RURAL INDUSTRIES RESEARCH
& DEVELOPMENT CORPORATION

Tasmannia Lanceolata

Developing a New Commercial Flavour Product

A report for the Rural Industries Research and Development Corporation

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FOREWORD

The essential oils research program in Tasmania has had a leading role in the development of quality extracts and has been closely involved in the promotion and marketing of many valuable essential oils. The essential oil and natural products industry of Australia contributes over $60m to export income.

In 1989 and 1990, in conjunction with industry partners, a selection of *Tasmannia lanceolata* extracts were introduced to the flavour and fragrance markets in Japan, Europe and the United States, with a view to establishing potential use in perfumes and flavourings.

Once market potential was established it was critical that reliability of supply, consistency of product and fulfilment of the legal requirements for exporting the oil be addressed.

The Horticultural Research Group, within the University of Tasmania, in consultation with Essential Oils of Tasmania (EOT), Natural Product Extracts (NPE) and RIRDC, identified the research need to:

- establish a database detailing plant variability within *Tasmannia lanceolata*
- specify the preferred chemical profile of the product
- identify clones with the desired characteristics
- standardise the extraction protocol
- address the legal impediments to development of an export market

This report, which details the progress achieved towards these objectives, is a new addition to RIRDC's diverse range of over 400 research publications. It forms part of our Essential Oils and Natural Plant Extracts R&D Program which aims to support the growth of a profitable and sustainable essential oils and natural plant extracts industry in Australia.

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Peter Core
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TECHNICAL SUMMARY

Survey - Extraction and Analysis for Database

Over ninety clonal selections were made during the initial stages of the study. These produced extracts with a wide range of characteristics. Detailed site and clone observations were compiled and used to establish a database which has already proven useful in identifying particular clone types.

Product Specification

At the present time, and without further input from potential users of the extract, the following general specifications have been established.

Low safrole content. GC MSD data was used to monitor the ions specific to safrole, (104 and 162). A large variation in safrole content was apparent. A standard curve for safrole has been established to aid in identifying low safrole extracts..

Low piperitone content.

High polygodial content.

The response factor of this analyte has been established previously and, like piperitone, is readily determined in routine analysis.

Spicy notes reminiscent of *Lantana* spp.

The Japanese client has brought this factor to our attention, and our experience has now been that we can organoleptically identify this quality.

Observations using gas chromatography interfaced with an olfactory detection unit have determined that the components most commonly described as lantana-like elute in the region commonly associated with the oxygenated sesquiterpenes. Percentage composition data, calculated for a range of extracts, confirm a large variability in the oxygenated sesquiterpenes, which may account for the difficulty associated with defining the 'essence' of *Tasmannia lanceolata* extracts.

Absence of sweet floral or fruity notes.

Terpenes contribute significantly in terms of composition of *T. lanceolata* extracts and are regarded in the essential oil industry as contributing a freshness and floral note to aroma profiles. Some have a slightly aggressive odour which is attributed to their unsaturated nature. Organoleptic assessment of a range of extracts with a high terpene content, has shown that these are regarded to be of poor quality. The fresh sweet floral notes are often identified by observers as eluting early in the chromatograms produced by GC and registered using olfactory and FID detectors. Low percent composition of these components is specified for quality products.

Concrete yield of 6% or more.

This level has been seen in many selections, and should be maintained to ensure commercial viability.

Clonal Selection

Several sets of extract fractions and whole extracts have been sent to Japan for evaluation. The feedback has been instructive and detailed assessment comments have been presented in the results section of this report.

Propagation

Tasmannia lanceolata semi-hardwood cuttings were struck in a sand/peat medium and generally produced a viable plantlet in 6-8 weeks. Most of the clones were comparably easy to strike.

Plantation Establishment

Two plantation sites were established, with a view to examining density, nutrition and canopy architecture issues. Subsequent to discussions with industry partners in October 1996, less emphasis was placed on the field trials, promoting the other aspects of the project. However, observations indicated clonal ranking with respect to survival rate, from best to poorest, was G6, W3, W5, H1, Q4, W1, Q4 and Q3. In addition, clone types W3, G6 and H1 proved to have almost double the growth rate of the other five clones.

Nutrition Trial

A factorial experiment with four levels of phosphorus (P) and nitrogen (N) applied in a solution culture situation was established.

The observed effects of low nitrogen levels (~2.5 mM) were reduced biomass, height and number of leaves. In addition, a high shoot to root ratio due to a lack of root growth was noted.

High phosphorus levels (~2.0 mM) resulted in a decrease in leaf number. In conjunction with either extreme of nitrogen level an increase incidence of apical death and no new growth were observed.

Healthy, vigorous growth occurred at the nitrogen and phosphorus levels, which coincide with full strength Hoaglands solution levels, namely 1.0 mM P and 10 mM N.

The Effect of Harvest Time on Extract Composition

The solvent extract of *Tasmannia lanceolata* was examined from a small community of plants over 230 days of the growing season. The results from gas chromatography were analysed using cluster analysis and principal co-ordinate analysis. Two distinct groups of plants were identified, based on polygodial content and the entire range of components detected. The use of as many components as possible is recommended for the analysis, in order to give the most complete representation of the composition of the extracts.

Within the low polygodial group, the tendency was for polygodial levels to remain constant over time, whereas, in the high polygodial group, concentrations varied.

Seasonal Variation in Clones

Levels of safrole do not vary significantly during the growth cycle. This implies that clones with low levels of safrole may be harvested at any time without the risk of high levels of safrole being present.

The analysis of the 23 major components of the 182 examined, showed that only eight of these varied significantly over the experimental period. However, the times at which a maximum was reached varied from one peak to another. In general, maxima were reached in spring rather than in the summer months. Peaks at retention times 11.28min (guaiol), 11.95min, 14.11min (218 diterpene), 15.26min, 16.13min (polygodial artefact) and 18.87min (drimenol related unknown), all fell in this category.

Chemical Characterisation

The complexity of the solvent extract of *T. lanceolata* is such that isolation and characterisation of each component was not possible within the scope of this project. However, the fractionation of the oil has allowed for the identification of many components by their mass spectra. The simplification of the aroma profile in these fractions has allowed for the assessment as to which chemicals contribute most to the quality of the typical *T. lanceolata* flavour.

> Polygodial was found to be stable in ethanol at room temperature over a period of almost 4 days. From this it may be assumed that an ethanol absolute may be prepared without significant loss of polygodial.

Trials showed that the decolourising ability of charcoal is superior to Tonsil (a bleaching earth), in combination with the solvents used.

Also, as polarity increases, the % volatiles decrease. The greatest amount of volatile material is retained with the use of hexane. Volatiles are also influenced by the quantity of charcoal.

Retention of polygodial is important since this component imparts the characteristic spicy nature to the extract. As the level of charcoal is increased, the polygodial content decreases. Ethyl acetate treatment removed the least polygodial, followed by the hexane/ethyl acetate mixture and hexane removed the most. Recovery fell from 98% to 80% as charcoal increased from 0.2 to 1.5 g.

The extraction of *Tasmannia lanceolata* berries produced a light yellow oil with a pleasant bushy green fragrance, reflecting the relatively high levels of monoterpenes present. A spice note was, none the less, quite strong in the back ground, with a citrus aroma persisting after the oil had been exposed for a few minutes. Polygodial constituted 45% of the volatile fraction and safrole was present at levels of 8ppm.

There may be opportunities for product diversification, with the inclusion of berry extracts and absolutes.

EXECUTIVE SUMMARY

The project is aimed towards the specification of the solvent extract as a new flavour product. This involves the survey, propagation, assessment and documentation of collected samples from around the state. Client preferences can be gauged through the evaluation of a range of extracts and fractions. The monitoring of safrole levels and the further chemical elucidation of the extract are important steps towards the registration of the product with an international body.

There has been international interest in the leaf extract of *T. lanceolata* since the late 1980's. A commercial extraction protocol has been developed and semi-commercial quantities of extract have been provided to a large Japanese flavour and fragrance company. To date, most of the leaf material used in the commercial scale extractions are collected from natural stands. The two areas of concern that have been identified are the ability to supply increasing quantities of product in the short to medium term, and the apparent product variability. In addition, there has been some difficulty with specifying market preferences.

Part way through this project, in July '97, discussions with growers highlighted the fact that further market penetration was being hindered through the lack of registration of the extract with any internationally recognised regulatory body. Consequently, field trial work was relegated to 'observation-only' status, and a greater emphasis was placed on chemical characterisation and safrole level investigations.

