CHAPTER FOUR

THE SELECTION PROCESS

4. 1 Introduction

The oil yield within a Tea Tree stand is highly variable. A single stand of trees can show a variation of up to 5. 5% oil content between high and low yielding trees (Bryant, 1950). The oil composition of the trees is also variable with high cineole, trees growing beside low cineole high terpinene-4-ol trees (Brophy, et al. 1989). The difference in yield and oil composition of Tea Trees can be selected for, and oil producers identify high oil trees when collecting seed for a plantation. This method improves the overall quality of the plantation but does not allow for genetic variability from the newly produced seed. To avoid variability of natural seed, clonal plants can be used. Before clonal plants can be propagated they must be identified as being the most suitable genotype within a population of trees.

To identify the ideal clonal Tea Tree selection criteria and methods of assessing those criteria have to be developed. In this chapter the methods required and problems associated with these assessments are outlined.

4. 2 Seed selection site

The plantation seed was collected from two sites on the eastern side of the Shark Creek Range in the Tyndale region, north eastern NSW. The sites were approximately 2km apart , on either side of the Shark Creek swamp. The locations were: site "I", grid ref 9538IVN228268 and site "II" from grid ref 9538IVN209262 to 211265.

Site "I" was the initial site where seed was collected. Five individual trees were sampled in 1985 and found to have high oil yields, > 3% on wet weight and an oil composition within the Australian Standard. The seed from these trees provided the source material for block B of the trial plantation.

Site "II" was larger than site I covering approximately 14 Ha. From within this site a number of trees were sampled and found to have high oil yields and good quality oil . The seed selection was not from individual trees but from the general population with more than 15 trees providing the seed. The seed from site B was collected in 1987 and was used as the source material for

block A of the trial plantation.

4. 3 Plantation Tree sites

The trial plantation blocks contained 5 year old Tea Tree seedlings. These trees had all been harvested at least once. The last harvest was 18 months prior to the commencement of the selection. Two thousand trees were used for the initial selection in eight 250 tree rows . Five rows were from trial plantation block A and 3 rows from plantation B. The initial selection used a visual assessment of plant height (trees greater than 1. 5m) and leaf bulk (full canopy no gaps between neighbouring trees) as criteria and reduced the population to 600 individuals.

The oil concentration within the plant may change from day to day and even hour to hour (Murtagh and Etherington, 1990). To avoid discrepancies in oil yield over time it was decided that sampling take place over one day with one reference tree being sampled hourly. Oil is not lost from the leaf once it is removed from the plant therefore collection of plants on one day and storage until distillation does not affect the oil yield (Murtagh and Curtis, 1991).

4. 4 Selection Methods

Five hundred, 200g leaf and twig samples were cut from the trees over a five hour period. The material collected consisted of leaves and fine twig (< 2mm) along with a limited amount of 5mm twig. Samples were of the total tree and came from all sides, top and bottom of the canopy. After collection samples were placed in plastic bags and stored in the shade. At the end of the collection period the plant material was laid in a drying tray and placed on an open drying frame (Plate 1 Section 3. 3). The drying frames allowed air circulation around the plant material and were covered with white Solarweave plastic film offering 50% shade (VP Industries Pty Ltd Q. L. D. Australia) (Plate 2 Section 3. 3)

The leaf material remained on the drying racks for 7 days. At the completion of drying the dry leaves were rubbed free from the stems, sieved and the pure leaf stored in paper bags ready for distillation. (Plates. 3 & 4 Section 3. 3)

Three, 10g samples of leaf were weighed; 2 samples were distilled for 1 hour in a modified Clevenger trap still (Section 3. 2) while the third sample was oven dried at 80°C for 24 hours to determine the dry weight (dw). The dry weight was used to calculate the percent oil volume per gram of oven dried leaf (% yield(v) /dw)

Yield results were plotted and trees with a yield % v/dw greater than 5. 5% were classed as very

high oil yield trees and trees above 5. 0% v/dw as high oil trees. Gas chromatography was used to examine these two tree classes. .

Analysis of the oil composition was performed as per (Section 3. 4. 1)

Results were analysed and ranked on the percent terpinene 4-ol and cineole. Plants ranked above the Australian Standard were then used for cloning.

The methods used for cloning are described in chapter 5

4. 5 Results

The oil yield from the trial seedling population showed a normal distribution around a mean of 4. 40% with a standard deviation of 0. 75%. This analysis is of the whole population including trees from both trial block A "Seedling source II" and B "seedling source I"

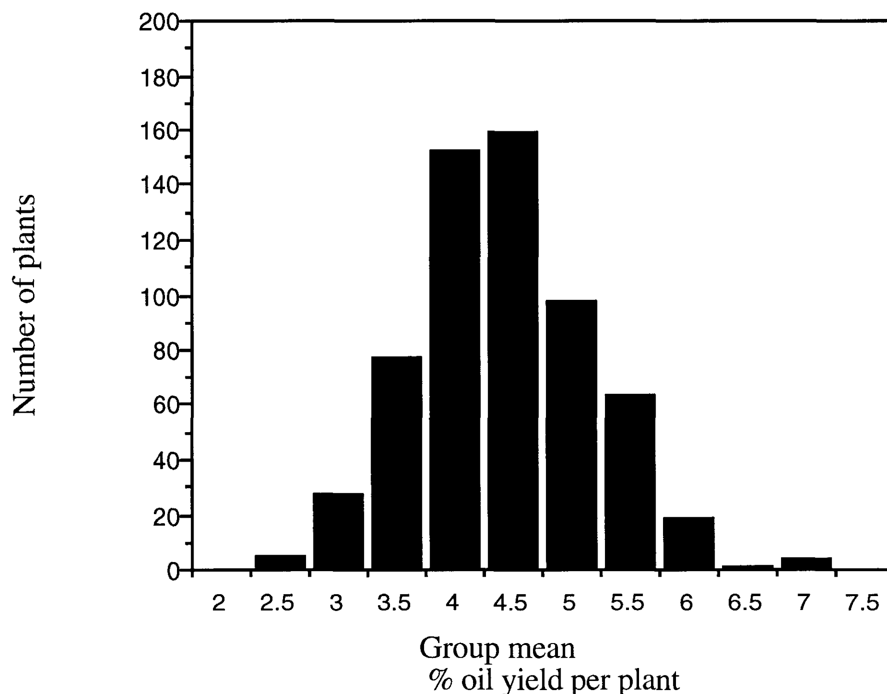

Fig. 7 Oil yield distribution of the seedling population. Plants were normally distributed around a mean of 4. 40% with a standard deviation of 0. 75%.

More samples were taken from block A than Block B owing to the greater number and more uniform age of the trees in block A. Block B had a slightly higher mean than block A although there was no significant difference between the two (Figs. 8 and 9).

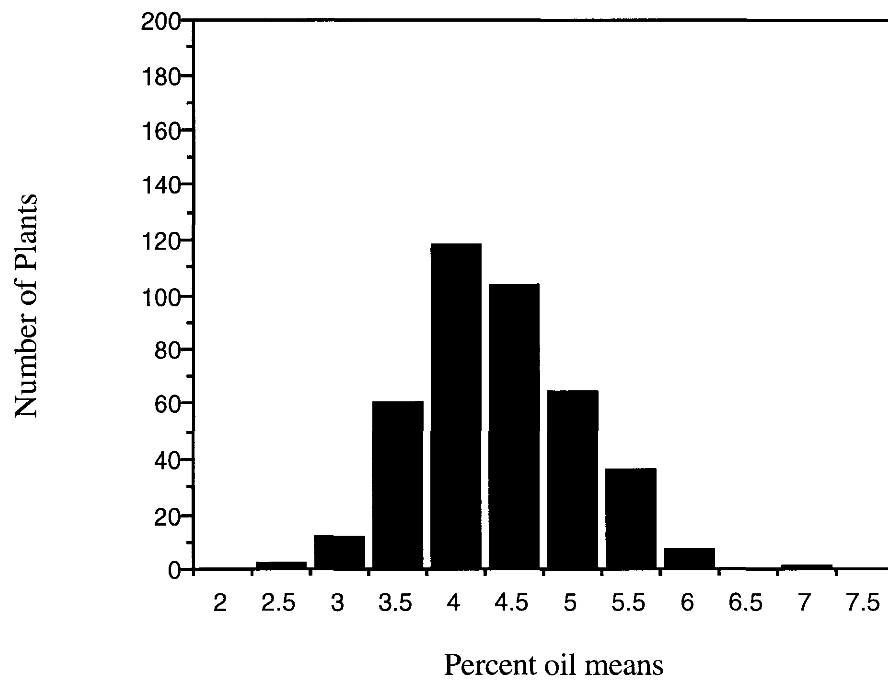

Fig. 8 Oil yield distribution of block A seedling population. Plants were normally distributed around a mean of 4. 34% with a standard deviation of 0. 67%.

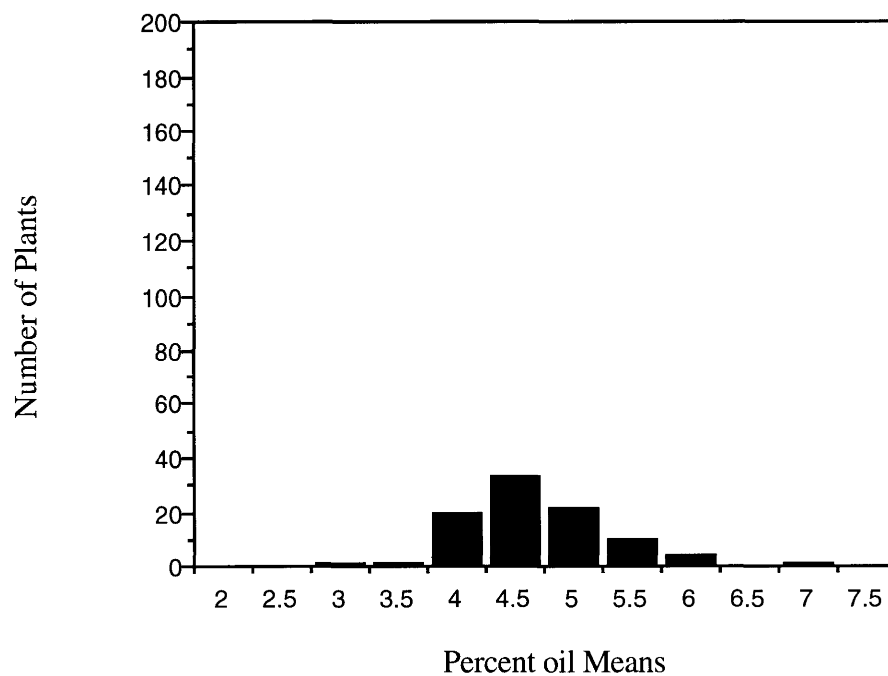

Fig. 9 Oil yield distribution of block B seedling population. Plants were normally distributed around a mean of 4. 65% with a standard deviation of 0. 62%.

From the sample population of 600 trees the 50 trees with the highest yield were analysed for quality. The quality of the trees with the highest yield varied enormously from trees well within the Australian standard 0. 96% cineole and 56. 94% terpinene-4-ol to an extremely high cineole level of 42. 89% and low terpinene-4-ol of 19. 72% (Table 8).

Table 8 The best yielding trees from the sample population with cineole and terpinene-4-ol concentrations.

