 1,8-cineole, or that 1,8-cineole acted as an irritant in these oils.

The Australian Standard for tea tree oil (AS 2782: 1985) required that the 1,8-cineole level should be below 15% and the terpinen-4-ol content should be above 30%. ISO 4730 set a standard of proportional requirements of compositions as follows:

| Australian Standard | | |
|----------------------------|--------------|--------------|
| component | % min | % max |
| α -pinene | 1.0 | 6.0 |
| Sabinene | Tr. | 3.5 |
| α -terpinene | 5.0 | 13.0 |
| limonene | 0.5 | 4.0 |
| p-cymene | 0.5 | 12.0 |
| 1,8-cineole | None | 28.0 |
| γ -terpinene | 10.0 | 28.0 |
| terpinolene | 1.5 | 28.0 |
| terpinen-4-ol | 30.0 | none |
| α -terpineol | 1.5 | 8.0 |
| Aromadendrene | Tr. | 7.0 |
| δ -cadinene | Tr. | 8.0 |
| globulol | Tr. | 3.0 |
| viridiflorol | Tr. | 1.5 |

Kawakami (1990) has already published analysis figures on eight tea tree oils produced by cloning showing large differences in composition. This might be considered slightly surprising since Willams L.R. (1997) had argued that natural variability factors had at least been reduced by cloning (see above), and that consistent oil composition was certainly the aim of the tea tree industry. However the individual development of the plant and the exact conditions of distillation may cause much influence in practice leading to some oil variability. Cornwell (1995) for example has shown the main components of tea tree oil (α -terpinene, γ -terpinene, p-cymene and terpinen-4-ol) are formed from pathways which involve cis-sabinene hydrate, and that terpinen-4-ol is at least formed in part as an artefact during distillation, by acidic conditions acting on sabinene hydrate. This would suggest that distillation conditions might be more important than hitherto thought.

Many companies now operated wholly mechanized harvesting and utilize heavy foliage harvesters to fill mobile bins with leaves, which are towed to the mobile distillation rig. Bins can be fitted with collecting pipes and condensers, and steam played underneath the grills holding the leaves. The bin contents (up to 2 tons)

can be stripped of oil in less than an hour, and distillation can be carried out on a 24 hour basis.

We also know that p-cymene can be formed from the action of light and oxidation on α -phellandrene and γ -terpinene. As selection has resulted in marketing of high terpinen-4-ol oils, so 1,8-cineole levels have come down. Note that total monoterpene hydrocarbon levels are relatively high in tea tree oil, as the combined α -terpinene and γ -terpinene levels alone can constitute over 30% of the total composition. For the holistic aromatherapists among us, it is of interest to note that Williams L.R. and Home V.N (1988) note that the major part of the sesquiterpene content is contained in the final 10% of the oil, which requires as much time and energy to collect as the first 90%. In the above paper he discusses whether this content should be left in the biomass, but suggests it is dependent on further investigation of cost and biocidal (or other) properties. Of further interest to the holistic practitioners may be the possible "sanitization" or cleaning up of tea tree oils. A statement on Main Camp's website owns up to using the technology to selectively remove detrimental components to yield consistent quality oil with a mild aroma.¹

Brophy (1989) has shown tea tree oil to be composed of over 100 components. Analysis of a 1999 batch of oil produced by Oilfields Ltd. by the author using GC/MS with some additional pre-preparative flash chromatography showed the following composition:

¹ <http://www.maincamp.com.au/austandardgrade.html>

| Tea Tree analysis: | |
|--------------------|--------------------------------|
| percent | Constituent |
| tr | α -thujene |
| 3.67% | α -pinene |
| 0.60% | β -pinene |
| 0.37% | Sabinene |
| 0.26% | α -phellandrene |
| 1.14% | Myrcene |
| 0.27% | α -terpinene |
| 0.46% | Limonene |
| 0.30% | β -phellandrene |
| 3.21% | 1,8-cineole |
| 21.08% | γ -terpinene |
| 2.90% | p-cymene |
| 3.29% | Terpinolene |
| tr. | <i>cis</i> -3-hexenyl acetate |
| 0.14% | Cyclohexanone |
| 0.12% | α -p-dimethyl styrene |
| 0.06% | <i>cis</i> -sabinene hydrate |
| 0.11% | α -gurjunene |
| tr | α -cubebene |
| 0.09% | Linalol |
| 0.44% | <i>tr</i> -p-menthen-2-ol |
| 0.20% | β -caryophyllene |
| 1.14% | Aromadendrene |
| 44.10% | terpinen-4-ol |
| tr | Allo-aromadendrene |
| 0.11% | Viridiflorene (= ledene) |
| 0.21% | <i>tr</i> -piperitol |
| tr | α -humulene |
| 3.07% | α -terpineol |
| | <i>cis</i> - β -guaiaene |
| 0.09% | β -elemene |
| | α -cedrene? |
| tr | <i>cis</i> -calamnene |
| 0.071% | p-cymen-8-ol |
| 0.04% | δ -cadinene |
| 0.03% | Spathulenol |
| 0.02% | Globulol |
| 0.03% | viridiflorol |
| tr | <i>tr</i> -cadinol |

Comments: The results carry few surprises, terpinen-4-ol concentrations are in excess of 40%, 1,8-cineole is low at 2.3%, and gamma-terpinene is over 20%. The p-cymene is moderate at 2.90%. The α -terpineol level (3.07%) may be

important for synergistic anti-microbial kill effects, but this is not likely to be joined in effect by linalol which is below 0.1%.

Microbiological Effects and Efficacy

According to earlier work by Williams *et al.* (1993) and others (Penfield and Grant, 1925; Carson and Riley, 1995) it was demonstrated that the component terpinen-4-ol is a significant contributor to the overall antimicrobial activity of the oil, whereas 1,8-cineole had insignificant activity. However, the activity of terpinen-4-ol seemingly varied according to the identity of the microorganism (Williams *et al.* 1993). Table 1.

| Table 1: Zones of inhibition of 1,8-cineole, terpinen-4-ol, and standard tea tree oil | | | |
|--|---------------|-------------|--------------|
| Radius of zone of inhibition in mm from 30 ul of test sample | | | |
| Test micro-organism | Terpinen-4-ol | 1,8-cineole | Tea tree oil |
| <i>Candida albicans</i> | 16.0 | 2.4 | 5.4 |
| <i>Pseudomonas aeruginosa</i> | 2.5 | 0.2 | 2.3 |
| <i>Escherichia coli</i> | 10.0 | 0.8 | 7.0 |
| <i>Staphylococcus epidermidis</i> | 8.0 | 0.0 | 7.0 |
| <i>Staphylococcus aureus</i> | 6.9 | 1.2 | 7.9 |

Thrush: Bioassay results of normal tea tree oil vs. No88 against *Candida albicans* showed comparable values when measuring the radii of the zones of inhibition of tea tree oil with 33% terpin-4-ol (radius 3.5mm) and oil of no 88 with 42% terpinen-4-ol (radius 9.0mm) clear evidence of the higher activity of the selected oil. MIC's of oils containing 1.5% and 20% 1,8-cineole were the same, confirming Southwell *et al.* (1993) statement that 1,8-cineole is not an antagonist. No synergist effect of 1,8-cineole was found however (Southwell *et al.* 1993) and Southwell has commented (Southwell *et al.* 1996) that this may be due to flawed experimental design or technique, where use of an emulsifier or poor oil dispersion through the agar may be to blame. Carson *et al.* (1995) were of the opinion that antifungal activity may be enhanced by 1,8-cineole.

For *Candida albicans*, the apparent activity of (a commercial sample of unknown chiral composition?) terpinen-4-ol was found to be greater than that of the oil. For *Staphylococcus aureus*, terpinen-4-ol was as equally active as the oil, so the author concluded there is obviously some other component in the oil contributing to the activity. (Of course, this reasoning ignores the fact that the activity of terpinen-4-ol may have been maximal at the level attained by oil dosing, and further increase in concentration may have been ineffective).