A widespread search of the state produced over 100 clones for assessment in terms of essential oil composition, organoleptic impact, yield and physical properties. The most promising clones have been propagated and a clone bank has been established to house these plants on a long term basis.

An integrated database has been set up, which gives ready access to valuable information concerning the clones that have been collected.
A time trial to monitor the level of safrole over the course of the season was initiated to investigate concerns over safrole accumulation patterns.

In order to further refine our target standard extract, fractions of the extract, a distilled essential oil and an absolute sample were sent to Japan for assessment.
The components of the extract have been largely identified through mass spectrometry, high performance liquid chromatography and gas chromatography, after initial fractionation by column chromatography.

The research findings showed that the levels of safrole in the extract do not vary significantly over the course of the growing season. The implication is that a low safrole content clone will not accumulate sufficient safrole to cause concern, irrespective of when samples are taken.

A clone bank which holds the most promising clones in terms of yield, organoleptic assessment and vigour now exists at the Horticultural Research Centre. The properties of these clones, and others, which were examined, are stored in a Filemaker Pro database.

The on-going work of registration of *Tasmannia lanceolata* extract with FEMA on the GRAS list is an involved one, and has to date been addressed through the intensive investigation of the chemical components of the extract, resulting in the identification of 98% of components. Other considerations such as toxicological studies are being addressed through liaisons with other institutions that specialise in this area, and will be addressed fully in future work.

BACKGROUND

Interest in leaf extract of *T. lanceolata* first arose with exposure during marketing visits by the company Essential Oils of Tasmania to international flavour and fragrance houses in the late 1980's. During the past two years, a commercial extraction protocol has been developed and semi-commercial quantities of extract provided to a large Japanese flavour and fragrance company for use in preparation of a commercial chewing gum product. During a recent visit to Tasmania, representatives of the company spoke enthusiastically of the prospects for this product and of plans to use the extract in several other confectionary lines. However, two areas of concern arose - the question of our ability to supply increasing quantities in the short to medium term, and the apparent product variability amongst our deliveries so far. At the same time the Japanese company has experienced difficulty in conveying, in technical terms, their requirements of the product, and simple technical analysis has proved inadequate in providing a workable guide. These issues are germane to commercialisation of any essential oil products, and similar questions have accompanied responses to *T. lanceolata* samples by European and North American companies.

Currently leaf material requirements (estimated at 1-5 tonnes per annum) are sourced from a small number of forestry sites upon which well established trees of unknown genetic heritage are present. This presents problems of two kinds.

Preliminary sampling demonstrated the wide variation in the Tasmanian population of the species with respect to yield, composition, physical properties and organoleptic impact of the leaf extract. While, with each successive harvest, refinement in the harvesting, drying and extraction technique improves the reliability of the product preparation, the major source of variation remains with the plant material itself.

Secondly, while the material has been collected from carefully selected, previously disturbed sites, or in advance of plantation forestry projects, use of a wild resource of this kind must be subject to scrutiny from an environmental impact point of view. As in forestry, exploitation must progress towards a fully managed and commercially sustainable basis if it is to satisfy the expectations of the wider community.

OBJECTIVES

1. To refine methods of specification for the new product. Parameters include yield, safrole concentration, polygodial levels and organoleptic properties.
2. To survey the available populations of the species, and select and propagate plants showing commercial promise.
3. To establish two pilot plantations of the species for development of management and harvest technologies.

In accordance with the recommendations agreed to by NPE, some alterations were made to the project objectives, tasks and their milestones, to reflect the change in emphasis from field trial work to registration of the product.

Subsequently the following objectives were identified.

4. To investigate the behaviour of safrole through the growing season.
5. Identify as many components of the extract as possible to aid in product registration.
6. Identify the remaining criteria for registration on the FEMA GRAS list and initiate the processes required for toxicological investigation.

INTRODUCTION

General

Tasmannia lanceolata (Poir.) A. C. Smith, (Mountain Pepper), is described as a much branched shrub, up to 5 m high, with dark green glabrous aromatic leaves and distinctive, crimson, young stems. This dioecious plant bears black, berry-like fruit, approximately 5mm in diameter, containing numerous small seeds [1]. The plant grows in cool wet habitats from sea level to about 1200 m in Tasmania, preferring disturbed sites in which it is an early coloniser, preceding wet eucalypt forest and *Nothofagus* rainforest [2]. It is also found in similar situations in Victoria and at high altitudes in New South Wales, as far north as the Hastings River.

The solvent extract of the leaves of *Tasmannia lanceolata* is being investigated as a potentially useful flavour product.

Safrole Investigation

In previous studies, a known carcinogen [3], safrole, was detected in the leaves and berries of *Tasmannia lanceolata.* [4]. For the product to be accepted on the world market, the concentration of this constituent had to be minimised. An initial investigation had been carried out, which examined the way in which safrole levels changed over the course of the growing season. That experiment used six plants in a localised area. The same extracts were used to determine changes in, and correlations between the components in the extracts.

One of the consequences of analysing for variation between plants was that there were distinct groupings of plants. Essentially, there was variation in the chemistry, even between so few individuals all within a 3.5 km stretch of roadway.

The present study eliminates that variable by using clonal material, currently held at the HRC. In addition, a more uniform growing environment can be provided, namely through potting media and shadehouse conditions.

Component Variation Investigation

For most essential oil crops, the timing of harvest and the selection of suitable populations of plants are two fundamentally important aspects of commercialisation. Both of these factors have a bearing on extract quality. Ideally, the extract of a commercial population would be chemically homogeneous and would be harvested at a time of optimal yield.

It is recognized that *T. lanceolata* has a great range of phenotypes from one area to another. Its range of extract compositions is likewise varied.
Some preliminary work has been done on seasonal changes in oil composition [4], and this has shown that there is little variation in oil quality due to the position of the leaf sampled.

The variation in oil quality due to plant gender was considered. Unpublished data has revealed that there is no significant difference between the extracts from male or female plants. Consequently, sex was not a factor that was taken into consideration in the selection of experimental plant material.

The focus of the present work was to examine the total extract composition in a group of plants from a localised geographic area, to determine the extent of compositional differences and their changes over time. Included were the levels of polygodial (the major contributor to the extract's peppery character), and percentage monoterpenes were studied over a period of 230 days. Cluster analysis of GC data has been used to select superior cultivars, rich in flavouring potency, in Japanese pepper [5].

Extract Decolourisation

An avenue of potential value-adding was identified, whereby small quantities of various extracts and essential oils are added to canola oil to give a flavoured cooking oil. *T. lanceolata* extract had to be decolourised before it could be used for this purpose. The limits set were that the end colour should lie between that of boronia absolute and canola oil. In addition, it was required that the level of polygodial remained high to retain the hot character of the extract, and that as much as possible of its base flavour was also present in the final product.

Plantation Establishment

A series of experiments were designed to investigate various aspects of management and crop agronomy. An experimental program was agreed upon, in conjunction with both North Eucalypt Technologies at Ridgley, and a local farmer, Mr Ian Farquhar, at Winnaleah.

The aim of the experiments at Winnaleah was to test the response of a single clone to canopy shape and nitrogen fertiliser treatment. A second trial was established to address the question of optimal spacing. The Ridgley site was used to test clonal differences in response to four harvest treatments. The slow growth habits exhibited by this species mean that special strategies for harvesting need to be determined.
This trial was established as an inter-eucalypt species in a regular eucalypt plantation area.

Experimental Program

As an adjunct to the plantation experiments, a solution culture experiment was performed to give some background information on adequate nutrition levels for the major nutrients.

METHODOLOGY

Survey Methods

A wide ranging field survey of *T. lanceolata* plants was carried during the first eight months of the project. Most areas of the state were visited and samples were taken for propagation and extraction. A database was established using FileMaker Pro, which incorporated information on each clones' characteristics. The database contains location, soil type, aspect, sex, yield, organoleptic assessment, meteorological, and compositional data.

Over one hundred new selections were made.

Establishment of Database

Laboratory Scale Extraction of Oils

Leaf samples from the survey of *T.lanceolata*, collected from native stands throughout Tasmania, were dried for 72 hours at 35°C were ground using a mortar and pestle. Each sample was extracted with 3 x w/v of petroleum ether, in a lidded vessel. Samples were sonicated for 10 mins and the solvent was filtered through a cotton wool plug into a pre-weighed round bottom flask. The leaves were washed a further 2 times and the combined filtrates were dried down on the rotary evaporator. The samples were subjected to a final dry down at 60°C for 5 mins before re-weighing. Each oil was subject to GC analyses and organoleptic assessment before being transferred to storage vials for future reference.