TREE NO	yield	1,8 cineole	terpinene 4-ol	ranked	cloned
B097	5. 25%	0. 96%	56. 94%	+	
U005	5. 30%	3. 37%	47. 52%	+	
F108	5. 32%	3. 79%	49. 85%	+	
C005	5. 32%	3. 77%	13. 59%	-	
F009	5. 40%	4. 96%	40. 16%	+	
C008	5. 44%	1. 78%	44. 66%	+	
B010	5. 44%	2. 49%	46. 98%	+	
D098	5. 45%	0. 53%	52. 96%	+	
B002	5. 47%	3. 30%	47. 60%	+	
E006	5. 49%	3. 27%	47. 27%	+	
A227	5. 49%	4. 15%	45. 92%	+	
A217	5. 50%	9. 25%	27. 97%	-	
C140	5. 52%	7. 41%	43. 53%	¥	
C001	5. 53%	4. 35%	40. 87%	+	
E165	5. 55%	7. 79%	42. 82%	¥	
C166	5. 55%	5. 66%	43. 68%	+	###
U049	5. 57%	4. 17%	48. 22%	+	
B011	5. 57%	2. 22%	42. 78%	+	###
A187	5. 58%	42. 89%	19. 72%	-	
C004	5. 58%	8. 23%	33. 81%	¥	
F002	5. 59%	3. 27%	41. 93%	+	###
D129	5. 61%	6. 86%	43. 63%	¥	
C006	5. 61%	3. 53%	37. 36%	+	
T009	5. 64%	4. 15%	43. 15%	+	
T117	5. 65%	6. 52%	43. 60%	¥	
E005	5. 66%	5. 87%	39. 93%	¥	
A165	5. 67%	6. 34%	43. 84%	¥	
D108	5. 75%	2. 72%	45. 21%	+	###
C042	5. 75%	8. 34%	42. 36%	¥	
E063	5. 75%	4. 47%	40. 90%	+	###
U053	5. 78%	3. 95%	45. 43%	+	
C001	5. 83%	4. 35%	40. 87%	+	###
T161	5. 83%	4. 06%	46. 64%	+	
E016	5. 87%	2. 03%	39. 67%	+	
U092	5. 88%	5. 73%	40. 67%	+	###
A190	5. 89%	17. 33%	21. 44%	-	
E054	5. 89%	41. 91%	18. 84%	-	
R	5. 90%	3. 10%	40. 20%	+	
A055	5. 92%	4. 79%	43. 22%	+	###
U014	5. 94%	4. 29%	43. 60%	+	
F011	6. 00%	6. 22%	38. 76%	¥	
E001	6. 09%	4. 22%	41. 24%	+	###
B057	6. 22%	7. 21%	46. 91%	¥	
A208	6. 62%	7. 11%	43. 84%	¥	
T024	6. 76%	8. 34%	35. 48%	¥	###
D014	6. 89%	6. 57%	40. 89%	¥	

DELTA	6. 97%	6. 96%	42. 68%	¥	###

(+) Within the Australian Standard and exceptionally high quality

(-) Outside the Australian Standard high level of cineole

(¥) Within the Australian Standard but not exceptionally high quality

4. 6 Discussion

The first selection criterion of plant yield identified 47 high oil trees; of these 28 showed an oil quality which could be classified as exceptionally high having a cineole content of below 5% . 42 trees met the Australian Standard and 5 were of poor quality having an exceptionally high level of cineole.

From this population of 47 high oil trees it can be seen that 10% of the trees were not suitable for commercial Tea Tree oil production based on oil quality. The fact that 10% of this sample produced an oil quality unsuitable for commercial production suggests that plantations established from wild seed populations will contain a proportion of low quality plants. Plantations comprising of clonal plants or selectively bred seed plants would improve the overall quality of plantation oil

The selection process outlined above was successful in identifying trees suitable for cloning and breeding. The plant population from which this selection occurred was only representative of two natural Tea Tree stands in one area. This limited pool of selection material, although yielding a high number of above average trees, is the major short coming of this study. With the selection being limited to two populations within one area only a small genetic base has been examined and the chance of tree loss through genetic disorders is high. Future work could improve this study by repeating the selection on other natural stands within the Tea Tree growing region. Clonal plantations established from the best trees in each region would supply high quality oil while providing genetic diversity, thus improving the plantation's defence against pests and disease.

The selection of high oil Tea Trees described here is an important step in the improvement of Tea Tree plantation productivity and the first step in a breeding strategy to improve the production of Tea Tree oil, industry wide.

CHAPTER FIVE
CLONAL PROPAGATION

5. 1 Introduction

To date clonal plantations of *M. alternifolia* do not exist. It is possible to propagate *M. alternifolia* but success is limited and variable. The first descriptions of *M alternifolia* as an essential oil producing plant were recorded by Penfold et al (1925,1948) and at that time attempts to clonally reproduce the plant were unsuccessful. Since then methods have been developed by Gardiner (1988) and Uebergang (pers comm) but these reports, although claiming to be successful, do not include expected strike rates. The work of Sachs, et al (1990) achieved a strike rate of 70% using 10cm softwood cuttings with a 10 sec dip in 4,000 mg per litre IBA, planted in a 50/50 mixture of vermiculite and perlite, under mist with bottom heat. However, some doubt does exists as to the identification of the stock plants in this experiment (Southwell pers com)

Micro propagation or tissue culture has also been used as a method for mass production of clonal plants. Successful protocols for the production of *M. alternifolia* have been developed by deFossard (1988) and Taji (unpublished) but these are not successful for the propagation of all genotypes (Hartney, pers comm).

Clonal propagation by cuttings is the cheapest and least infrastructure dependent of the propagation methods. Successful cutting propagation requires the addition of hormone to stimulate root production and the correct environmental conditions to maintain plant survival before and after root initiation. Propagation techniques vary between plant genotypes and propagators. The measure of success in plant propagation is dependent on the use of the propagated plant. Domestic and specialty propagators measure success as the ability to clone an individual while commercial propagators require a success rate of 80% for the production to be commercially viable. Tea Tree is planted in high densities, thus if clonal plants were to be used a great many plants would be needed. Therefore the clonal propagation of Tea Trees must be

efficient requiring a success rate of at least 80%.

5. 2 Propagation of selected plants

Three methods of culturing improved plant material exist, these are; selected seed, cuttings or micropropagation.

5. 2. 1 Selected Seed

Selected seed is produced from clonal trees grown in seed orchards. The seed from these trees is on average of higher quality than the original seed collected from natural tree stands. Genetic variation will still exist and assessment of the progeny will be necessary to continually improve the seed quality.

The cost of establishment and maintenance of a seed orchard and breeding program can be extremely high. In New Zealand it is cheaper to produce cuttings than grow forest plants from selected seed (Menzies and Arnott, 1992). The degree of improvement and type of genotypic variation being bred for can vary the cost immensely. The information to date suggests that a breeding program for Tea Trees would not be extremely expensive and for this reason would be one of the best methods with which to improve plant quality (Doran et al 1992).

5. 2. 2 Cutting production

Cutting production would be an essential component of a seed breeding strategy but could also be used as an improvement strategy in its own right and may be cheaper and faster than improved seed. The production of successful Tea Tree cuttings is relatively straight forward provided the propagation infrastructure is available. The major cost in the production of clonal Tea Trees is the maintenance of the stock plant nursery. The most successful Tea Tree cuttings are produced from juvenile regrowth. The use of this material limits the number of cuttings available; each stock plant stump produces a maximum of 50 cuttings twice a year and over harvesting causes stock plant death.

Tea Tree plantations are an intensive form of agriculture with high plant densities. These high plant densities mean large stock plant nurseries would be required to provide sufficient cutting

material to stock the plantations. The efficiency of the stock plants may be improved by increased plant spacing, continual irrigation and fertigation along with shade and polyhouse coverings. Improved health of the stock plants would increase cutting production and survival. Further work in these areas would be required before a cost benefit analysis could determine the risk of cutting production and expense of stock plant maintenance.

5. 2. 3 Micro propagation

The use of micro propagation allows for large numbers of plants to be produced from a limited number of stock plants, all year round. New cultivars can be rapidly multiplied and released long before seed or cuttings. Micro propagation is an expensive form of clonal culture ranging between 2 and 3 times the cost of cutting production (Menzies and Arnott, 1992). With respect to Tea Trees the cost of micro propagation per plant has the potential to be less. Tea Trees have been reported to multiply quickly and require only one medium during production (Hartney, pers com; de Fossard, 1988) During the final stages of production root initiation and acclimatization can be combined with the plantlets planted out as micro cuttings (Hartney et al, 1990). The fewer production steps required reduces the production costs and may make tissue culture a viable means for clonal production of Tea Tree.

5. 2. 4 Seed Production and hardening of Tea Trees prior to field planting

Two main nursery systems are recommended for the growing of Tea Trees. These are open rooted production and containerised (Colton and Murtagh, 1990). Within the industry individual containerised production has become the standard method. Some growers favour open root production but the response of the plants after planting out was poor compared with containerised plants. Open rooted plants could still be a useful source of material but production methods would need to include periodic undercutting and wrenching to condition the plants prior to planting, thus reducing the transplanting stress.

Single cells or plugs are the ideal method for producing plants for plantations, although a number of disadvantages exist. The capital cost of setting up facilities for the production of individual containerised plants is high particularly in harsher climates. With the small

containers rapid moisture loss can occur necessitating expensive watering systems. Fixed watering systems in polyhouses may not provide adequate water cover to the plants. Many containerised plants are held in trays of plastic or styrofoam cells, these containers do not allow horizontal water movement and drying of cells can easily occur. The most suitable watering systems are overhead booms which move over the plants wetting every cell (Menzies and Arnott, 1992). The use of under cell watering trays can avoid the problems of overhead watering but the trays stop the plants from being air pruned and root damage can occur during transplanting. The water trays also increase the humidity around the plant thus increasing the risk of disease.

The disadvantages of single cells aside, plants produced in this manner are of high quality. Individual cell systems maximise seed use, have fewer weed problems, are easier and faster to plant, and provide greater control and flexibility of production (Menzies and Arnott, 1992).

Individual cells would be the most suitable method for the production of clonal Tea Tree plants. The potting medium should have a high moisture holding capacity and be light. A 3:1 mix of peat and vermiculite would be ideal. The difficulty with using such a mix is supplying adequate water. Peat and vermiculite have a good water holding capacity but if the peat is allowed to dry out it is very difficult to re-wet (Menzies and Arnott, 1992) emphasising the need for an efficient watering system.

5. 3 Experiment one: Potting media

5. 3. 1 Introduction

Media type can play an important role in the success of cutting production. The correct balance between water retention and gas exchange must be achieved and different combinations of media can manipulate these components to suit the plant species or genotype.

For Tea Trees a number of media have been successful for different propagators. The aim of this experiment was to evaluate these media and determine the most suitable medium for the plant material.

5. 3. 2 Methods

The media used were a 1:1 mixture of perlite and vermiculite (Sachs et al 1990); 1:1:1 Peat Sand Perlite (Sheather, pers com) and 1:1:1 perlite sand and vermiculite. These media were placed in 5cm diameter pots.

Softwood cuttings were cut to 10 cm and $^2/_3$ of the basal leaves removed prior to a 10 sec dip in 4000ppm IBA hormone solution. 5 cuttings were placed in each pot and the pots were placed under mist with a bottom heat of 30°C. Plant health and root production were measured after four weeks.

5. 3. 3 Results

Table 9 shows the first treatment of 50:50 perlite and vermiculite to be the most successful, producing the highest root initiation and the healthiest plants. Treatments two and three showed signs of over watering with the stem rotting by the third week

Table 9 Effects of media on survival and rooting of *M. alternifolia* cuttings

TREATMENT	Dead	With roots	Healthy without roots
1perlite 1 vermiculite	12	7	23
1peat 1sand 1perlite	34	5	3
1perlite 1sand 1vermiculite	42	0	0

5. 3. 4 Discussion

Treatments two and three have a fine porosity which increases the water holding capacity of the media and reduces the air space within. The result of the increased water holding capacity was plant death caused by stem rot. Over misting is often the cause of stem rot however, a reduction in misting caused dehydration of plant tops and again death.

The perlite and vermiculite (1:1) medium was the most suitable medium for the cutting

production of Tea Trees. The medium has good water holding capacity without affecting the porosity.

The hormone treatment of 4000ppm IBA was not a suitable dip only producing a low rooting percentage.