Later the above view was admitted to be too simplistic (see Chambers N. 1998). Further work by Williams (1996), Southwell (1997), and Markham (1996) had already established that further increasing terpinen-4-ol above a certain value

does not further enhance anti-microbiological effects as expected. Markham (1996) alludes to a possible synergistic effect (without actually using the term) which may be caused by minor components present in tea tree oil such as 1,8-cineole, α -terpineol and linalol, and study of these factors was considered a way forward to improve anti-microbiological efficacy. Previously Southwell (1993) had determined that 1,8-cineole is not an antagonist (i.e. reduces the activity) to the action of terpinen-4-ol against micro-organisms, provided that the concentration of the latter is kept above 30%. Further 1,8-cineole could act as a synergist when the concentration of reached 20-30%. The situation had therefore come full circle, with the original reason for developing high terpinen-4-ol oils now questionable.

Unfortunately however, all the work referred to in this presentation does not take into account chiral purity of the essential oil components which appears to be a common basic oversight by workers in this field. Instead of isolating individual components from the oil (by preparative GC or by fractional/ adiabatic distillation), or matching the chiral purity of individual enantiomers involved, synthetic commercial samples of the component constituents are ordered in from a supplier of organic chemicals, in the misguided belief that these items will do the same job. Impurities in commercial chemicals can easily cause misinterpretations of data in these circumstances, as we have already seen with the alleged toxicity of anethole. Leach (1993) found the following chiral components in tea tree oil: (see Box 1.)

| Box 1 | |
|---|--------|
| Chiral components in tea tree oil: | |
| (-)- α -Pinene | 0.18% |
| (+)- α -Pinene | 1.68% |
| (-)- α -Phellandrene | tr |
| (+)- α -Phellandrene | tr |
| (-)-limonene | tr. |
| (+)-limonene | 0.51% |
| (-)- β -phellandrene | |
| (+)- β -phellandrene | |
| (-)-linalol | tr. |
| (+)-linalol | tr. |
| (+)-terpinen-4-ol | 24.73% |
| (-)-terpinen-4-ol | 13.13% |
| (-)- α -terpineol | 0.69% |

Ravid (1995) found the chiral purity of terpinen-4-ol in tea tree oil to be (+)-(4R)- α -Terpineol 75%: (-)-4S- α -Terpineol 25%. Main Camp Pharmaceutical Grade tea tree oil is quoted as having a range of +/- isomeric ratio of 1.0 –2.4: 1 on their website.

Antimicrobial studies

Once sufficient quantities of the cloned oil were made available, direct comparisons could be made with commercial tea tree oil. The agar plate method was used to compare oil from No 88 with four other test oils or individual components. The usual procedure was adopted, of measuring the relative zones of inhibition against cultured microorganisms implicated in acne (*Propionibacterium acnes*), dandruff (*Pityrosporum ovale*) and tinea (*Trichophyton mentagrophytes*). No reference to strain or collection numbers could be found quoted in the experimental details. Additionally, it must be remembered that there is a difference between activity found in agar plate tests, and efficacy under clinical conditions.

Acne is typically treated with oral or topical retinoids (which may have adverse side effects), or the tetracycline group of antibiotics, which may often lead to GI upsets, vaginal candidiasis and phototoxic problems. Benzyl peroxide has also been used as an active ingredient. Figures in Table 2 show how micro-organisms are susceptible to the higher levels of terpinen-4-ol, with No88 showing a higher level of activity.

Table 2
Comparison of the zones of inhibition of test oils and component

| Component | Zone of inhibition (mm) | | |
|-------------------------|--------------------------------|---------------------------|------------------------------------|
| | <i>Propionibacterium acnes</i> | <i>Pityrosporum ovale</i> | <i>Trichophyton mentagrophytes</i> |
| Terpinen-4-ol | 9.0 | 9.5 | 30.0 |
| No 88* | 6.0 | 7.0 | 24.0 |
| Standard Tea tree oil # | 4.0 | 4.0 | 20.0 |
| Eucalyptus oil | 0.8 | 1.5 | 0.5 |
| 1,8-cineole | 0.4 | 0.0 | 0.4 |