Organoleptic Assessment

A 1% extract in ethanol solution was prepared from each oil, which was added drop wise to 100mL of distilled water. The mixture was evaluated for initial impact and persistence.

Gas Chromatographic Analyses

Aliquots of between 10 to 20 mg of the oils were accurately weighed into 3 mL vials. Approximately 1mg of the internal standard octadecane was added. The samples were dissolved in 1mL hexane and analysed using gas chromatography (GC).

Oil Composition

The composition of the oils were determined using GC, with detection by flame ionisation (FID). These analyses were performed using a Hewlett Packard (Palo Alto Ca. USA), HP 5890 unit fitted with an HP 7673A automatic injector and an FID detector, with control and data analysis by HP/Chemstation 3365 software. The carrier gas was high purity nitrogen, run at a pressure of 17 psi. The column flow rate was 2 mL/min. The injector temperature was 250°C and the detector temperature was 280°C. The temperature program was 50°C (1 min), ramping by 20°C/min to 150°C, then 5°C/min to 260°C (5 min). The injection volume was 1 µL, with a split ratio of 50:1.

Oil compositions were calculated relative to the internal standard, octadecane, with each component presented as a percentage of total volatiles.

Determination of Safrole levels

Safrole levels within the sample were too low for accurate assessment using the results obtained by GC FID. A separate analysis was performed on a HP 5890 GC, coupled via an open split interface to a HP 5970B mass selective detector (MSD) in the selective ion monitoring mode (SIM). 1µl automatic splitless injections analysed on a 15 m HP1 column (cross-linked methyl silicone gum), USA, i.d. 0.22 mm, phase thickness, 0.33 µm). The injector and detector temperatures were 250°C and 290°C respectively, with the oven temperature gradient rising from 50°C (1min) to 290°C at 10°C/min. The ions monitored were 77, 131, 104 and 162 for safrole and 254 for the internal standard, octadecane.

A standard curve was established by spiking sub samples of an extract of *T. lanceolata*, which had been previously shown to have naturally low levels of safrole, with a commercial preparation of the analyte. Seven sub samples of 10 to 20 mg of this oil were fortified with a range of safrole concentrations from 1.5×10^{-3} to 7.6µg. A response factor was calculated relating the areas of the safrole peak 162 relative to the areas of octadecane.

Product Specification

Product specification required the bringing together of many aspects of the work on this product. Desirable types were distinguished through analytical and organoleptic assessment work. Also of importance, were the detection of possible problem components in the extract, such as piperitone and safrole, and the development of screening procedures for these compounds.

Clonal Selection

In the field situation, plants were selected as potential stock specimens by making a visual assessment of vigour, including leaf colour and degree of branching. An impression of the essential oil was obtained by crushing and smelling some of the fresh leaves. The degree of 'hotness' was not assessed in the field through taste, since the senses become saturated and unresponsive after the first exposure to polygodial, making subsequent judgements unreliable.

Propagation

Tip cuttings were taken from selected plants. Each cutting was approximately 5-6 cm in length. The base of the cutting was scored and dipped in rooting hormone. The rooting medium was a 1:1 mixture of sand and aged pine-bark. Up to a dozen cuttings were placed in each punnet and put on a heated sand bed with misting. Generally, the cuttings had developed roots within 6-8 weeks, after which time they were potted on using the following mix:

1:4 sand : pine-bark

200g dolomite

200g osmocote

25g $FeSO_4$

25g micromax

Plantation Establishment

Canopy Management (Winnaleah)

The W3 clone was planted at a spacing of 800mm x 3m in four blocks with six plants per treatment. Three fertiliser schedules and three canopy shapes were used. The N fertiliser (as ammonium nitrate) was added at the rate of 0, 10 or 20 g/plant (Treatments N_0, N_1 and N_2). In conjunction with these, three canopy shapes (square,

flat and pyramid, (Treatments S, F and P) were applied, in the following combinations:

1	N_0S	2	N_0F	3	N_0P
4	N_1S	5	N_1F	6	N_1P
7	N_2S	8	N_2F	9	N_2P

Spacing Experiment (Winnaleah)

Four blocks of eight plants per treatment were used. Distances of 60cm, 80cm and 120cm were used, in combination with either a single row or double row arrangement.

At the end of the first year, an estimate of vigour of plants in both trials was to be made through height and width measurements. When harvest was possible, (year 2 onwards), an estimate of dry matter production was to be made through harvest weight. The canopy shapes were to be introduced after the first years growth.
The Winnaleah site was left under the supervision of Mr Ian Farquhar.

The trial site at Winnaleah was inspected in November 1996. These plants had grown some 5-6cm since bud burst in late October. Their survival rate was assessed as 85%. The major contributors to the death rate was the competitive effect of weed species.

Clonal Management (Ridgley)

At the Ridgley site, four harvest treatments were envisaged. These were:

a) Removal of the current years growth,
b) Removal of half of current years growth,
c) No harvest until year 3, then a)
d) No harvest until year 3, then b)

This treatment strategy was adopted since the greater the supporting network of branches, the greater the number to possible new shoots.
Treatments a) and b) examine the level of harvest, while c) and d) deals with canopy density and level.
The same measurement would be made as in the Winnaleah trials.

A set spacing of 3m between plants, being the inter Eucalypt distance, with an inter-row spacing of 3m was used. The pre-planting preparations included laying down of weed mat to control weeds which would otherwise be controlled through a spray regime.

Four blocks, each containing eight plants of each of eight clones was planted out. The clones were: W3, W4, W5, G6, Q3, Q1, Q4 and H1. The areas from which the clones were collected were:

ID	**LOCATION**
W	Mt.Wellington
G	Guildford (Talbots Lagoon)
H	Hellyer
Q	Queenstown

The observations performed on the Ridgley trial sites included two height measurements in Nov. 96 and June 97.

Height difference and survival data from the two sampling times was analysed using the NPAR1WAY procedure of the SAS statistical package, and the output is shown in Appendix 1.

Subsequent to discussions with industry partners in October 1996, the milestones were modified decreasing the emphasis was placed on the field trials, promoting the other aspects of the project.

Nutrition Trial

The effect on growth of various levels of P with a range of N levels was investigated.

T. lanceolata plants were grown in glasshouse solution culture. A factorial experiment was set up, with four levels of each of phosphorus (P) and nitrogen (N) were applied to 4 replicates, in blocks.

Other nutrients were supplied as in half strength Hoaglands solution. The micronutrient concentrations are shown in Table 1. The 0.5 mM P and the 5 mM N treatments correspond to half strength Hoaglands solution concentrations (in bold type face).

This approach allowed a check on high P level tolerance, and occurrence of significant growth increases with increased N.

Treatment number, levels of N and P and the N:P ratio are shown in Table 2.

Table 1

Micronutrient Concentrations

Micronutrients	Concentration
Fe as FeEDTA	2ppm Fe
B as H_3BO_3	0.25 ppm
Mn as $MnSO_4$**.**	0.25ppm
Cu as $CuSO_4$**.**$7H_2O$	0.01ppm
Mo as $NaMoO_4$**.**$7H_2O$	0.0055ppm
Zn as $ZnSO_4$**.**$7H_2O$	0.0005ppm

Table 2
Treatment Numbers And N:P Ratios

Trt No	P Level	N Level	N:P ratio
1	0.25	2.5	10
2	0.25	5.0	2
3	0.25	10.0	4
4	0.25	20	8
5	0.50	2.5	5
6	**0.50**	**5**	**10**
7	0.50	10	20
8	0.50	20	40
9	1.0	2.5	2.5
0	1.0	5	5
11	1.0	10	10
12	1.0	20	20
13	2.0	2.5	1.25
14	2.0	5	2.5
15	2.0	10	5
16	2.0	20	10

The parameters measured were number and length of laterals, number of leaves per lateral, plant height, shoot and root weight. Subjective assessments were also made of the negative symptoms seen in each treatment. These were lack of growth, reddening of older tissue, purple colouration, necrosis, paleness/chlorosis, apical death, leaf scorch, distortion of leaves and leaf loss. These factors were scored as 1 for present or 0 for absent. A total score was then produced for negative effects. In addition, each symptom was analysed separately for statistical significance. The SAS statistical package was used for the analyses.

The Effect of Harvest Time on Extract Composition

Six plants were selected at random at the Arve Loop site. The plants were located along a 3.5 km length of the roadway. Plants 1, 4, 5 and 6 were of roughly the same age and were ~2 m in height. Plants 2 and 3 were about half the height of the other

four. Plant number 6 was not as vigorous as the other five, being infected with sooty mould, with its habitat being more shaded and cooler than the more exposed sites of the other five plants. Leaf position does not affect oil quality [4], but for reasons of uniformity of physiological age, three recently fully expanded leaf pairs were taken on seven separate occasions [16 Aug ‘96 (0 days), 14 Nov ‘96 (91 days), 04 Dec ‘96 (111 days), 30 Dec ‘96 (137 days), 16 Jan ‘97 (154 days), 20 Feb ‘97 (189 days) and 02 Apr ‘97 (230 days)].