5. 4 Experiment 2: Root initiating hormones

5. 4. 1 Introduction

From experiment one it can be seen that the high levels of IBA did not promote root development. The poor success of this hormone may be attributed to the high levels used. Sachs et al (1990) used 4000 mg per litre and achieved a strike of 70% but the identification of their stock plant is questionable (Southwell pers comm.).

The age of the plant material may be another reason why root initiation was not successful. The age of a cutting can have a marked effect on the cuttings ability to produce roots. The formation of roots is more successful with juvenile material.

During this experiment a range of hormones were tried to see which individual hormone or hormone combination was most suited to the cutting propagation of *M. alternifolia.* The age of the plant material and cutting type were also examined.

5. 4. 2 Methods

Experiment A

Eight hormone treatments were tried as per Table 10

Table 10 Hormones and hormone concentrations trialed to initiate roots on *M. alternifolia* cuttings

Concentration of each Hormone ppm	Hormone Type	Total Hormone Concentration ppm
500	IBA	500
500	NAA	500
500	IBA and NAA	1000
500	IBA and NOA	1000
500	NOA and NAA	1000
1000	IBA and NAA	2000
1000	IBA and NOA	2000
1000	NOA and NAA	2000

Two age treatments were included, "Juvenile" cuttings taken as new growth from the base of the plant below 50cm and "Mature" cuttings taken from the tip growth of a mature tree above 1m from ground level. Finally two different cutting types were used; 10cm softwood tip cuttings or

tip cuttings with the apex removed.

For uniformity the cuttings were all collected from one tree. The tree, which was branched at ground level, had been pruned to one stem. This stem was then bent down to help stimulate coppicing of the cut stump. Once coppicing had occurred the branch was returned to its natural position, and 4 weeks later the cuttings were collected. 10cm cuttings were collected and two thirds of the leaf material was removed. All hormone treatments were for 10 seconds. The cuttings were planted in a 1:1 mixture of perlite and vermiculite then placed under mist with 30°C bottom heat for 4 weeks. The success of the cuttings was measured by the percent root initiation and the type of roots produced.

Results

Juvenile plant material is clearly the best material to strike cuttings of *M. alternifolia* (Table 10). The assessment of plant hormones shows some interesting trends. The plants with the highest rooting percentage were all dipped in NAA, or combination including NAA. Apex removal was less successful than the entire cuttings. A hormone treatment of 500ppm IBA+ 500ppm NAA appears to have been the most successful treatment producing a greater percentage of fine roots compared with the other treatments.

Table 11 Effects of Hormone Solution on Root Initiation of *M. alternifolia* cuttings

Hormone Treatments	Cutting age	Apex removed	Number of dead plants	Root type		% cuttings rooted	% cuttings with fine roots
				thick	fine		
500 IBA NAA	juvenile	**	5	3	13	**72. 7**	61. 9
500 IBA NAA	juvenile			2	10	**80. 0**	66. 6
500 IBA NAA	Mature			5	7	60. 0	35. 0
1000 IBA NAA	juvenile	**	6	3	3	28. 5	14. 2
1000 IBA NAA	juvenile				7	46. 6	46. 6
1000 IBA NAA	Mature			11	3	63. 6	13. 6
500 IBA	juvenile			9	1	66. 6	6. 6
500 NAA	juvenile			6	6	**80. 0**	40. 0
500 IBA NOA	juvenile			4	5	60. 0	33. 3
500 NAA NOA	juvenile			13		**92. 8**	0. 0
1000 NOA NAA	juvenile			8	2	66. 6	13. 3
1000 NOA IBA	juvenile			4	2	40. 0	13. 3

To determine the most suitable hormone treatment the three most successful treatments were repeated using juvenile intact cuttings (Table 11). Again the results show no difference between the treatments of 500ppm NAA+ 500ppm IBA and 500ppm NOA+ 500ppm NAA.

Table 12 Effect of NAA on Root Initiation of *M. alternifolia* cuttings

Treatment	% rooting
500 NAA 500 NOA	80
500 NAA	63
500 NAA 500 IBA	80

5. 4. 3 Discussion

The results show that for the cutting propagation of *M. alternifolia*, young juvenile material should be used. The cuttings should be 10 cm long, have the apex removed, and receive a 10 sec dip in a hormone solution of either 500ppm NAA+ 500ppm IBA or 500ppm NOA+ 500ppm NAA. These two hormone combinations produce similar results but 500ppm NAA+ 500ppm IBA is cheaper and therefore would be the preferred choice.

The assessment of root type (Table 10) shows the hormone solution containing NOA, although initiating the highest rooting percentage, did not produce the fine fibrous root system considered desirable (Menzies and Arnott, 1992). The production of an evenly spread fibrous root system is considered essential for Tea Trees as a balanced root system prevents the uprooting of trees during mechanical harvest. Plate 5 shows the root formation of a 16 week old Tea Tree cutting. Roots were initiated by the method described and the hormone mixture was 500ppm NAA+ 500ppm IBA.

5. 5 Tissue culture.

Micro propagation or tissue culture has also been used as a method for mass production of clonal plant material. Successful protocols for the production of *M. alternifolia* have been developed by (deFossard, 1988) and Taji, (unpublished) but they have not been successful for the propagation of all genotypes (Hartney pers comm). A new protocol has apparently been developed which successfully cultured those plant genotypes unsuccessful by the de Fossard and Taji methods but the details are not available (Hartney pers comm). This new method is still untested over a range of genotypes and laboratory environments, so may, like the other methods, favour particular genotypes. Until these production methods can be evaluated one would have to assume that the production of *M. alternifolia* by micropropagation is genotypically dependent.

5. 5. 1 Introduction of *M. alternifolia* into tissue culture.

The first stage of micro propagation is the introduction of plant material to culture. This is an important stage in plant micropropagation as the plant must be disinfected without affecting its potential to grow and multiply within the culture environment. In this experiment a range of techniques were assessed for their ability to disinfect the plant material and their effect on the growth and well being of the plant.

5. 5. 2 Methods

Preparation of field plant material

3-4 weeks prior to inoculation the stock plant shoots were cut back to stimulate new growth by encouraging the development of axillary buds. After pruning, fungicide and insecticide were sprayed on these new shoots and they were covered with a glacine bag of the type used by plant breeders for controlled pollination's. The new growth was harvested and treated by the following disinfestation procedures.

Inoculation into tissue culture

The shoots were partially defoliated then placed under running water for one hour . Following this initial removal of surface microbes, the shoots were divided and subjected to the following disinfectant treatments.

(A) Effect of Alcohol,

(1) 70% Ethanol for 60 seconds followed by treatment in sodium hypochlorite (1% w/v) + wetting agent for 20 minutes. (wetting agent 1-2 drops of triton)

(2) 70% Ethanol for 30 seconds rest as in A

(3) 70% Ethanol for 10 seconds rest as in A

(4) No Alcohol only sodium Hypochlorite as in A

All specimens were then rinsed in sterile water

(B) Effect of Sodium hypochlorite concentration; specimens were not dipped in Alcohol

(1) 2% w/v Sodium Hypochlorite for 20 minutes

(2) 3% w/v Sodium Hypochlorite for 20 minutes

(3) 4% w/v Sodium Hypochlorite for 20 minutes

Then rinsed in sterile water

(C) Effect of decreasing concentration of sodium hypochlorite prior to inoculation : Specimens dipped in Alcohol for 10 sec, followed by

(1) 1% Sodium Hypochlorite for 15 minutes, 0. 5% Sodium Hypochlorite for 5 minutes, and finally 0. 1% Sodium Hypochlorite prior to inoculation:

(2) 2% Sodium Hypochlorite for 10 minutes, 1% Sodium Hypochlorite for 10 minutes, and 0. 1% Sodium Hypochlorite prior to inoculation:

(3) 3% Sodium Hypochlorite for 10 minutes, 2% Sodium Hypochlorite for 5 minutes, 1% Sodium Hypochlorite for 5 minutes, and 0. 1% Sodium Hypochlorite prior to inoculation:

(D) Effect of decreasing concentration of sodium hypochlorite prior to inoculation: Specimens not dipped in Alcohol

(1) 1% Sodium Hypochlorite for 15 minutes, 0. 5% Sodium Hypochlorite for 5 minutes, and 0. 1% Sodium Hypochlorite prior to inoculation:

(2) 2% Sodium Hypochlorite for 10 minutes, 1% Sodium Hypochlorite for 10 minutes, and 0. 1% Sodium Hypochlorite prior to inoculation:

(3) 3% Sodium Hypochlorite for 10 minutes, 2% Sodium Hypochlorite for 5 minutes, 1% Sodium Hypochlorite for 5 minutes, and 0. 1% Sodium Hypochlorite prior to inoculation:

5. 5. 3 Results

Table 13 Initiation success of Tea Tree

TREATMENT	SURVIVAL*	COMMENTS
A1	1/10	1plant phenolic
A2	2/10	1 phenolic
A3	3/10	1 phenolic
A4	0/10	
B1	0/10	one plant healthy little infestation
B2	2/10	1 phenolic
B3	2/10	2 phenolic
C1	5/10	5 phenolic most dead after 2 weeks
C2	0/10	
C3	5/10	4 phenolic
D1	**21/25**	**good healthy plants no phenolic**
D2	10/25	low infestation high phenolic
D3	0/25	no infestation all plants dead

* Explants healthy and free of infestation

The results show a range of responses to the severity of the disinfection process. The use of alcohol as a surface steriliser was found to increase phenolic production and impede survival. The chlorine dilution series were the most successful with 1% chlorine for 15 min, 0. 5% chlorine for 5 min and 0. 1% chlorine prior to inoculation being the most suitable for Tea Tree

5. 5. 4 Discussion

The use of a chlorine dilution series disinfected the plant specimen without excess damage to the plant. The high level of initiation is unusual for field plants and could be improved further if cuttings of the explant were taken and maintained in a glasshouse with no overhead watering prior to culture initiation.

5. 6 Multiplication of Tea Trees in culture

Multiplication of plant material is an important step in micro propagation. During this project a range of media and production methods have been trialed (Table 14) to access their multiplication success on the selected Tea Tree plants. Multiplication rates of X3 and X4 over a 4 week period have been achieved but the plant growth rate is low making these protocols unsuitable for commercial production. The multiplication trial results are inconclusive with a high degree of variation present.

Table 14 Multiplication media for the multiplication of shoots on tissue cultured *M. alternifolia.*

Minerals *	**Growth factors***	**Agar g/l**
Medium	zero	8
Medium	zero	6
Medium	zero	4
Medium	low	8
Medium	low	6
Medium	low	4
Medium	medium	8
Medium	medium	6
Medium	medium	4
Medium	high	8
Medium	high	6
Medium	high	4
High	zero	8
High	zero	6
High	zero	4
High	low	8
High	low	6
High	low	4
High	medium	8
High	medium	6
High	medium	4
High	high	8
High	high	6
High	high	4

* de Fossards system of mineral and growth factor concentrations was used see Appendix B

5. 7 Formation of Roots

All explants produced roots in the standard basal media so the formation of rooted plantlets is not difficult in tissue cultured Tea Trees. Once root initiation had occurred the plantlets were easily acclimatised under intermittent mist and reducing shade (Whish, 1992).

5. 8 Tissue cultured Tea Trees

This incomplete development of a tissue culture protocol shows that the shoots can be easily introduced into culture, roots initiated and the plantlets finally acclimatised out of culture. The multiplication stage of the protocol needs further research before the protocol can be used commercially. At this point in time the commercial multiplication of Tea Tree by means of tissue culture is not an option however the above protocol may be used to produce micro cuttings from limited source material and in this way increase the stock plant populations for

standard cutting propagation.

5. 9 Conclusion.