* No 88 – 1,8-cineole 1.0 % and terpinen-4-ol 44%
standard tea tree oil - 1,8-cineole 5.5% and terpinen-4-ol 35.5%

The yeast *Pityrosporum ovale* (*Malassezia furfur*) is considered the pathogen responsible for seborrheic dermatitis and dandruff and it also possibly plays a role in inflammatory dermatitis of the sebaceous areas. Typical treatments include shampoos containing ketoconazole, zinc pyrithione and selenium sulfide. Because this microorganism is susceptible to terpinen-4-ol, tea tree oils with high specific levels of this component should be more active. From the results it would appear reasonable to formulate products with 2-5% of this type of tea tree oil for the prevention of dandruff.

Tinea pedis, or ringworm, is due to an infection of the fungus *Trichophyton mentagrophytes*. Often this condition may be accompanied by candidiasis or other bacterial infections. Typical treatments include Tolnaftate (Tinaderm) as topical ointment, and if severe, with oral antifungals such as Griseofulvin or ketoconazole. Side effects include the possibility of toxicity to the liver toxicity by Ketoconazole in view of the long term needed for treatment (3-5 months). In the table, the activity of No88 was higher than standard oil, and should ultimately provide a more active formulation with a broad spectrum of antimicrobial activity.

MIC's of oils containing 1.5% and 20% 1,8-cineole were the same, confirming Southwell *et al.* (1993) statement that 1,8-cineole is not an antagonist. No synergist effect of 1,8-cineole was found however (Southwell *et al.* 1993) and Southwell has commented (Southwell *et al.* 1996) that this may be due to flawed experimental design or technique, where use of an emulsifier or poor oil

dispersion through the agar may be to blame. Carson *et al.* (1995) were of the opinion that antifungal activity may be enhanced by 1,8-cineole.

The work of Stockley *et al.* (1999) working with *Staphylococcus aureus* gives further insight. Concern over the appearance of multi-resistant strains of this bacterium in many parts of the world have resulted in hopes that tea tree oil might be effective as a control agent for the elimination of this bacterium in sensitive situations (i.e. hospitals etc.).

| Oil type | MIC% | MBC% |
|---------------------|-------------|-------------|
| TTO Clone 88 | 0.25 | 1.0 |
| STTO | 0.25 | 1.0 |
| LSTTO | 0.12 | 0.12 |
| 10%LSTTO + Clone 88 | 0.12 | 0.25 |
| 20% LSTTO Clone 88 | 0.12 | 0.25 |

The above Table 3 shows the MIC (minimum inhibitory concentration) and MBC (minimum bactericidal concentration to kill 99.9% of the organisms) for a range for *Melaleuca* oils against *S. aureus*, using Tween as a surfactant. It can be noted that tea tree oil is not the most active oil, but its combination of broad-spectrum activity and non-irritating action on the skin offer convincing advantages. Several points emerge from this work. This first is that the MIC of tea tree oil is several thousand times less than that of antibiotics. Secondly the kill time is very long: 240 minutes for 99.9% kill in the case of standard tea tree oil. The kill time of LSTTO was found to be 10 minutes: far more impressive. A 20:80 mixture of LSTTO: gave a 60-minute kill time.

Mann *et al.* (1998) critically reviewed the techniques used for determining the comparative anti-microbial properties of tea tree oils. They note that most methods rely on Tween as a dispersant, which underestimates the MIC of tea tree oil. Since producing reliable data in this area is required for registration of the oil as a therapeutic agent, they developed a new micro-dilution method based on the redox dye reazurin using 0.15% w/v agar to stabilize and provide adequate contact between the oil and test micro-organisms.

Insight into the method of kill of tea tree oil was investigated by Gustafson *et al.* (1998) who established that autolysis in exponential growth and stationary phase cells was manifested by loss of electron dense material, coagulation of cell cytoplasm and the formation of extracellular blebs. Differences in response were noted in stationary phase cells, which demonstrated less autolysis and greater

tolerance to the oil. Further work by Cox S.D. *et al.* (1998) on the inhibition or decrease of growth of *E. coli* by tea tree oil has shown inhibition of glucose-dependent respiration and leakage of potassium ions. An explanation of membrane changes which occur in stationary phase cells which make tea tree oil less effective as a membrane-damaging agent is also given by the authors.