Extraction and Analyses

The extraction method used was that developed by Read [4] as being efficient for small scale applications. The fresh leaves were dried at 35 °C in a thermostatically controlled oven for 48 h. The leaves were then ground to a fine powder with a mortar and pestle in preparation for extraction. Leaf samples of (150-200mg) were transferred to 20 mL glass vials, and 5 mL redistilled petroleum ether (Shell Australia Ltd. bp 40-60 °C) containing 1 mg octadecane (Sigma, 99%) as an internal standard. The vials were placed in a Branson 5200 sonication bath for 20 min. The samples were allowed to settle before transferring the solvent solution to a GC vial for analysis.

All samples were analysed using GC FID for determination of the percent composition and GC MSD for safrole assessment, as described previously.

Integration of the peaks used a rejection area of 500. The analysis of the extract from each plant, at each sampling time, resulted in a chromatogram that consisted of between 70 and 120 peaks. The complete profiles from all 42 of these runs were consolidated into a single file that represented each of the components as a number from 1 to 183. Where there were peaks that were not present in other traces, they were assigned a unique number.

Compositional data are reported as peak area percents, and as such, one may assume that the detector response is equivalent from one component to the next [6].

Variability of the above extraction and analysis method was determined through four repetitions of one sample. The standard error of the polygodial peak area percent was 0.099.

The techniques used for analysis of the data included clustering, principal co-ordinate (PC) analysis and correlation coefficient determinations. The package TAXON [7] was used for cluster and principal co-ordinate work. Similarity coefficients were calculated using standardised Euclidean distance. Clustering was performed by an agglomerative hierarchical procedure using the 'incremental sum of squares' sorting strategy [8]. The principal co-ordinate analysis was applied to the 42 member matrix of [6 plants × 7 times], each with their 183 components [9].

SAS v 6.12 (SAS Institute Inc., Cary, NC, USA) was used for correlation coefficients and regression analysis of the peak areas of polygodial, with time being the explanatory variable.

Seasonal Variation of Components in a Single Clone

Thirty W3 plants of uniform age, cultivated at the Horticultural Research Centre, were used. In order to check for similarity between the plants, a random sample from twelve of the individuals was taken for micro scale extraction and analysis. Thereafter, samples of the most recently fully expanded leaves, consisting of three leaf pairs, were taken at regular intervals. The sampling protocol followed ontogenetic events such as budburst, onset of flowering, the flush of growth and leaf maturation.

The uniformity of the experimental material means that samples may be taken from a subset of the total at any one time, in order to maintain enough material for sampling throughout the year. The plants were divided into seven blocks of four plants. Each block being sampled sequentially. That is, the first block provided four reps of (3x2) leaf samples for the first sample. Block two was used for the next harvest.

Samples, collected between the 29 August 1997 and 2 February 1998, were dried at 35°C for 72 hours then ground with mortar and pestle.

The trials monitoring the effect of harvest time in native stands, conducted in 1995, had shown that the level of safrole dropped below the detection limit of the analytical method described previously. As such, a supercritical fluid extraction method was developed to pre-concentrate the analytes before analyses.

Extraction and Analyses

Method development

A bulk sample of dried *T. lanceolata* leaves was mixed to uniformity. Approximately 200-300mg sub-samples were weighed into 10mL stainless steel SFE extraction tubes. 1g of hydromatrix was added and the tubes were spiked with sufficient safrole (4.88 µg) to allow detection using the standard GC FID method. Each were extracted under a range of conditions of temperature, pressure and solvent composition using a Varian Star SFE and modifier pump, coupled to a Varian Autoprep 44 Accutrap. Samples were analysed using GC FID to determine the optimal conditions for reproducible extraction of the leaf components.

The basic conditions common to all the SFE methods trialed had static mode of 5 minutes and a dynamic mode of 20 minutes at 2mL/min flow rate. The static and dynamic temperatures were both 40˚C. The trap was set at –10 ˚C, with a collection volume of 1.8 mL of methanol. The extraction chamber conditions for each sample were as follows.

Once conditions were optimised a repeat experiment was conducted to determine the reliability of the extraction.

Analyses of Samples

Approximately 200-300 mg of leaves, collected from propagated plants, over specified time intervals, were weighed into 10mL stainless steel SFE extraction tubes along with ~ 1g of hydromatrix. The samples were extracted under the following SFE conditions.

Conditions of SFE Extraction

Pressure	350 atm
Modifier	10% methanol
Static mode	40°C held for 5 minutes
Dynamic mode	40°C for 20 minutes at 2g/min CO_2
Restrictor temperature	35°C
Trap temperature	-10°C
Collection solvent	acetone
Collection volume	1.8 mL
Wash volume	5 mL

The sample extracts were collected in GC vials that had been spiked with 10 µL of a 40 mg/mL octadecane solution. The samples were analysed by GC MSD in the selected ion-monitoring mode for safrole, and by GC FID for volatiles.

A standard curve for safrole was constructed by spiking 10 – 20 mg of *T. lanceolata* oil, previously extracted, and shown to be low in endogenous safrole with 100, 10 and 1 µL of a 1.004 µg/mL solution of safrole. Each standard was also spiked with octadecane internal standard and made up to 1mL with acetone in 3mL GC vials.

A precision experiment was conducted to ensure the reliability of the extraction method wherein five repeats of a uniform, ground leaf sample were sub sampled, extracted and analysed. These samples and the extracts of the time series samples were analysed consecutively.

The information from these runs was used to perform statistical analyses of the components in the extract with the SAS statistical package.

The FID runs were used to assess the variation of 23 major peaks through the experimental period. The SAS statistical package (Proc GLM and Proc Npar1way) was used to analyse differences in component percentages and yields between sample times.

Chemical Characterisation & Product Development

A range of techniques were employed to further elucidate the chemically complexities of the extract. These included fractional distillation, column chromatography and high performance liquid chromatography (HPLC). As well, a range of different product presentations were investigated and their potential for acceptance on the world market assessed.

Column Chromatography

Crude separations of fractions were made with a column, slurry packed with 120g silica (activated for 24 hours at 110°C). The methanol fraction was run with a hexane/ether gradient, yielding many fractions with interesting aromas, including coconut and caramel.

The hexane fraction was separated into eight fractions, of which the last two to elute were assessed as being of interest organoleptically.

Isolation of Polygodial and Fractionation by Column Chromatography

The following procedure was developed which yielded a range of fractions and polygodial (98% pure). The methanol fraction was put through the silica column using combinations of hexane and ether, beginning at 50% through to 100% ether.

Column Fractionation Conditions

Mobile Phase:	50 : 50 hexane / ethyl ether.
Gel type:	Merck Silica Gel 60 for column chromatography
	0.040-0.063 mm (230-400 mesh)
Bed Volume:	~200 mL
Id:	41.4±0.3 mm
Wall thickness:	2.3mm (medium wall)
Gel height:	16 cm

Three runs were completed.

TLC plates were run of individual fractions in order to determine similarities between them, with similar fractions then being combined: A previous crude preparation of polygodial was used on TLC to identify those fractions that contained polygodial.

Approximately 130 mL of solvent flowed through the column before the first fractions were collected.

Run 1: A1 (30mL), B1 (40mL), C1 (40mL), D1 (60mL), E1 (80mL) and F1 (80mL).
Run 2: A2 (30mL), B2 (40mL), C2 (40mL), D2 (50mL), E2 (80mL) and F2 (80mL).
Run 3: A3 (30mL), B3 (40mL) C3 (30mL), D3 (30mL), E3 20mL), F3 (40mL), G3 (80mL) and H3 (60mL).

Of these, D1, E1, C2, C3 and D3 contained polygodial, according to TLC checks. These fractions were consequently combined. The sample was re-applied to the column. A solvent gradient going from 10:90 ether/hexane (150 mL), 15:85 (50 mL), 20:80 (50 mL) to 30:70 (250 mL) ether/hexane was used to elute the sample. After an initial fraction of 100 mL was discarded, 47 x 10 mL fractions were collected and analysed by TLC. Nothing was present in the eluent until fraction 9. Fractions 9-48 were then recombined according to their degree of similarity. The following combinations were used:

A	9-11	E	25-26	I	33-34
B	12-16	F	27-28	J	35-36
C	17-19	G	29-30	K	37-40
D	20-24	H	31-32	L	41-44
				M	45-48

Of the above combinations, fractions H, I, J and K formed white-cream crystals on drying down. The product (polygodial), was impure, so all four fractions were redissolved in chloroform, combined and dried down. Hexane was added, the solution warmed and left to cool gradually to re-crystallise the product.