The production of clonal Tea Trees by cuttings would be the cheapest form of production provided adequate healthy stock plant material was available and production facilities designed for high volume clonal production were provided. The use of contract nurseries specialising in cutting production would be one method of reducing the infrastructure costs. Tissue cultured plants could also be used to develop stock plant nurseries over a short time

Tissue culture is a more expensive form of clonal production, yet it may be more suitable to *M. alternifolia* production. The clonal plants produced from cuttings tend to branch very early sending out 2-5 shoots from low down the stem. This branching form is encouraged in older Tea Trees but in nursery plants may prove a problem during field planting. Tissue cultured plants follow the form of seedlings; when potted out the main stem grows straight with little branching. This form is easily handled during field planting, and the straight stems allow the plants to slide easily down the planter shute and stand straight in the cultivation slot. Branched stem plants such as those produced by cuttings (Plate 5) may block the shute causing misses. The low branching stems of the cuttings may also be buried in the planting slot damaging the plants and reducing survival.

The production of tissue cultured plants would have plants ready for production in a much shorter time than from cutting culture. Within 12 months of a suitable micro propagation protocol being tested on a specific cultivar a limited number of plantations could be stocked. The equivalent production by cuttings could take 24-36 months due to first having to establish enough stock plants.

For the production of seed nurseries either method of clonal production would be suitable with the understanding that successful micro propagation techniques could supply a variety of plant cultivars in less time than cutting production but at a greater cost.

Plate 5. A 16 week old high oil yielding Tea Tree (F011) produced by softwood cuttings and featuring a well developed root mass.

CHAPTER SIX

EFFECT OF AIR DRYING LEAF PRIOR TO DISTILLATION

6. 1 Experiment one : effect of drying time on oil yield

6. 1. 1 Introduction

To sample essential oil plants accurately only the plant organ that contains the oil should be tested. With reference to Tea Tree the majority of the oil is found in the leaf (Murtagh , 1988; Doran and Bell, 1991) so distillation of the stem material can only work to bias the results and change the oil to dry matter ratio (Bryant, 1950; Fluck 1960).

Tea Trees are fine leafed, the average leaf size is 1. 5 cm long (Bodkin, 1986) and weight as little as 1 mg (Southwell and Stiff, 1988). The removal of leaves in sufficient number for an adequate distillation is a difficult task and for this reason has been neglected for convenience in Tea Tree research (Murtagh and Curtis, 1991).

Air drying of leaf and twig material prior to distillation enables the leaves to be removed by simply rubbing. This method saves time and avoids the discrepancies caused by the distillation of twig material. Drying the leaf may however, introduce a new source of error. During drying oil can be lost by volatilisation as in the case of nutmeg and sage (Murtagh and Curtis, 1991). Tea Tree is a member of the Myrtaceae family, which typically have sub epidermal oil glands (Fahn, 1979). These may be less vulnerable to oil loss (Murtagh and Curtis, 1991). Some Eucalypts (also members of the Myrtaceae) have been observed to lose no oil over a one month drying period (Bryant, 1950; Zrira and Benjilali, 1991). Tea Trees have shown a similar response with Murtagh and Curtis (1991) reporting no oil loss or chemical change from Tea Tree foliage for up to 13 days after harvest.

In response to the work of Murtagh and Curtis (1991) an experiment was designed to examine leaf drying as a method for distillation sample preparation.

6. 1. 2 Method

Foliage cut from three Tea Trees with approximately 12 months regrowth was treated to a range of drying exposures. 1500 grams (fresh weight) of leaf and fine twig material was removed from each tree, mixed and divided into five groups of approximately 300g. Four of the five groups were then placed on a drying frame under white "Solarweave" covers (VP Industries Pty Ltd Q. L. D. Australia) (Plates 1 and 2 Section 3. 3) providing 50% light reduction, for 1,2,4 or 6 weeks. The fifth treatment was used as a control and extracted within one hour of harvest. After drying the leaf material was separated from the stems by rubbing. Four 10g leaf samples from each treatment were distilled by the method already described (Section 3. 2) and a fifth oven dried for a dry weight comparison. This process was repeated for each of the three individual trees.

6. 1. 3 Results

As with the results of Murtagh and Curtis (1991), no oil loss occurred during the drying phase of the experiment (Fig. 10). In fact there was an increase between the initial sample and the first week. The yield then stabilised for 4 weeks and finally showed a slight decline after six.

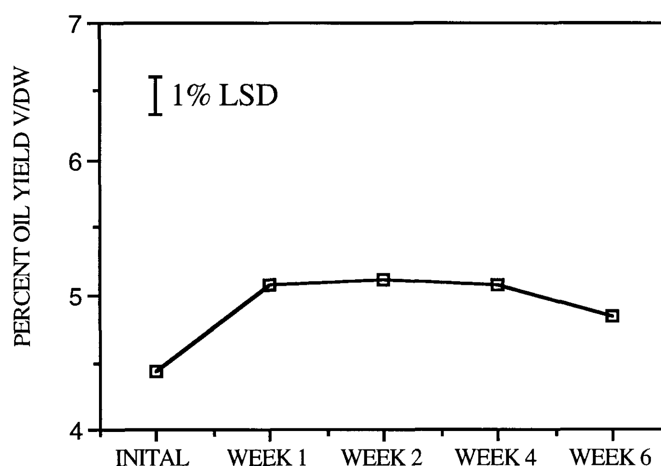

Fig. 10 The effect of pre-distillation drying on oil yield

6. 1. 4 Discussion

The increase in oil from the initial samples to the first week of drying is an unusual result. This increase in oil may have been as a result of continual metabolic activity within the plant or an error in the methodology of the experiment (Murtagh pers com). No replicate samples of the oven drying stage were used in this experiment and the dry weights were taken as single representative samples from weekly material not paired samples of the individual distillations. However, despite this possible error source, a clear trend shows that some change in the oil yield is occurring between the initial harvest time and week 1. The idea of an oil yield increase as a result of air drying prior to distillation is supported by Zrira and Benjilali, (1991) who observed a 0. 66% yield increase when *Eucalyptus camaldulensis* leaves were dried.

6. 2 Experiment two: post harvest metabolism of oil yield in drying plants

6. 2. 1 Introduction

To test the hypothesis that oil yields increase as a response to drying a second experiment was designed. This experiment replicated the treatments and distillations to eliminate error . The aim was also to determine where the oil increase came from. For this reason both unstripped shoots and stripped leaf samples were dried to see if a corresponding oil increase occurred. Other samples of stem and leaf material were sealed in double plastic bags to stop moisture loss during the treatment period. If the increase in yield is as a result of continual post harvest oil metabolism the plants maintained at their original moisture content due to being stored in plastic bags will also have an improved yield. Should the yield of the plants stored in the plastic bags not increased then the increase in yield must be a result of the drying process and not continued metabolism.

6. 2. 2 Methods

Three clonal trees were cut 10cm above ground level on 2nd April 1992. The stems and large branches were separated from the crowns and the remaining material was thoroughly mixed. Forty eight 150g samples were taken and sealed in plastic bags. The bags were mixed and then separated into four treatments of 4 reps, each rep required 3 distillations ie 3 bags of material. Each bag provided one 10g distillation, and a paired 10 g dry weight sample.

The four treatments are described below. The numbers in the treatments refer to the leaf weight in each sample bag; three sample bags =1 rep, 4 reps =1 treatment.

(A). The control (IL): from each bag, 20g of fresh leaf was removed from the fine twigs and branches, 10g of this leaf was immediately distilled while 10g was oven dried.

(B)Stripped and dried leaf (SDL): from each sample bag 20g of fresh leaf was removed from the fine twigs and branches this leaf was placed in plastic weighing trays and left to air dry for 20 days. After air drying 10g was oven dried and 10g distilled.

(C) Leaf dried on the Stem, (LDOS): 150g of leaf and twig was placed in a drying tray and allowed to air dry for 20 days (Same environmental conditions as treatment B (SDL)). After 20 days of drying the leaf was rubbed free from the stems and twigs, sieved and 10 g taken for distillation and 10g oven dried.

(D) Leaf stored in a Bag(LSIB): 150g sample of leaf and twig was sealed in two plastic bags and stored with treatments (B&C) for 20 days. The leaf was then removed from the stems and 10g distilled and 10g oven dried.

The drying of the plant material took place in open trays on the laboratory bench, there was no direct sun light and the maximum temperature was 28°C.
The 10g leaf samples were distilled in a semi-micro hydro still (Section 3. 2). The dry weight was used to calculate the percent oil volume per gram of oven dried leaf (% yield(v) /dw) (Murtagh and Curtis, 1991)

6. 2. 3 Results

A significant difference exists between those plants stripped within one hour of collection (IL & IDL) and those stripped 20 days later (LDOS & LSIB) (Fig. 11). The control distillation (IL) and the distillation of the leaf stripped and dried(IDL) show no significant difference. There is also no significant difference between the leaf dried on the stem (LDOS) and the stem and leaf stored in plastic bags (LSIB).

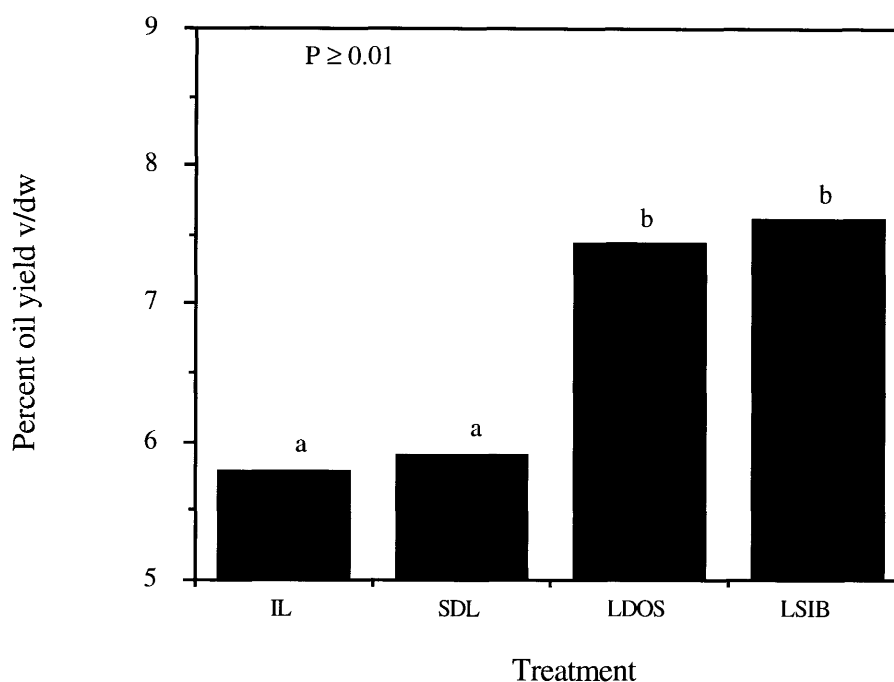

Fig. 11 The effect of post harvest leaf treatment on oil yields

(IL = Initial leaf sample distilled within one hour of collection; SDL = Stripped and Dried leaf, leaf stripped from the stem within one hour of collection and dried for 20 days; LDOS = Leaf dried on the stem for 20 days then stripped and distilled; LSIB Stem and leaf stored in a bag for 20 days then stripped and distilled

6. 2. 4 Discussion

The results show the dried stem and leaf material gave more oil than the dried leaf or the initial fresh leaf sample. Storage of the leaf and stem material in a plastic bag gave a similar yield as drying the stem and leaf material. An explanation of these results may be an interaction between oil production in the leaf and oil movement through the vascular system of the stem. The production of essential oil is considered to be a metabolic process (Murtagh and Etherington, 1991). If this is the case then one can assume that production of the metabolite is continued after harvest. The additional oil is stored in the subcutaneous glands of the leaf. The period of storage without loss is not known but from the results of experiment 1 and the work of Murtagh and Curtis (1991) would be longer than 6 weeks.

The actual cause of the increased oil in the stored leaf material is not known, however, the phenomena of increased oil yields in stored leaf material may be a feature of the Myrtaceae as similar findings to those reported here have been found in *Eucalyptus camdulensis* in Morocco (Zrira and Benjilali, 1991).