Discussion

For the three particular microorganisms of the study, both tea tree oils showed greater antimicrobial activity than 1,8-cineole and eucalyptus oils alone. For all micro-organisms, oil from No 88 was more inhibitory than standard tea tree oil.

While these results may appear to put clone No 88 in a good light, other results dilute the significance of the findings. Overall we have the picture that tea tree oil is a moderately good anti-microbial agent, markedly less effective than antibiotics, and less effective than many other essential oils. STTO's kill time borders on the unacceptable. Its' odor is not pleasant to most, although not outwardly objectionable. Its' lackluster action against *S. aureus* lead to Stockley *et al.* to look at synergistic relationships with other oils, especially *Leptospermum petersonii*.

One area of future study could be in the geriatric population, since the above mentioned conditions are frequent and spread rapidly in this population sector. Since this population has fragile skin, many common medications are inappropriate. The above-mentioned benefits of tea tree oil are appropriate therefore to this sector.

New oils: *Melaleuca ericifolia* Smith ("Rosalina oil") from northern NSW was mentioned by Daniel Penoel in his book *Natural Home Health Care Using Essential Oils*, has a linalol content of some 48% (range 35-55%), and a 1,8-cineole content of 23% (range 18-26%), α - pinene to 10% and aromadendrene to 4%. As might be expected it is believed to be mildly anti-infectious and expectorant. Its pleasant odor is expected to be more universally-appealing than tea tree medical-spicy earthy character. Its suggested topical uses include application for acne, boils, tinea and herpes. Microbiological support data is required to fully evaluate the properties of this oil, together with full safety testing.

Leptospermum petersonii Bailey (lemon tea tree) is also of potential anti-microbial interest. Already mentioned in the work of Stockley above, it has an aldehyde content of 70-85% comprised of largely of citral with some citronellal. Its pleasant citral-lemon odor confer an easy acceptability in use, however it has, at the time of writing, no supporting formal safety testing. Its potential skin irritancy and instability problems, due to high levels of aldehydes have lead some workers to considering using a synergistic blend of it, with standard tea tree oil. Further work is needed to optimize broad-spectrum microbiological activity of these blends, provide toxicological data, and to determine efficacy against target

organisms. A synergistic blending approach of tea tree oils however, may be the way forward for the tea tree industry.

Conclusion:

In conclusion, the prospects for Tea tree oil have changed remarkably in the last decade with the establishment of a large market which has not enjoyed recent stability as production volumes have increased. In addition, the demand for high terpin-4-ol oils have bred-out those synergistic compounds which could have enhanced tea tree oil's ultimate microbiological potential. Attempts to back-track on this policy by blending in other oils with tea tree which contain synergistic compounds such as linalol and alpha-terpineol, are still to prove their economic effectiveness & appeal in the market-place.

Clone 88 is part of the story for this drive for high terpinen-4-ol oils. As a plant, allegations of the weakness of its' root structure are as yet undocumented. In other ways it seems to have been a successful development experiment: initial development and yields had been satisfactory, and only the quirks of the marketplace had lead to the decline of the company that pioneered the development. The future at the time of writing (winter '99) is less certain for the tea tree industry, but clonal development is probably here to stay, mirroring experiences in other essential oil marketing areas i.e., for vetiver and peppermint. We have not heard the last, we are sure, of this model of plant development.

REFERENCES

Anonymous (1997) "Tea Tree Oil – Universal Agent" SPC May 1997 pp49-52.

Brophy J.J. *et al.* (1989) "Gas chromatographic quality control of oil of *Melaleuca terpinen-4-ol* type (Australian tea tree)." *J. Agric. Food Chem.* **37**: 1330-5.

Byrnes N.B. (1986) "A revision of *Melaleuca* L. (*Myrtaceae*) in northern and eastern Australia. Part 1, 1, 65-76; Part 2, 2, 131-146; Part 3, 3, 254-273.