Production of Fractions of T. lanceolata for Market Assessment.

To develop a standard extract, with qualities favoured by the market, fractions of *T. lanceolata* were prepared for market assessment. It must be emphasised that the fractions were not presented as a viable commercial product. All samples were presented to obtain feedback from the market and determine the qualities and organoleptic profiles favoured by overseas clients.

Initially 21.105 g of the petroleum ether extract of *T. lanceolata* was partitioned into two fractions. The extract was dissolved in dichloromethane (50 mL) and hexane (100mL) in a separatory funnel. Methanol/water (50 mL of 80/20) was added and the mix was vigorously shaken. The aqueous phase was drained into a separate vessel and the non polar fraction was washed with a further 50mL of the aqueous methanol before being transferred to a pre weighed round bottom flask.

The aqueous layers were combined and dried by RVE. Dichloromethane (50mL) was added to the water suspension remaining, to back extract the relatively polar components in solution. The DCM was carefully removed with a pipette to a pre-weighed, round bottom flask.

Weight of hexane fraction : 15.0225 g
Weight of methanol/water fraction : 5.1595 g

Both samples were subject to analysis using GC FID.

Column Chromatography of the Hexane Fraction

The hexane fraction (0.553 g) was dissolved in hexane/ether (10mL of 50/50) and carefully placed on a 14.7 x 4 cm Merck silica gel F60 column with a bed volume of diethyl ether/hexane (~ 300 mL of 50/50). The sample was eluted with

1. 120 mL 50/50 hexane/ether
2. 70mL 36/64 hexane/methanol
3. 200mL 100% methanol

Twelve fractions were collected of varying volumes. These were assessed by TLC and combined to from four fractions which were analysed by GC FID.

The column chromatography of the hexane fraction was repeated with varying proportions of the three solvents hexane, diethyl ether and methanol as the mobile phase. After quality assessment the fractions were combined to produce four samples.

Fraction 2 was reapplied to the silica column and eluted with 100% methanol. Indistinct banding resulted and the fractions collected were recombined and re-chromatogrammed with 5% methanol in 50/50 hexane/ether. Four main bands were collected. Band 2a was discarded, as it had no distinctive qualities.

Column Chromatography of the Methanol Fraction

A portion of the methanol fraction (~1 g) was placed in a beaker and dissolved in a minimum amount of diethyl ether. Dry Merck F60 silica gel was added and well mixed with the extract. The mix was placed a stream of air until a dry yellow powder remained. The powder was carefully placed on top of a dry 14.7 x 4 cm silica gel column and eluted with 400mL of dichloromethane. The column was washed with 200mL of methanol.

Sixteen fractions combined after TLC assessment and four final fractions resulted.

HPLC Methods

Several methods were used in the attempt to identify the characteristic *T. lanceolata* components, including any 'Lantana-like' fractions.

Fractions from the hexane portion of the extract were further separated using the following general method.

Analytical C_{18} column 5µ Radial Pak cartridge, 5mm i.d.

Conditions: 55-100% MeOH (15mins) 2ml/min Detection at 233nm.

Semi-preparative conditions: Same column as above, 40-100% MeOH (15mins) 2ml/min.

Injection volume 80-250µL

The sample was 20mg extract fraction dissolved in 2ml MeOH.

Fractions were collected and tested for organoleptic properties. None were lantana-like, and insufficient quantities were recovered for further elucidation work.

Fractions from the methanol portion of the extract were further separated using a silica column (5µm Rad-Pak 5mm i.d). A range of solvent systems was tested

including hexane/chloroform/ether. Some interesting sub-fractions were collected, but again, insufficient quantities were recovered for further analysis.

Identification of Components

The chemical composition of the T. lanceolata solvent extract is extremely complex. It includes a large number of unknown compounds, often present in very small concentrations and it is beyond the scope of this project to identify every component. However initial investigations had determined the identity of the majority of components using a range of techniques including isolation and purification, and the use of GC MS to characterise the components by comparison of the Kovat indices and mass spectra.

However, the sheer complexity of the extracts meant that many of the mass spectra could not be determined, as multiple components eluted within the time periods of those under investigation. Often the chemicals were present in such low concentration that it was not possible to obtain a reliable mass spectrum. The isolation and complete characterisation of these components was not feasible with the resources available. However the chromatographic experiments described previously, provided fractions with dramatically fewer components. These samples were subject to GC MSD and the simplified profiles allowed for a large number of reliable mass spectra to be determined and the chemicals identified. The relative retention times were used to identify these chemicals within the profile of the non-fractionated extract.

The Stability of Polygodial in Ethanol over Time

It had been assumed that the dialdehyde moiety of polygodial would be susceptible to degradation in an ethanol solution. On this assumption, the preparation of an absolute on a commercial scale had previously been considered unfeasible. To determine the behaviour of polygodial in an ethanol solution, a time series experiment was undertaken.

Initially a hexane solution of semi pure polygodial was analysed to estimate the time periods required to effect the breakdown of polygodial. Aliquots of the 4.796mg/mL polygodial solution (2 x 300µL) were placed in GC vials. Vial 1 was made up to

1mL with 700µL of hexane whilst 700µL of ethanol was added to vial 2. Octadecane was added (25µL of a 41 mg/mL solution) to each vial as an internal standard. Immediately after addition of the ethanol, vial 2 was analysed by GC FID. Both vials were analysed at 3, 38, 67 and 109 minutes.

Preliminary results indicated that purified polygodial did not degrade in ethanol solution. A more extensive study was undertaken to determine if degradation of the dialdehyde occurred when polygodial was dissolved within the matrix of *T. lanceolata* extract in solution in ethanol.

Three separate standard extracts were warmed and 20mg of each were transferred into four GC vials. Hexane (1mL) was added to vial 1 and ethanol (1mL) added to the remaining three vials. Immediately on addition of the solvent, each vial was analysed by GC FID. Repeat analyses were conducted at time intervals. The percentage polygodial detected was recorded for each over the time series.

GC FID Conditions

Instrument : HP 5890.
Injection Mode : 1µL automatic injection.
Injector Temp.: 250˚C
Column : 0.52µm 15m HP1, 17psi.
Oven Temp : 50˚C (1) - 20˚C/min - (150˚C)-5˚C/min - (215˚C)-15˚C/min -(260˚C)
Detector : FID
Detection Temp : 280˚C

The Preparation of an Absolute from *T. lanceolata* Concrete

Previous experiments have shown that the dialdehyde moiety of polygodial does not degrade in ethanol solution. The preparation of an absolute from the *T. lanceolata* extract was undertaken.

A sample of *T. lanceolata* extract from Clone W3 (10.5699g), was warmed to 40˚C in 100mL of redistilled ethanol for 10 minutes. The flask was then placed in the chill room (2˚C) for 2 hours then transferred to a -12˚C freezer for 2 days.

A Büchner filter holder, receiving vessel, ethanol and filter paper were placed in the freezer for 2 hours. The chilled extract was vacuum filtered through the chilled Whatman No.4 filter paper. The waxes were washed with three aliquots of chilled ethanol. The filtrate was transferred to a pre-weighed round bottom flask and the solvent removed by RVE. The extract underwent a final RVE at 50˚C for 5min and then the flask was re-weighed. The waxes were dried, scrapped off the filter paper and also weighed.

The 15.5mg of the original W3 extract concrete and 14.6mg of the absolute were weighed into GC vials and dissolved in 1mL of hexane. Octadecane was added (25µL of 1.323mg/25µL solution) to each and analysed by GC FID.

GC FID Conditions

Instrument : HP 5890.
Injection Mode : 1µL automatic injection.
Injector Temp.: 250˚C
Column : 0.52µm 15m HP1, 17psi.
Oven Temp : 50˚C (1) - 20˚C/min (150˚C) - 5˚C/min (215˚C) - 15˚C - (260˚C)
Detector : FID
Detection Temp : 280˚C

Extract Decolourisation

Samples of commercial *T. lanceolata* extract were obtained from EOT. Aliquots of extract were used with a variety of solvent and decolourant combinations, as shown in Table 3. The decolourants used were charcoal and Tonsil (an activated bleaching earth, manufactured by Süd-Chemie, München). The extract was placed in a flask with the decolourant and the solvent was added. The mixtures were shaken for 1 hour, then filtered through Whatman No.42 filter paper using a Büchner funnel and flask. The filtrate was dried down on the rotary evaporator. Final dry down was at 60°C for 5 min.