6. 3 Conclusion

The effect of drying Tea Tree plant material for the purpose of easy removal of leaves had no detrimental effect on the oil yield. In fact drying leaf on the stem increased the oil yield. This increase is not a result of changing moisture content but appears to be due to an active form of transport from the stem into the leaf. It is not known if the oil is synthesised within the leaves or the stem after harvest or is already in existence and transported from the stem to the leaves during drying.

The value to oil producers of drying and storing leaf prior to distillation is a more efficient distillation system. At present distillation occurs as close as possible to foliage harvest with the aim of reducing any chance of oil volatilisation. The results of the above experiment show that immediate distillation of cut foliage is unnecessary and furthermore a delay would actually improve the yield rather than reduce it. For the industry these results could change the accepted methods of production. To date producers have had to have their own distillation plant as a way of avoiding distillation delays and the need for carting wet leaf material. Since drying leaves on the stems of the tree increases the oil yield, and since the removal of leaves from the stem is more easily accomplished when the leaves are dried, producers need only have storage and leaf stripping facilities. Thus the pure leaf can be easily transferred to a distillery throughout the season.

Further research is required to determine why the increase in yield of stored material occurs and whether manipulation of the post harvest environment can further increase the additional yield.

CHAPTER SEVEN

THE EFFECTS OF NITROGEN AND PHOSPHORUS ON THE PRODUCTION AND QUALITY OF TEA TREE OIL

7. 1 Introduction

No published information has been found on the effects of mineral nutrition on Tea Tree oil production, but evidence from other essential oil crops suggests that nutrition is not only important to promote plant growth but it can also influence the oil quality (Section 2. 24). Current advice to Tea Tree producers is to select fertile soils and ensure the plants have adequate moisture (Colton and Murtagh, 1990).

The harvesting of Tea Tree requires the removal of all the above ground plant. In some cases the distilled leaf is returned to the plantation as a mulch, but in recent years this material has been bought by landscape gardeners and has become a valuable by-product. The harvesting of Tea Tree therefore also removes nutrients from the plantation soil. For sustainable production these nutrients must be replaced, however the effect of fertilizer application on Tea Tree growth and oil production is not known.

The natural habitat of Tea Tree is along the north eastern coast line of New South Wales. The composition and quantity of the plants oil can exhibit a high degree of natural variation even within seedlings propagated from one parent (Section, 4. 5). The genetic variability within current seedling plantations makes it difficult to accurately interpret nutrition data or to draw conclusions from pot trials. The use of clonal cuttings (Section, 5. 3) has reduced this source of error and has enabled nutrient studies within pots under controlled environmental conditions.

7. 2 Methods

7. 2. 1 Clonal plants:

Clonal plants were produced using the cutting propagation methods previously described (Section, 5. 3). These plants were struck in a 64 cell plug tray (Ritegrow Australia) using a 1:1 v/v vermiculite : perlite medium. During root initiation the plants were fed with a dilute nutrient solution (0. 25g. l^{-1} N:P:K, 23:4:18). After a root ball had developed the plants were transferred into the experimental pots.

7. 2. 2 Potting medium

The clonal plants were potted into 15cm diameter plastic pots (Ritegrow Australia) using a UC potting medium (3:1 v/v peat : sand) This medium was adjusted to pH 6 by additions of agricultural lime. Basal nutrients were added and spread evenly through the medium while the medium was being revolved in a cement mixer. The medium was then divided into 16 equal samples and randomly allocated a nutrient treatment. The experiment was a 4x4 factorial (Table 15). Pots were lined with plastic bags to avoid nutrient leaching. After 2 months plant growth the plastic bags were removed and the pots placed in individual saucers to reduce water stress.

Table 15 Nutrient rates applied Kg ha^{-1}

Nitrogen		Phosphorus
13		0
90	X	45
180		90
360		180

Pots were randomly arranged on four benches within a "tropical" glasshouse (30°c day 20°c night). Harvesting occurred 7 months after potting on by cutting the stem off at ground level and placing the stem and leaves in a paper bag. The paper bags were then air dried for 5 days. Dry leaves were removed from the stems by rubbing the stems over a 5 mm sieve, further sieving removed all the stems leaving clean leaf and stem samples.

The dry samples were weighed to give an air dry weight and the leaf samples were distilled.

5g leaf samples were hydro distilled in a semi micro distillation unit (still type 2) for 2 hours . Following distillation a 50μl oil sample was collected, diluted in 1ml of ethanol and analysed using a Varian Star 3400 Gas Chromatograph (Table 16) fitted with an 8200 autosampler and star workstation computerised integrator.

Table 16. G. C. Parameters

Stationary phase column	Econo Cap SE54
Length	30m
Internal diameter	0. 32ml
Film thickness	0. 25μ
Detector	FID
Oven temperatures Initial	50°C hold 3 minutes
Ramp	4°C / minute to 100°C
Hold	100°C for 3 minutes
Ramp	15°C / minute to 200°C
Hold	200°C for 1 minute
Ramp	20°C / minute to 250°C
Hold	1 minute Stop.
Injector temp	150°C
detector temp	265°C
carrier gas	Helium
retention time cineole	10. 88 minutes
retention time terpinene 4-ol	16. 77minutes

All results were analysed by a 2 way analysis of variance using the statistical software package "Neva".

7. 3 Results

The oil concentration in the leaf samples was not affected by increasing nitrogen levels (Fig. 12). Plant dry matter did however show a significant increase from $180kgha^{-1}$ to $360kgha^{-1}$

(Fig. 13). The increase in dry matter production, combined with the unchanged concentration of oil within the plant, as the nitrogen levels increased from 180kgha^{-1} to 360kgha^{-1}, increased the total oil yield per plant (Fig. 13). Nitrogen applications less than 180kgha^{-1} had no affect.

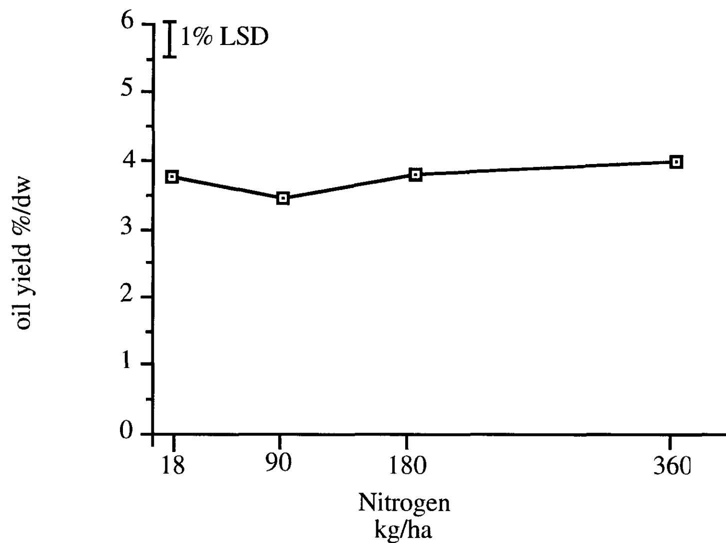

Fig. 12. Effect of increasing nitrogen on the oil concentration within the leaf

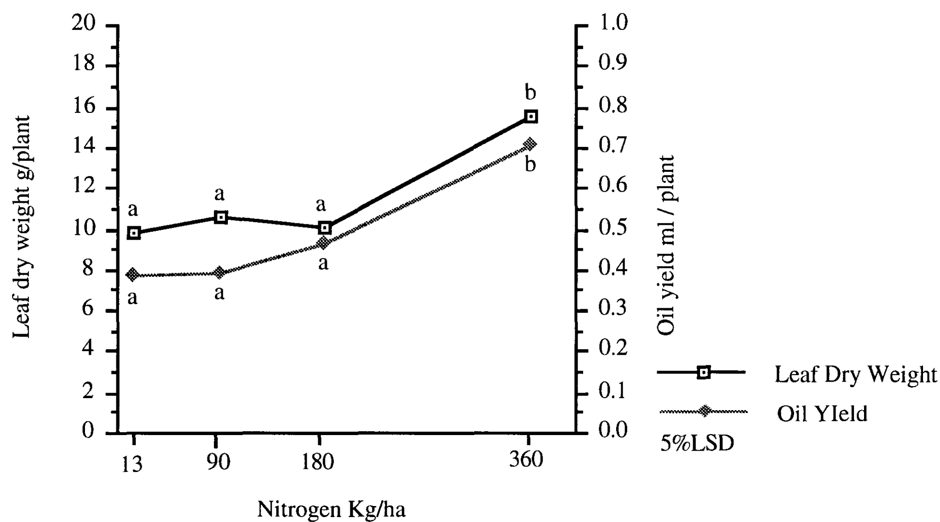

Fig. 13 Effect of nitrogen on growth, oil content and oil yield.

The application of phosphorus had no effect on the oil concentration (Fig. 14). Dry matter production was increased with 45kgha^{-1}, but not at higher or lower application rates. Thus, as with nitrogen application, the same oil concentration but increased dry matter production at 45kgha^{-1} resulted in a significant increase in Tea Tree oil yield per plant (Fig. 15)

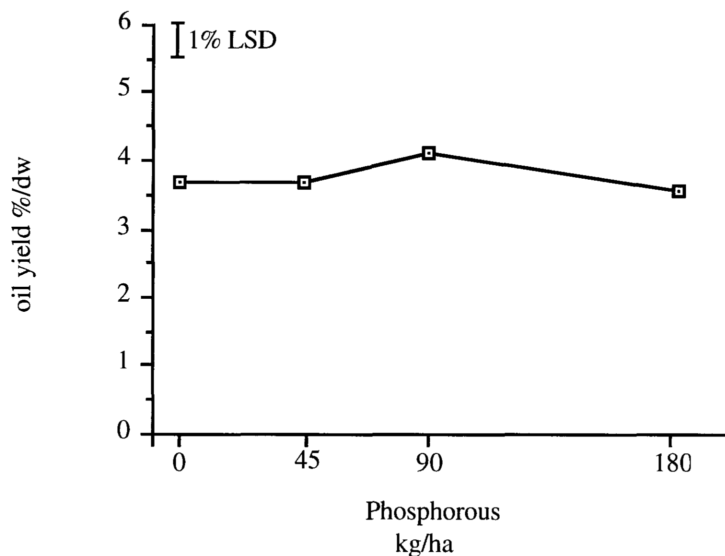

Fig. 14. Effect of increasing phosphorus on the oil concentration within the leaf

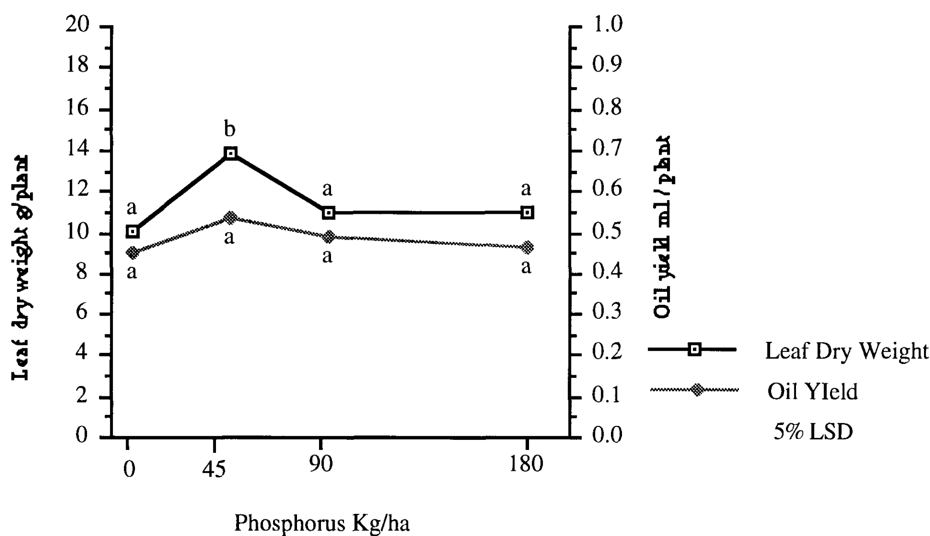

Fig. 15. Effect of phosphorus on growth, oil content and oil yield.