Carson C F, Riley T. V. (1995) "Antimicrobial Activity of the major components of the essential oil of *Melaleuca alternifolia*". *Journal of Applied Bacteriology* **78**(3);264-269.

Cornwell C.P., Leach D.N. and Wyllie S.G. (1996) "Incorporation of Oxygen 18 into terpinen-4-ol from the H₂¹⁸O steam distillates of *Melaleuca alternifolia* (tea tree)." *J. Essent. Oil Res.* **7**, 613-620

Cox S.D., Gustafson J.E. Mann C.M., Markham J.L., Liew Y.C., Hartland R.P., Bell H.C., Warmington J.R., Wyllie S.G. "Tea tree causes K⁺ leakage and inhibits respiration in *E. coli*" *Letters in Applied Microbiology* **26**, 355-8.

Chambers N. "Understanding Potentiation – Why more of a good thing doesn't always mean better" a sub-section in : Dean C. "Australian Tea Tree Oil" , paper presented to the Australasian Aromatherapy Conference, Sydney 1998.

Gustafson J.E., Liew Y.C., Chew S., Markham J., Bell H.C., Wyllie S.G and Warmington J.R. "Effects of Tea tree oil on *Escherichia coli*." *Letters in Applied Microbiology* **26**, 194-198.

Jarmyn R. (1999). Private communication.

Kawakami W., Sachs R.M. and Shibamoto T. "Volatile constituents of essential oils obtained from newly developed tea tree (*Melaleuca alternifolia*) clones." *J. Agric. Food Chem.* **38**, 1657-1661 (1990).

Lassak E.V. "Bacterial oils of *Melaleuca*. A chemical re-investigation of the essential oil of *M. alternifolia* Cheel {Fam. *Myrtaceae*)." In Troisieme Symposium sur les substances naturelles d'intérêt biologique de la region de la region Pacifique Asie. Edits., C. Debitus, P. Amade, D. Laurent and J.P. Cosson pp177-181, Noumeu, Nouvelle Caldonie (1991).

Leach D.N., Wyllie S.G., Hall J.G., Kyratzis I. (1993) "Enantiomeric composition of the principal components in the oil of *Melaleuca alternifolia*." *J. Agric. Food. Sci.* **41**, 1627-1632.

Mann C.M. and Markham J.L. "A new method for determining the minimum inhibitory concentration of essential oils" *Journal of Applied Microbiology* 1998, **84**, 538-544.

Markham J. "Anti-microbial effectiveness of Tea Tree Oil" AusTTeam Conference paper (1996).

Penfold A R, Grant R (1925) "The germicidal values of some Australian essential oils and their pure constituents - together with those for some essential oil isolates and synthetics." *Journal of Proceedings of the Royal Society (NSW)* **59**;346-350

Redl R. (1996) "Safety Profile of Tea Tree ...Proceedings of the AusTTeam Conference Sydney Australia.

Southwell I.A., Hayes A.J., Markham J. and Leach D.N. (1993) "The search for optimally bioactive Australian tea tree oil" *Acta Horticulturae* **334**, 256-265.

Southwell I.A., Markham J., and Mann C. (1996) "Is 1,8-cineole detrimental to tea tree oil?" *Perfum. and Flav.* **21** (5) 7-10.

Stockley J.K., Chuan-Han Chan and L.R. Williams (1999) "The anti-microbial activity of cloned tea tree oils" *Cosmetics, Aerosols and Toiletries in Australia* **12**(4) 14-18

Williams L.R., Home V.N. (1988). "Plantations of *Melaleuca alternifolia* – A revitalised Australian Tea Tree Industry". *Search* **19**, 294-7.

Williams, L. R., Home V. N., Lusanzi I (1993) "An evaluation of the contribution of 1,8-cineole and terpinen-4-ol to the overall antimicrobial activity of tea tree oil." *Cosmetics, Aerosols and Toiletries in Australia* **7**(3); 25-28, 34

Williams, L.R. "The selection of superior trees of *Melaleuca* species to Provide Tea Tree Oil for Therapeutic Use" Abstract, IFEAT conference, Seville, 1997