Sample 19 attempted to use very polar solvents and both decolourants for the removal of pigments. After dry down, the mixture was back extracted with hexane and separated with a separatory funnel. The resultant hexane solution was divided into two parts. The first was filtered again and dried down (sample 19a). Tonsil (0.75 g) was added to the other, filtered and dried down (sample 19b).

Samples were prepared for GC analysis (30-50 mg in DCM, with 7.15 µg C_{18} internal standard) and organoleptic assessment, (1% extract in ethanol solution, which is added dropwise to 100mL of distilled water).

Table 3
Decolourisation Combinations

No	Solvent	Decolourant	Weight (g) Decolourant	Weight (g) Extract
1	Hexane	Charcoal	0.2	3.0
2	Hexane	Charcoal	0.6	3.0
3	Hexane	Charcoal	1.5	3.0
4	Hexane	Tonsil	0.2	3.0
5	Hexane	Tonsil	0.6	3.0
6	Hexane	Tonsil	1.5	3.0
7	Ethyl Acetate	Charcoal	0.2	3.0
8	Ethyl Acetate	Charcoal	0.6	3.0
9	Ethyl Acetate	Charcoal	1.5	3.0
10	Ethyl Acetate	Tonsil	0.2	3.0
11	Ethyl Acetate	Tonsil	0.6	3.0
12	Ethyl Acetate	Tonsil	1.5	3.0
13	Hex/EtOAc (1:1)	Charcoal	0.2	3.0
14	Hex/EtOAc (1:1)	Charcoal	0.6	3.0
15	Hex/EtOAc (1:1)	Charcoal	1.5	3.0
16	Hex/EtOAc (1:1)	Tonsil	0.2	3.0
17	Hex/EtOAc (1:1)	Tonsil	0.6	3.0
18	Hex/EtOAc (1:1)	Tonsil	1.5	3.0
19a	MeOH/H_2O/EtOAc (1:1:1)	Charcoal	1.5	3.0
19b	MeOH/H_2O/EtOAc (1:1:1)	Tonsil	1.5	3.0

Production of an Extract of *T. lanceolata* Berries

A sample of berries had been collected from King Island in Tasmania. Two sub samples were dried at 35°C for 72 hours and reweighed. Two samples of approximately 17g of the berries were frozen in liquid nitrogen and ground with a

mortar and pestle. Petroleum ether was added (3 x w/v) and the vessels were placed on a shaker at room temperature for 2 hours. The solvent was filtered through sintered glass funnels and the berries further extracted with 50 mL of solvent for a further 10 minutes. The filtrates were combined, transferred to a pre-weighed round bottom flask and dried using RVE.

The two oils produced were sub-sampled into GC vials (10-20mg) and spiked with 13.2 µg of octadecane and analysed for safrole by the method detailed previously.

Product Registration

Contact was made with FEMA to determine the requirements for registration of natural extract products. Enquiries were also made to various European agencies for guidelines. This consultative portion of the project was managed by EOT, and by Mr Witold Petrusewicz and Mr Tim Smith, in particular.

The registration requirements, as set out in the documents that were received from FEMA were addressed through the chemical elucidation work and by making contacts with toxicological institutes.

RESULTS

Survey - Extraction and Analysis for Database

A wide ranging survey of the state has been undertaken. Some ninety selections have been made to date. Extractions have being performed and assessments completed. A database and processing methodology has been established with yield and composition data being entered. In addition to documenting results from recent screenings, data from previous studies has been incorporated. The organoleptic properties of each selection were also recorded. The database will be a valuable resource from which suitable types can be identified.

The appendices give a comprehensive listing of the information gathered for each selection.

Appendix 2 Meteorological data

Appendix 3 Site data

Appendix 4 Summary of chemical data and organoleptic assessment

Appendix 5 Detailed chemical data

The considerable clonal variation is highlighted by comparisons amongst the clones.

The range of % yield (dry matter basis), % polygodial, % monoterpenes, % sesquiterpenes and ppm safrole are shown below.

	Minimum	**Maximum**
% Yield (dmb)	1.1	7.6
% Polygodial	0.0	67.3
ppm Safrole	0.7	662.5
% Monoterpenes	1.6	13.8
% Sesquiterpenes	86.3	98.5

Product Specification

The constraints identified for the product include:

a) Low safrole content:

Safrole is a suspected carcinogen and is endogenous in the leaves of plants closely related to *T. lanceolata*. GC MSD data has been reprocessed from files stored from the analyses conducted in 1995 and the ion currents specific to safrole, ions 104 and 162, extracted.

Safrole was detected at a significant level in one of the extracts. A range of *T. lanceolata* samples was analysed, and a large variation in safrole content was obvious. Selection criteria for propagation and extraction material will specify low safrole content. This will be determined by establishing a standard curve for safrole and calculating a response factor for the analyte.

b) Low piperitone content:
Analysis of this component requires no special technique as piperitone is usually present at high enough concentrations in *T. lanceolata* extracts to be readily measured from the GC FID trace used to assess major components in routine screening.

c) High polygodial content:
The Japanese market has determined that the polygodial content is an important factor in the assessment of product quality. The response factor of this analyte has been established previously and is readily determined in routine analysis.

d) Spicy notes reminiscent of *Lantana* spp.
Terminology to describe odours can be quite different depending on operator experience, and is related to environments encountered by individual observers. This is particularly pertinent in respect to the smell described as lantana-like (referring to the smell exuded from crushed leaves of *Lantana camara*), which is usually associated with extracts particularly favoured by the Japanese market. After becoming familiar with the aromatic qualities of crushed lantana leaves observers can readily identify the relative similar stimuli found in the *T. lanceolata* aroma profile.

Observations using gas chromatography interfaced with an olfactory detection unit have determined that the components most commonly described as lantana-like elute in the region commonly associated with the oxygenated sesquiterpenes. Percentage composition data, calculated for a range of extracts, confirm a large variability in the oxygenated sesquiterpenes, which may account for the difficulty associated with defining the 'essence' of *Tasmannia lanceolata* extracts.

e) Absence of sweet floral or fruity notes:

Terpenes contribute significantly in terms of composition of *T. lanceolata* extracts and are regarded in the essential oil industry as contributing a freshness and floral note to aroma profiles. Some have a slightly aggressive odour which is attributed to their unsaturated nature. Organoleptic assessment of a range of extracts with a high terpene content, has shown that these are regarded to be of poor quality. The fresh sweet floral notes are often identified by observers as eluting early in the chromatograms produced by GC and registered using olfactory and FID detectors. Low percent composition of these components is specified for quality products.

f) Concrete yield of 6% or more:
This level has been seen in many selections, and should be maintained to ensure commercial viability.

The 'essence' of *Tasmannia lanceolata* extracts is not easily defined with the proportion of any one component not necessarily reflecting the contribution made to the oil character.

The characteristics and hence 'quality' of *Tasmannia lanceolata* extracts are variable. The complexity of the chemistry incorporating a plethora of monoterpenes, sesquiterpenes and oxygenated sesquiterpenes makes the specification of a preferred commercial standard difficult. However, guidelines have now been established for screening of potential commercial clones.

Clonal Selection
The clones in the collection presented a range of flavour and aroma impacts. Selections were made on the basis of their organoleptic properties. One group bears a woody character, which may be accompanied by other qualities. These were available for the European perfumers to examine, and are shown in Table 4.
Another group of twelve were selected because they exhibit some unusual aroma quality, and lantana impact may not be the primary one. Therefore, diverse notes, for example, reminiscent of mint, raspberry, floral and others were sought, as shown in Table 5.