The leaf to stem ratios were unaffected by phosphorus (Table 17) while the nitrogen results show a small and inconsistent variation (Table 18)

Table 17 Effect of Phosphorus on stem dry weight and the leaf to stem ratio

nutrient	P 0	P 45	P 90	P 180
leaf to stem ratio	1. 10 : 1 a	1. 09 : 1 a	1. 06 : 1 a	1. 13 : 1 a

Table 18 Effect of Nitrogen on stem dry weight and the leaf to stem ratio

nutrient	N 13	N 90	N 180	N 360
leaf to stem ratio	1. 18 : 1 b	1. 02 : 1 a	1. 05 : 1 a	1. 12 : 1 ab

The components of Tea Tree oil respond independently to the changing nutritional status of the plant. The cineole content of the oil increased with nitrogen application from 13 to 90 $kgha^{-1}$ whereas terpinene-4-ol decreased with nitrogen application up to 180 $kgha^{-1}$ (Fig. 16).

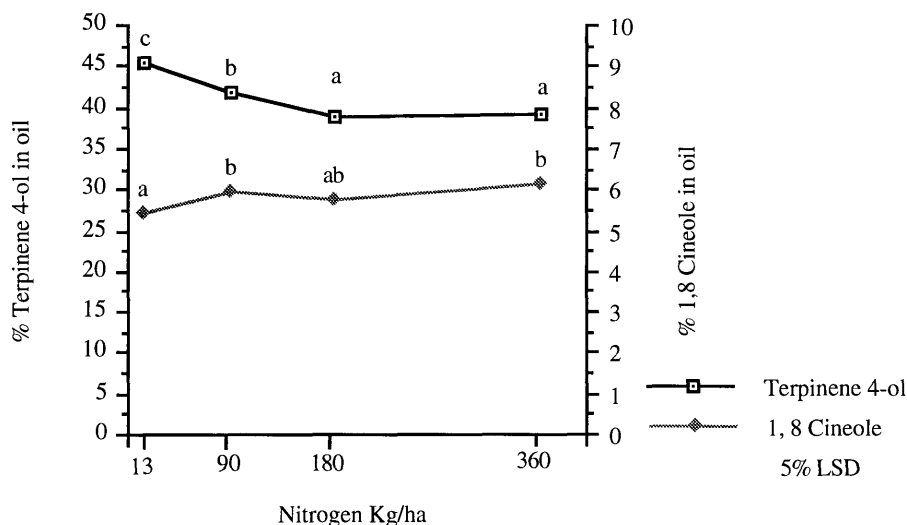

Fig. 16 The effect of increasing nitrogen content on the primary components of Tea Tree oil

The level of phosphorus had no effect on either the terpinene 4-ol or 1,8, Cineole content of the oil (Fig. 17)

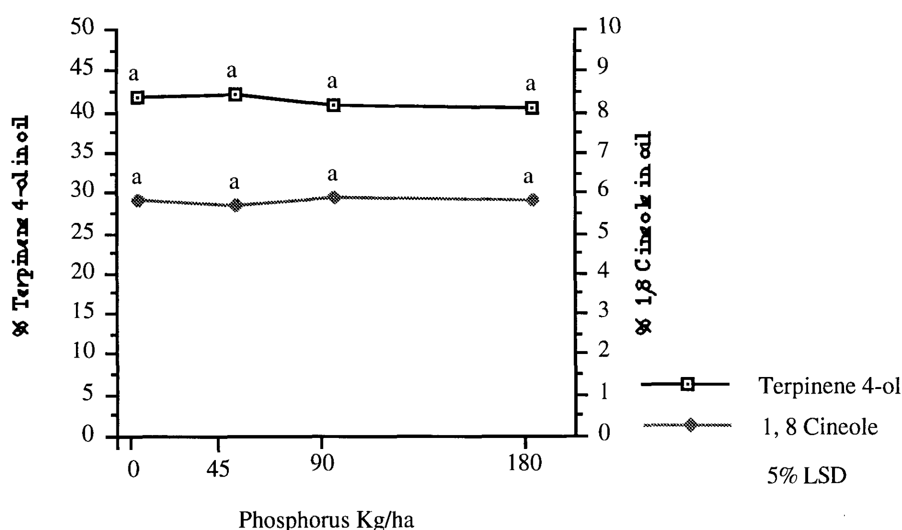

Fig. 17. The effect of increasing phosphorus content on the primary components of Tea Tree oil

7. 4 Discussion

7. 4. 1 Oil yield.

Increasing applications of nitrogen and phosphorus had no effect on the concentration of oil within the plant leaves. This response is similar to Caraway (*Carum carvi*) as reviewed by Dachler (1992), Wormseed (*Chenopodium ambrosioides*) which recorded a 0. 63% oil concentration when either 20 or 40 $kgha^{-1}$ of nitrogen was applied (Vomel, 1984) and Geranium, (*Pelargonium graveolens*) which showed no oil concentration change after being grown with nitrogen additions of 0,60,120 $kgha^{-1}$ (Rao, et al, 1990). The addition of nutrients did however cause an increase in leaf dry matter production resulting in an increase in total oil yield from each plant.

7. 4. 2 Dry matter

A significant effect on dry matter production occurred when 360$kgha^{-1}$ of nitrogen was applied. The lower application rates had no effect at harvest even though clear differences in growth could be observed between all nitrogen treatments during the early stages. Inadequate pot size may have restricted plant growth in the later part of the experiment. Tea Trees produce a massive root system and the plants soon filled the 15 cm pots. A better response may have been achieved by using larger growing containers or performing the experiments in the field.

7. 4. 3 Oil Composition

The relative proportion of the two primary components in Tea Tree oil, cineole and terpinene-4-ol, where affected by the addition of nitrogen but not phosphorus. The addition of some nitrogen caused an increase in the production of cineole but the effect was lost once the nitrogen reached moderate levels as was the case with the cineole content in *E. torquatta* and *E. angulosa* (Mahdi, et al, 1987).

The Terpinene-4-ol content of the oil showed a significant decrease as the nitrogen application increased up to 180$kgha^{-1}$. The reduction of terpinene-4-ol as the plant nitrogen status increased, was similar to the response of mint and basil where other monoterpinene alcohols, menthol and linalool, decreased in concentration as the available nitrogen was increased (Hornok, 1983).

The addition of nitrogen and phosphorus to Tea Tree will increase the dry matter yield of the plant thus increasing the total oil yield per plant. The composition of the oil may also change. In respect to the Australian Standard, these changes reduce the oil quality. However, provided the oil is well within the standard before the nutrient is added, the changes will not be sufficient to downgrade the product quality and there will be an overall benefit from increased oil yield.

Further research is required to look at the effects of nutrition in the field. This would give a better guide to the growth response of Tea Tree by avoiding pot root restrictions.

CHAPTER EIGHT

CONCLUSIONS

This study has provided a significant advance in the technology of Tea Tree oil production and research. A major limitation in the past has been the difficulty in obtaining and analysing the required number of Tea Tree leaf samples. The semi-micro distillation units developed here have largely overcome this limitation. These units require only small quantities of plant material to give an accurate reading making them suitable for large scale screenings of individual plants. Efficient sample preparation methods were also developed. The technique of pre-drying shoots to facilitate leaf removal not only simplifies sample preparation, it can also be applied to full scale production thereby increasing flexibility and efficiency of post harvest processing. An actual increase in oil yield during drying would be an added bonus.

The semi-micro stills have already enabled the identification of superior individual trees. A group of high quality, high oil yielding trees, with good plantation growth characteristics, have been selected from a seedling trial plantation. The results also illustrate the large variation in oil yield and quality even within selected seedling plantations; hence the need for clonal propagation, at least until more uniform inbreed lines can be produced.

While this method of assessing and selection was successful, the small number of original stock plants used reduced the genetic diversity available. To improve this selection a larger number of stock plants from geographically different environments would need to be examined. Such a screening would ensure the selected trees came from as wide a genetic base as possible. Again the technology developed here now makes this feasible.

Some progress was made with the clonal propagation of Tea Tree. The feasibility of cutting propagation or micropropagation was demonstrated but further work is needed. The supply of suitable cutting material remains a limitation. Large numbers of stock plants would be needed to supply sufficient cuttings for large scale planting's. Tea Tree was shown to be amenable to micropropagation giving an acceptable 3-4 fold shoot multiplication rate with ready rooting and acclimatisation. The high cost of micropropagated plants remains a limiting factor. This work

was not continued because it was understood that a successful micropropagation system has been developed elsewhere, although the details have not been published.

Nitrogen and phosphorus applications generally increased dry matter production without reducing oil concentration or lowering the quality below the Australian Standard, giving an overall increase in oil yield per hectare. However the responses to fertiliser application in this study may have been limited due to the restrictions of root growth due to the size of the pot. Further investigations using larger pots or under field conditions are needed to verify these results.

The production of Tea Trees within Australia is still in its infancy. For the industry to expand successfully the planting material used to establish plantations must improve. Selection and clonal propagation of high oil yielding good quality trees from a diverse genetic base would be the most efficient and cost effective means of this improvement. Application of the work presented here should help this process.

APPENDIX A
OIL YIELDS FROM THE SAMPLED TREE POPULATION

TREE NO	DATE	yield	1-8 cineole	terpinene 4-ol
A031	7/5/91	2. 30%		
C026	26/7/91	2. 31%		
F038	26/7/91	2. 48%		
A001	30/4/91	2. 50%		
A052	7/5/91	2. 62%		
B047	26/7/91	2. 84%		
GAMMA	8/5/91	2. 89%		
F117	26/7/91	2. 89%		
T003	26/7/91	2. 90%		
A015	30/4/91	2. 94%		
A035	7/5/91	2. 97%		
A002	30/4/91	3. 00%		
bulk	20/11/91	3. 03%		
C072	26/7/91	3. 04%		
BULK	3/10/91	3. 05%		
F275	26/7/91	3. 06%		
A016	30/4/91	3. 08%		
A001-1	7/5/91	3. 10%		
F075	26/7/91	3. 12%		
F235	26/7/91	3. 13%		
C191	26/7/91	3. 13%		
B035	26/7/91	3. 13%		
B131	26/7/91	3. 15%		
BULK	9/10/91	3. 17%		
A023	30/4/91	3. 17%		
B083	26/7/91	3. 21%		
A001-1	7/5/91	3. 23%		
A073	7/5/91	3. 23%		
BETA	17/5/91	3. 23%		
A037	7/5/91	3. 24%		
F115	26/7/91	3. 25%		
F023	26/7/91	3. 25%		
B021	26/7/91	3. 25%		
bulk	13/11/91	3. 27%		
B027	26/7/91	3. 27%		
A061	7/5/91	3. 28%		
A152	28/5/91	3. 34%		
F095	26/7/91	3. 34%		
F161	26/7/91	3. 34%		
F213	26/7/91	3. 34%		
F226	26/7/91	3. 34%		
A226	5/6/91	3. 36%		
C052	26/7/91	3. 36%		
E058	26/7/91	3. 36%		
F086	26/7/91	3. 36%		
E020	26/7/91	3. 37%		
F140	26/7/91	3. 38%		
A033	7/5/91	3. 38%		
F005	26/7/91	3. 38%		
E061	26/7/91	3. 38%		
BULK	16/8/91	3. 39%		
E162	26/7/91	3. 39%		
F100	26/7/91	3. 39%		
F205	26/7/91	3. 40%		
E161	26/7/91	3. 40%		
B172	26/7/91	3. 41%		
F070	26/7/91	3. 42%		
C201	26/7/91	3. 43%		
E071	26/7/91	3. 43%		
T043	26/7/91	3. 43%		
BULK	18/9/91	3. 44%		
F162	26/7/91	3. 45%		
B055	26/7/91	3. 46%		
C025	26/7/91	3. 47%		
BULK	14/8/91	3. 47%		
BULK	4/9/91	3. 48%		
F019	26/7/91	3. 48%		
F127	26/7/91	3. 49%		
F124	26/7/91	3. 49%		
D177	26/7/91	3. 50%		
BULK	25/9/91	3. 52%		
E009	26/7/91	3. 52%		
D145	26/7/91	3. 55%		
F102	26/7/91	3. 55%		
B177	26/7/91	3. 55%		
BULK	25/7/91	3. 56%		
A051	7/5/91	3. 56%		
E002	26/7/91	3. 56%		
C066	26/7/91	3. 57%		
BULK	16/10/91	3. 58%		
F047	26/7/91	3. 58%		
E218	26/7/91	3. 59%		
BULK	11/9/91	3. 60%		
E174	26/7/91	3. 60%		
B091	26/7/91	3. 61%		
F022	26/7/91	3. 62%		
D028	26/7/91	3. 63%		
F230	26/7/91	3. 63%		
B145	26/7/91	3. 64%		
B158	26/7/91	3. 64%		
C013	26/7/91	3. 64%		
B133	26/7/91	3. 65%		