Table 4

Tasmannia lanceolata Aroma Profiles (Woody)

Clone	Impression	%Polygodial
AL1	Strong lantana, spicy, **woody**, balanced, powerful	20.14
FG6	sharp **sweet raspberry**, dusty cinnamon, fresh **floral**, lantana spice **woody**	0.00
HY7	spicy **woody** slight lantana; lemony cinnamon	55.89
HZ1	**woody** sassafrass, strong, sweet lantana pepper	32.74
NW2	**woody** sassafrass pine, pineapple, fish oily, slight lantana	54.88
UB4	**sweet fruity** spicy, bitter strong, **woody**/spice some lantana	63.99
WH4	fruity sweet, weak, spicy **woody**	16.43

Table 5

Tasmannia lanceolata Aroma Profiles (Unusual)

Clone	Impression	%Polygodial
FG1	**mint**, very strong Tasmannia spice, lantana	0.25
FG2	strong lantana, **limey green** note	0.00
GL6	spicy, citrus, clear **green** note, **lime**	49.65
HY9	fruity sweet persistent, **floral**, herby	47.79
MB2	**tropical fruit** spicy, strong lantana (high volatiles) very fruit	23.68
MB7	strong **heavy** sharp notes	48.78
PP1	strong lantana, very **fruity (tropical)**, cloves	59.24
PP2	floral **(lavender)** lantana, strong lantana and spice	16.88
PP3	Tasmannia; strong to medium **lantana** and spice	50.64
RR1	**aromatic** spicy **lantana**	37.77
TR3	sharp **spicy**, **strong lantana** fruity, lantana, bushy	44.83
TR5	**spice and lantana**	60.56

Client Assessment

Table 6

Assessments Made By Client

Clone	Evaluation of 1% Ethanol solution in water
FG1	spicy, sharp taste
FG2	clean like lemon lime, not as spicy as FG1
GL6	citrus smell, pepper like taste, hot
HY9	herb spicy note, spicy, hot, long lasting
MB2	spicy, but taste weak
MB7	heavy, weak smell
PP1	white pepper note, strong taste, long lasting
PP2	slightly floral note, not spicy, long lasting
PP3	green, herbal spicy note, lacking strong spice
RR1	green, spicy note, lacking strong spice
TR3	green, spicy note, spicy enough- stronger, sharp
TR5	strong top note, strong taste
Clone	**In gum base:**
GL6	cool impression, but not enough
HY9	quiet top note, herbal spicy bottom note
MB7	herbal spicy impression, increase coolness
PP1	sharp, and spicy, helps mint well, long lasting
TR5	green, herbal, sharp, enhance coolness, long lasting

Propagation

Sufficient numbers of plants were successfully propagated from semi-softwood cuttings to enable the establishment of a clone bank. The survival rates varied considerably between clones, though the precise data cannot be recounted, since distortions arose due to mist-bed failure on one occasion.

Plantation Establishment

The planting of *T. lanceolata* clones at the Ridgley site coincided with the planting of *Eucalyptus nitens* as the intra-row species. Planting was completed on 28 June 96. The site is located at Surrey Hills, where the elevation is approximately 600m. The plot was inspected

in Nov '96. Due to the high elevation and consequent temperature depression, bud burst had not yet occurred, but was imminent. At that time the site was free of competing weed species and held ample moisture reserves.

The growth data was statistically analysed to determine which clones were the most vigorous. Details of the statistical analysis are given in Appendix 1.

No other clone management treatments were applied since the project direction was altered in October 1996. Clone types W3, G6 and H1 proved to have almost double the growth rate than the other five clones (W5, Q1, W4, Q4 and Q3). In addition, the statistical analysis showed that there were significant differences in survival rates between clones. Clonal ranking with respect to survival rate, from best to poorest, was G6, W3, W5, H1, Q4, W1, Q4 and Q3.

Nutrition Trial

A general view of treatment effect can be gained by examining the total biomass produced. The total biomass of the plants was taken as the weight of shoot + root material. The level of nitrogen was significant in determination of the total biomass. However, the effect of nitrogen and phosphorous (N*P) interaction was also significant. Figure 1 shows the treatments as ranked by biomass production.

The effect of nitrogen alone can be seen in Figure 2. At 10 mM N there is the greatest accumulation of total biomass. The same pattern is present for the increase in height over the trial period, as shown in Figure 3. In addition, the same pattern was observed for the three parameters in Figures 4-6. The number of leaves produced and the total lateral length produced per plant, with respect to nitrogen levels, show a similar trend to the effect of phosphorus treatment on number of leaves produced.

The results of the cumulative negative effect score is shown in Figure 7. No significant differences were noted between the treatments, however, there is some benefit in noting that a trend exits. Some treatments produce fewer negative effects than others. Treatments from the middle of the range tested showed fewer deleterious effects.

The relationship between apical death and no growth with treatment is shown in Figure 8.

These two parameters were significant in the statistical analysis. Again, there are treatments which produced neither of these effects, such as 11 and 12, which are near to full strength Hoaglands solution concentrations.

The relationship between the shoot to root ratio and treatment is given in the three parts of Figure 9. A highly significant treatment effect was observed. A greater proportion of shoots were produced in treatments 9, 14, 1, 5 and 13. Now, the appearance of the lowest levels of N and P used, in treatment 1, suggests that there was little if any root growth in these plants. The other treatments promoted shoot growth with balance between N and P levels. The N/P ratios in these treatments was 1.25 and 5. Treatments with higher N/P ratios produced less shoot material.