E059	26/7/91	3. 65%		
D043	26/7/91	3. 65%		
E097	26/7/91	3. 65%		
F051	26/7/91	3. 65%		
B151	26/7/91	3. 66%		
F224	26/7/91	3. 67%		
D022	26/7/91	3. 67%		
E175	26/7/91	3. 67%		
B106	26/7/91	3. 68%		
B039	26/7/91	3. 68%		
C082	26/7/91	3. 69%		
C190	26/7/91	3. 70%		
E033	26/7/91	3. 70%		
F182	26/7/91	3. 71%		
F203	26/7/91	3. 73%		
BETA	1/5/91	3. 74%		
BULK	29/8/91	3. 75%		
A001-1	7/5/91	3. 76%		
F060	26/7/91	3. 76%		
F066	26/7/91	3. 76%		
C046	26/7/91	3. 76%		
C030	26/7/91	3. 76%		
F150	26/7/91	3. 77%		
U068	26/7/91	3. 77%		
B242	26/7/91	3. 77%		
E017	26/7/91	3. 77%		
A086	7/5/91	3. 77%		
A183	4/6/91	3. 77%		
B062	26/7/91	3. 77%		
GAMMA	17/5/91	3. 78%		
C106	26/7/91	3. 78%		
D039	26/7/91	3. 78%		
B143	26/7/91	3. 78%		
C057	26/7/91	3. 78%		
D025	26/7/91	3. 78%		
E184	26/7/91	3. 79%		
LOW	7/5/91	3. 80%		
B120	26/7/91	3. 80%		
B051	26/7/91	3. 80%		
F029	26/7/91	3. 81%		
B049	26/7/91	3. 81%		
T163	26/7/91	3. 82%		
ALPHA	17/5/91	3. 82%		
B182	26/7/91	3. 82%		
E178	26/7/91	3. 83%		
B044	26/7/91	3. 83%		
F184	26/7/91	3. 84%		
F058	26/7/91	3. 84%		
B183	26/7/91	3. 84%		
F131	26/7/91	3. 84%		
B045	26/7/91	3. 84%		
F179	26/7/91	3. 84%		
F017	26/7/91	3. 84%		
D020	26/7/91	3. 85%		
A018	30/4/91	3. 85%		
B036	26/7/91	3. 85%		
B100	26/7/91	3. 86%		
F037	26/7/91	3. 86%		
T016	26/7/91	3. 86%		
B122	26/7/91	3. 86%		
E203	26/7/91	3. 86%		
B087	26/7/91	3. 88%		
T075	26/7/91	3. 89%		
B031	26/7/91	3. 89%		
E073	26/7/91	3. 89%		
E087	26/7/91	3. 89%		
BULK	8/7/91	3. 89%		
F190	26/7/91	3. 90%		
C032	26/7/91	3. 90%		
F202	26/7/91	3. 90%		
F015	26/7/91	3. 91%		
F093	26/7/91	3. 92%		
T160	26/7/91	3. 92%		
F135	26/7/91	3. 92%		
U040	26/7/91	3. 92%		
D070	26/7/91	3. 92%		
C146	26/7/91	3. 92%		
U011	26/7/91	3. 93%		
LOW	30/4/91	3. 93%		
F142	26/7/91	3. 94%		
U126	26/7/91	3. 94%		
D042	26/7/91	3. 94%		
F277	26/7/91	3. 94%		
D095	26/7/91	3. 95%		
D003	26/7/91	3. 96%		
E245	26/7/91	3. 96%		
F098	26/7/91	3. 96%		
F001	26/7/91	3. 97%		
F091	26/7/91	3. 98%		
B063	26/7/91	3. 98%		
D173	26/7/91	3. 98%		
E050	26/7/91	3. 98%		
C162	26/7/91	3. 99%		
F233	26/7/91	3. 99%		
B140	26/7/91	3. 99%		
T171	26/7/91	3. 99%		
F030	26/7/91	3. 99%		
D143	26/7/91	4. 01%		
F264	26/7/91	4. 01%		
E029	26/7/91	4. 01%		

C058	26/7/91	4.02%		
F018	26/7/91	4.02%		
U006	26/7/91	4.02%		
B147	26/7/91	4.03%		
E157	26/7/91	4.03%		
D113	26/7/91	4.03%		
F096	26/7/91	4.03%		
D046	26/7/91	4.04%		
C051	26/7/91	4.05%		
E064	26/7/91	4.05%		
D112	26/7/91	4.05%		
D132	26/7/91	4.05%		
T007	26/7/91	4.05%		
U100	26/7/91	4.06%		
D032	26/7/91	4.06%		
E237	26/7/91	4.06%		
D160	26/7/91	4.06%		
D081	26/7/91	4.07%		
T035	26/7/91	4.07%		
F079	26/7/91	4.07%		
B090	26/7/91	4.08%		
U127	26/7/91	4.08%		
F089	26/7/91	4.09%		
D080	26/7/91	4.10%		
U123	26/7/91	4.10%		
B092	26/7/91	4.10%		
U039	26/7/91	4.11%		
D150	26/7/91	4.11%		
D086	26/7/91	4.11%		
E128	26/7/91	4.12%		
C076	26/7/91	4.12%		
D034	26/7/91	4.12%		
B169	26/7/91	4.13%		
C153	26/7/91	4.14%		
BULK	17/7/91	4.14%		
D103	26/7/91	4.14%		
E041	26/7/91	4.15%		
E186	26/7/91	4.15%		
C053	26/7/91	4.15%		
T017	26/7/91	4.16%		
C084	26/7/91	4.17%		
B069	26/7/91	4.18%		
A124	28/5/91	4.18%		
F114	26/7/91	4.19%		
D124	26/7/91	4.19%		
A222	5/6/91	4.19%		
U136	26/7/91	4.19%		
C091	26/7/91	4.19%		
C174	26/7/91	4.19%		
E096	26/7/91	4.19%		

E079	26/7/91	4.20%		
F171	26/7/91	4.20%		
F153	26/7/91	4.20%		
A082	7/5/91	4.20%		
E023	26/7/91	4.20%		
E069	26/7/91	4.21%		
F136	26/7/91	4.21%		
T056	26/7/91	4.21%		
BULK	20/6/91	4.22%		
E150	26/7/91	4.22%		
B246	26/7/91	4.24%		
D050	26/7/91	4.24%		
T010	26/7/91	4.24%		
E039	26/7/91	4.25%		
D026	26/7/91	4.25%		
D167	26/7/91	4.25%		
C038	26/7/91	4.25%		
B215	26/7/91	4.25%		
F085	26/7/91	4.25%		
C079	26/7/91	4.25%		
E022B	26/7/91	4.25%		
BULK	31/5/91	4.26%		
E193	26/7/91	4.26%		
CHAS 1	28/5/91	4.26%		
E015	26/7/91	4.26%		
F174	26/7/91	4.26%		
B217	26/7/91	4.26%		
B146	26/7/91	4.26%		
T001	26/7/91	4.27%		
U001	26/7/91	4.27%		
F084	26/7/91	4.28%		
D082	26/7/91	4.29%		
T022	26/7/91	4.29%		
B076	26/7/91	4.29%		
F134	26/7/91	4.29%		
A005	30/4/91	4.29%		
F021	26/7/91	4.29%		
F137	26/7/91	4.30%		
T150	26/7/91	4.30%		
U023	26/7/91	4.30%		
BULK	14/6/91	4.30%		
F026	26/7/91	4.30%		
B019	26/7/91	4.30%		
D012	26/7/91	4.31%		
CHAS 1	28/5/91	4.31%		
B232	26/7/91	4.32%		
U002	26/7/91	4.32%		
F049	26/7/91	4.32%		
U031	26/7/91	4.33%		
F177	26/7/91	4.33%		

B209	26/7/91	4. 34%		
F116	26/7/91	4. 34%		
D128	26/7/91	4. 34%		
D133	26/7/91	4. 34%		
D048	26/7/91	4. 34%		
F227	26/7/91	4. 35%		
U013	26/7/91	4. 35%		
E110	26/7/91	4. 35%		
A205	5/6/91	4. 35%		
E038	26/7/91	4. 35%		
A158	28/5/91	4. 35%		
U152	26/7/91	4. 36%		
F197	26/7/91	4. 36%		
BULK	1/8/91	4. 36%		
B017	26/7/91	4. 36%		
F207	26/7/91	4. 36%		
C070	26/7/91	4. 37%		
C138	26/7/91	4. 38%		
U009	26/7/91	4. 38%		
T120	26/7/91	4. 38%		
T034	26/7/91	4. 38%		
A149	28/5/91	4. 38%		
E149	26/7/91	4. 38%		
B067	26/7/91	4. 39%		
D038	26/7/91	4. 39%		
E092	26/7/91	4. 39%		
T006	26/7/91	4. 39%		
E133	26/7/91	4. 39%		
U056	26/7/91	4. 39%		
U004	26/7/91	4. 39%		
F087	26/7/91	4. 39%		
F081	26/7/91	4. 39%		
B102	26/7/91	4. 40%		
C093	26/7/91	4. 40%		
D202	26/7/91	4. 41%		
U020	26/7/91	4. 41%		
F238	26/7/91	4. 41%		
B171	26/7/91	4. 41%		
E177	26/7/91	4. 41%		
U012	26/7/91	4. 41%		
C050	26/7/91	4. 41%		
A093	28/5/91	4. 42%		
BETA	8/5/91	4. 43%		
C087	26/7/91	4. 44%		
F077	26/7/91	4. 44%		
C127	26/7/91	4. 44%		
E137	26/7/91	4. 45%		
T005	26/7/91	4. 45%		
T167	26/7/91	4. 46%		
T146	26/7/91	4. 46%		

B132	26/7/91	4. 46%		
LOW	28/5/91	4. 46%		
U054	26/7/91	4. 47%		
B129	26/7/91	4. 47%		
C011	26/7/91	4. 47%		
T028	26/7/91	4. 48%		
T018	26/7/91	4. 49%		
D005	26/7/91	4. 50%		
F126	26/7/91	4. 50%		
D008	26/7/91	4. 51%		
B075	26/7/91	4. 52%		
E027	26/7/91	4. 52%		
F274	26/7/91	4. 52%		
B073	26/7/91	4. 53%		
B004	26/7/91	4. 53%		
C230	26/7/91	4. 54%		
ALPHA	8/5/91	4. 55%		
T004	26/7/91	4. 55%		
E169	26/7/91	4. 55%		
C074	26/7/91	4. 55%		
A017	30/4/91	4. 57%		
C012	26/7/91	4. 57%		
B110	26/7/91	4. 57%		
T143	26/7/91	4. 57%		
F113	26/7/91	4. 57%		
E088	26/7/91	4. 57%		
C002	26/7/91	4. 58%		
T027	26/7/91	4. 58%		
D075	26/7/91	4. 58%		
C113	26/7/91	4. 58%		
T019	26/7/91	4. 58%		
T105	26/7/91	4. 58%		
F020	26/7/91	4. 58%		
B185	26/7/91	4. 58%		
bulk	14/1/92	4. 59%		
E158	26/7/91	4. 59%		
F069	26/7/91	4. 59%		
D035	26/7/91	4. 59%		
E075	26/7/91	4. 59%		
D011	26/7/91	4. 60%		
BULK	17/5/91	4. 62%		
A189	5/6/91	4. 62%		
F193	26/7/91	4. 62%		
E004	26/7/91	4. 63%		
C083	26/7/91	4. 63%		
B192	26/7/91	4. 64%		
F143	26/7/91	4. 64%		
B224	26/7/91	4. 65%		
U143	26/7/91	4. 65%		
T020	26/7/91	4. 66%		

D170	26/7/91	4. 66%		
CHAS 1	28/5/91	4. 67%		
A176	4/6/91	4. 68%		
C059	26/7/91	4. 68%		
F065	26/7/91	4. 68%		
C016	26/7/91	4. 69%		
A197	4/6/91	4. 69%		
B179	26/7/91	4. 69%		
C077	26/7/91	4. 70%		
B117	26/7/91	4. 70%		
D125	26/7/91	4. 70%		
ALPHA	1/5/91	4. 70%		
C041	26/7/91	4. 70%		
E102	26/7/91	4. 70%		
E152	26/7/91	4. 71%		
A053	7/5/91	4. 71%		
U101	26/7/91	4. 72%		
T157	26/7/91	4. 72%		
C179	26/7/91	4. 73%		
B173	26/7/91	4. 73%		
T127	26/7/91	4. 74%		
F170	26/7/91	4. 74%		
D023	26/7/91	4. 74%		
D104	26/7/91	4. 74%		
C062	26/7/91	4. 75%		
C155	26/7/91	4. 75%		
D114	26/7/91	4. 75%		
A089	28/5/91	4. 75%		
D193	26/7/91	4. 75%		
B187	26/7/91	4. 76%		
B165	26/7/91	4. 77%		
F110	26/7/91	4. 77%		
E013	26/7/91	4. 77%		
D049	26/7/91	4. 77%		
T064	26/7/91	4. 77%		
E135	26/7/91	4. 78%		
C197	26/7/91	4. 79%		
C036	26/7/91	4. 79%		
D010	26/7/91	4. 79%		
F139	26/7/91	4. 79%		
C048	26/7/91	4. 79%		
E007	26/7/91	4. 80%		
E093	26/7/91	4. 80%		
D088	26/7/91	4. 81%		
A168	28/5/91	4. 81%		
C020	26/7/91	4. 81%		
B204	26/7/91	4. 82%		
U034	26/7/91	4. 82%		
?	26/7/91	4. 84%		
T113	26/7/91	4. 84%		

A156	28/5/91	4. 85%		
U125	26/7/91	4. 85%		
T135	26/7/91	4. 86%		
C015	26/7/91	4. 86%		
B013	26/7/91	4. 86%		
U051	26/7/91	4. 86%		
D077	26/7/91	4. 86%		
B207	26/7/91	4. 87%		
ALP1	5/6/91	4. 87%		
U019	26/7/91	4. 87%		
E154	26/7/91	4. 87%		
T152	26/7/91	4. 87%		
B162	26/7/91	4. 87%		
F159	26/7/91	4. 88%		
F167	26/7/91	4. 88%		
E148	26/7/91	4. 88%		
F016	26/7/91	4. 88%		
C199	26/7/91	4. 88%		
A081	7/5/91	4. 89%		
C171	26/7/91	4. 90%		
B204	26/7/91	4. 90%		
B157	26/7/91	4. 90%		
F061	26/7/91	4. 91%		
A084	7/5/91	4. 92%		
F121	26/7/91	4. 92%		
U072	26/7/91	4. 92%		
F151	26/7/91	4. 92%		
D120	26/7/91	4. 93%		
C010	26/7/91	4. 93%		
D019	26/7/91	4. 93%		
E057	26/7/91	4. 94%		
U137	26/7/91	4. 94%		
B078	26/7/91	4. 95%		
F053	26/7/91	4. 96%		
T126	26/7/91	4. 96%		
A170	28/5/91	4. 96%		
T142	26/7/91	4. 98%		
C160	26/7/91	4. 99%		
T141	26/7/91	4. 99%		
F031	26/7/91	4. 99%		
B071	26/7/91	5. 00%	2. 07%	50. 56%
U008	26/7/91	5. 00%		
D097	26/7/91	5. 00%		
C029	26/7/91	5. 02%		
C109	26/7/91	5. 03%		
A091	28/5/91	5. 03%		
E066	26/7/91	5. 03%		
C141	26/7/91	5. 04%	2. 07%	52. 37%
A098	28/5/91	5. 04%		
A133	28/5/91	5. 05%		

T015	26/7/91	5. 05%		
D007	26/7/91	5. 05%		
C018	26/7/91	5. 05%		
D068	26/7/91	5. 07%		
T107	26/7/91	5. 07%	3. 73%	48. 24%
E067	26/7/91	5. 08%		
LOW	26/7/91	5. 08%		
BULK	8/5/91	5. 09%		
C009	26/7/91	5. 10%		
F025	26/7/91	5. 10%	1. 78%	51. 41%
U124	26/7/91	5. 11%		
A108	28/5/91	5. 11%		
E100	26/7/91	5. 12%		
h103	26/7/91	5. 13%		
D002	26/7/91	5. 14%		
U003	26/7/91	5. 15%		
B221	26/7/91	5. 16%		
U050	26/7/91	5. 16%		
C104	26/7/91	5. 18%		
F106	26/7/91	5. 18%	4. 35%	46. 39%
B015	26/7/91	5. 20%		
T031	26/7/91	5. 22%		
E012	26/7/91	5. 23%		
C003	26/7/91	5. 23%		
T151	26/7/91	5. 23%		
B097	26/7/91	5. 25%	0. 96%	56. 94%
U032	26/7/91	5. 25%		
C128	26/7/91	5. 25%		
B239	26/7/91	5. 26%		
C001	30/4/91	5. 26%		
BULK	1/5/91	5. 26%		
T139	26/7/91	5. 26%		
A090	28/5/91	5. 27%		
E094	26/7/91	5. 27%		
A213	5/6/91	5. 28%		
U005	26/7/91	5. 30%	3. 37%	47. 52%
F180	26/7/91	5. 30%		
B218	26/7/91	5. 30%		
C022	26/7/91	5. 31%		
DELTA	8/5/91	5. 32%		
F108	26/7/91	5. 32%	3. 79%	49. 85%
C005	26/7/91	5. 32%	3. 77%	13. 59%
C088	26/7/91	5. 32%		
A106	28/5/91	5. 34%		
C069	26/7/91	5. 36%		
A097	28/5/91	5. 37%		
D064	26/7/91	5. 38%		
D045	26/7/91	5. 38%		
T025	26/7/91	5. 38%		
D001	26/7/91	5. 39%		

D053	26/7/91	5. 40%		
F009	26/7/91	5. 40%	4. 96%	40. 16%
BULK	21/8/91	5. 41%		
B005	26/7/91	5. 41%		
U007	26/7/91	5. 42%		
U151	26/7/91	5. 44%		
C008	26/7/91	5. 44%	1. 78%	44. 66%
B010	26/7/91	5. 44%	2. 49%	46. 98%
A177	4/6/91	5. 45%		
C149	26/7/91	5. 45%		
D098	26/7/91	5. 45%	0. 53%	52. 96%
D139	26/7/91	5. 45%		
U052	26/7/91	5. 47%		
B002	26/7/91	5. 47%	3. 30%	47. 60%
E006	26/7/91	5. 49%	3. 27%	47. 27%
A227	5/6/91	5. 49%	4. 15%	45. 92%
A217	5/6/91	5. 50%	9. 25%	27. 97%
C125	26/7/91	5. 51%	6. 02%	43. 69%
C140	26/7/91	5. 52%	7. 41%	43. 53%
C001	26/7/91	5. 53%	4. 35%	40. 87%
BULK	29/1/92	5. 54%		
E165	26/7/91	5. 55%	7. 79%	42. 82%
C166	26/7/91	5. 55%	5. 66%	43. 68%
E022	26/7/91	5. 56%		
U049	26/7/91	5. 57%	4. 17%	48. 22%
R	17/5/91	5. 57%		
B011	26/7/91	5. 57%	2. 22%	42. 78%
A187	4/6/91	5. 58%	42. 89%	19. 72%
C004	26/7/91	5. 58%	8. 23%	33. 81%
F002	26/7/91	5. 59%	3. 27%	41. 93%
BULK	23/5/91	5. 59%		
D129	26/7/91	5. 61%	6. 86%	43. 63%
C006	26/7/91	5. 61%	3. 53%	37. 36%
T009	26/7/91	5. 64%	4. 15%	43. 15%
T117	26/7/91	5. 65%	6. 52%	43. 60%
E005	26/7/91	5. 66%	5. 87%	39. 93%
A165	28/5/91	5. 67%	6. 34%	43. 84%
R	1/5/91	5. 68%		
D108	26/7/91	5. 75%	2. 72%	45. 21%
C042	26/7/91	5. 75%	8. 34%	42. 36%
E063	26/7/91	5. 75%	4. 47%	40. 90%
U053	26/7/91	5. 78%	3. 95%	45. 43%
C001	30/4/91	5. 83%	4. 35%	40. 87%
T161	26/7/91	5. 83%	4. 06%	46. 64%
E016	26/7/91	5. 87%	2. 03%	39. 67%
U092	26/7/91	5. 88%	5. 73%	40. 67%
A190	5/6/91	5. 89%	17. 33%	21. 44%
E054	26/7/91	5. 89%	41. 91%	18. 84%
R	26/7/91	5. 90%		
A055	7/5/91	5. 92%	4. 79%	43. 22%

U014	26/7/91	5.94%	4.29%	43.60%
F011	26/7/91	6.00%	6.22%	38.76%
DELTA	1/5/91	6.01%	6.96%	42.68%
bulk	16/1/92	6.07%		
E001	26/7/91	6.09%	4.22%	41.24%
R	30/1/92	6.16%		
B057	26/7/91	6.22%	7.21%	46.91%
GAMMA	1/5/91	6.24%		
A208	5/6/91	6.62%	7.11%	43.84%
T024	26/7/91	6.76%	8.34%	35.48%
BULK	5/2/92	6.85%		
D014	26/7/91	6.89%	6.57%	40.89%
DELTA	17/5/91	6.97%		
R	8/5/91	7.27%		

APPENDIX B

Media codes used in tissue culture practice to denote the various constituent categories and their concentration levels. (From de Fossard 1976).

Four digit code referring to constituent category : $X_1 X_2 X_3 X_4$

where X_1 = Inorganic Nutrients

X_2 = Auxins

X_3 =Cytokinins

X_4=Organic nutrients (vitamins and amino acids)+ sucrose

X_4 may be divided into two parts $X_1 X_2 X_3 (X_{4a} X_{4b})$

Where X_{4a}= Organic nutrients (vitamins and amino acids)

X_{4b}= sucrose

Four standard concentration levels are used: Z= zero, L=low, M=medium, H=high

Examples

Basal medium	MZZM	medium inorganic nutrients zero auxins zero cytokinins medium organic nutrients and sucrose.
Rooting Medium	LHZL	lowinorganic nutrients high auxins zero cytokinins low organic nutrients and sucrose
Multiplication Medium	MZHM	medium inorganic nutrients zero auxins high cytokinins medium organic nutrients and sucrose
Sucrose Only Medium	ZZZ(ZM)	zero inorganic nutrients zero auxins zero cytokinins zero organic nutrients and medium sucrose

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