Figure 1

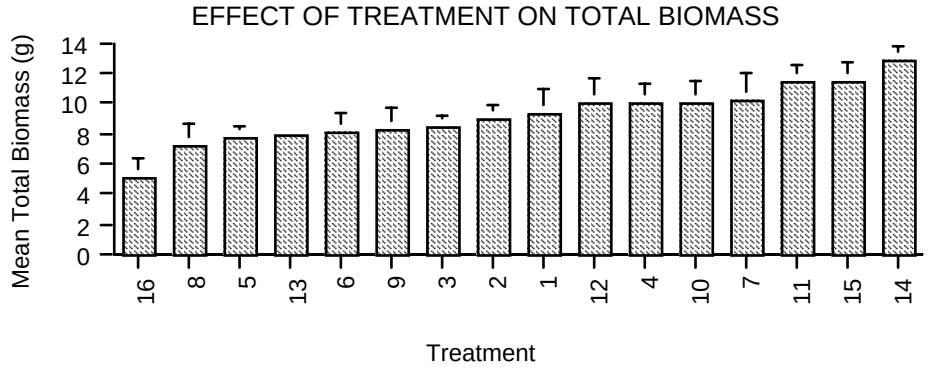


Figure 2

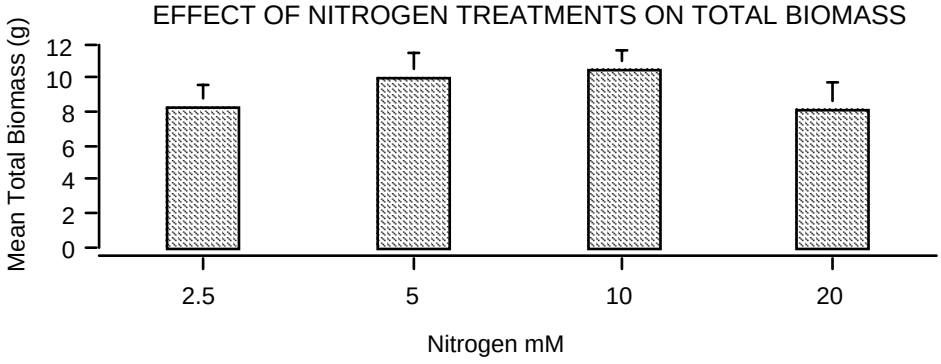


Figure 3

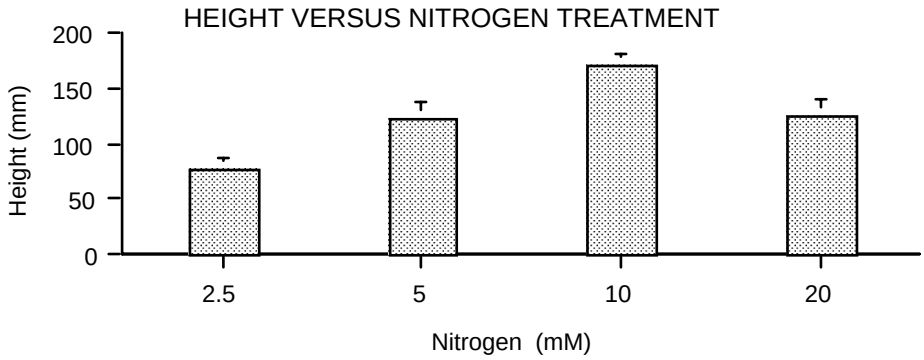


Figure 4

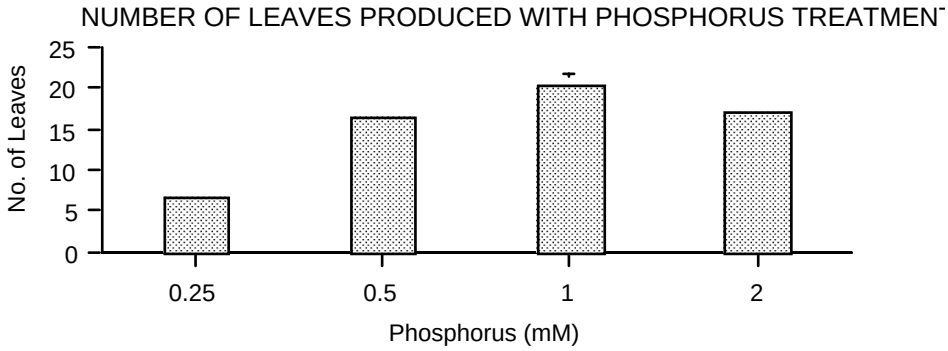


Figure 5

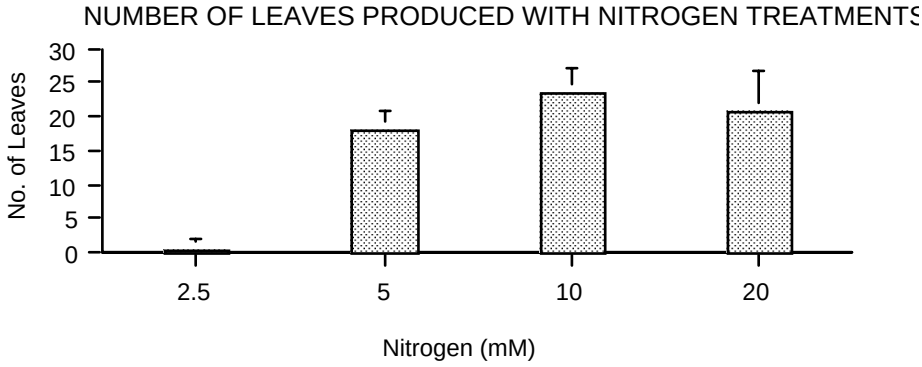


Figure 6

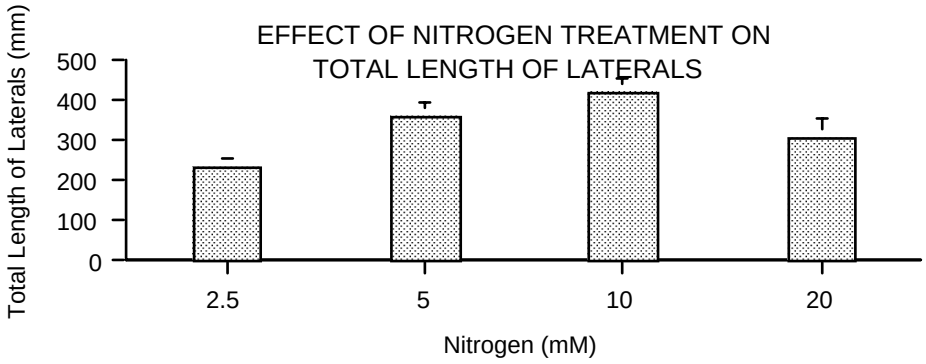


Figure 7

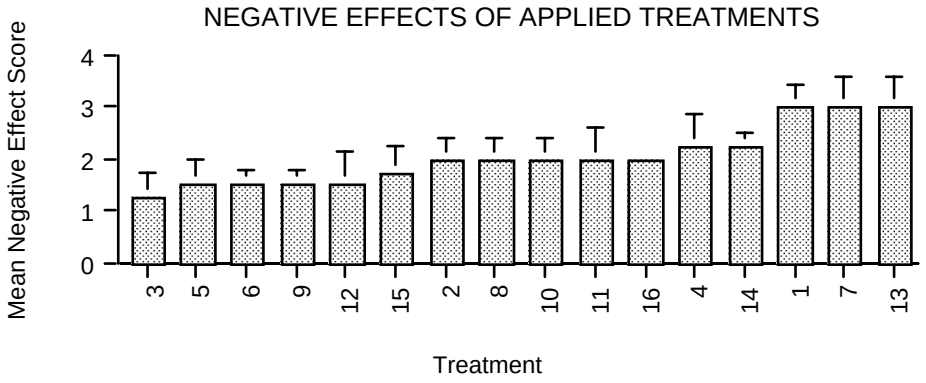


Figure 8

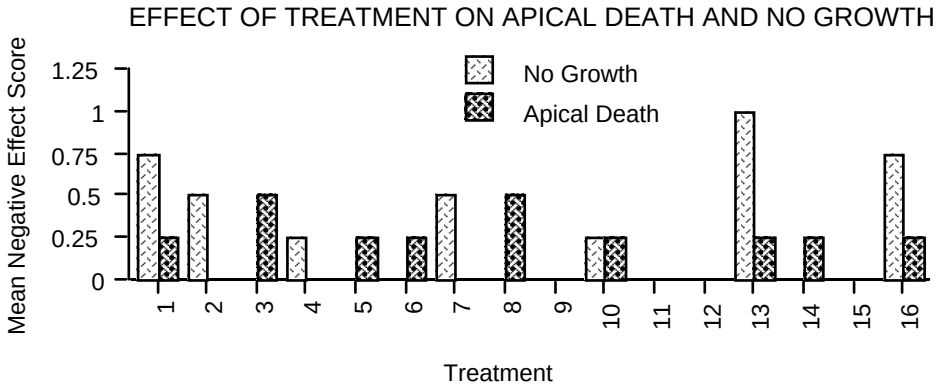


Figure 9(a)

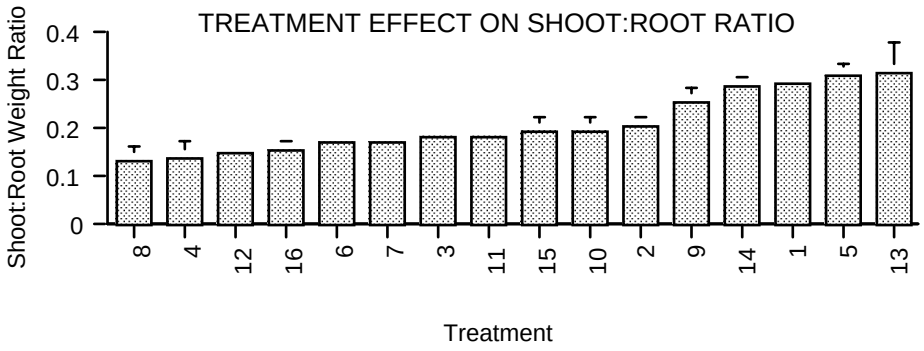


Figure 9(b)

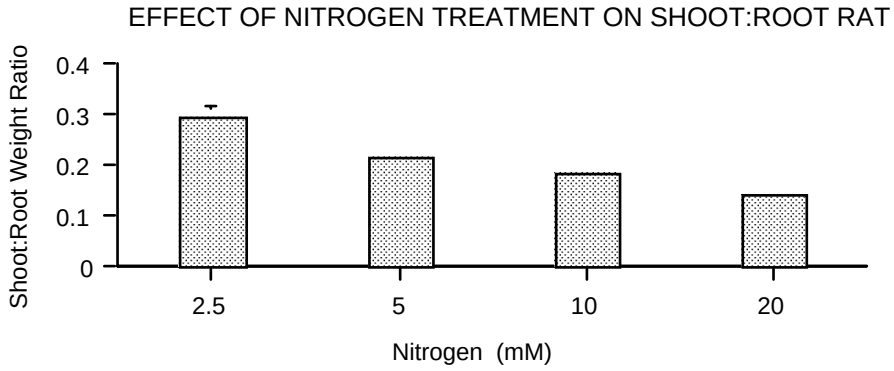
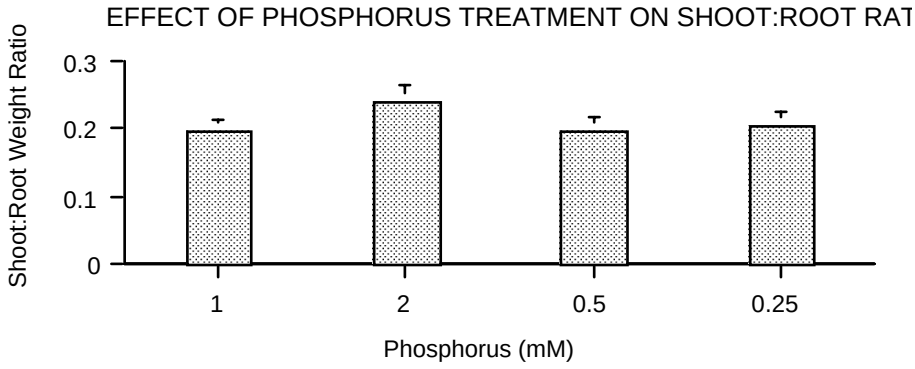


Figure 9(c)



The Effect of Harvest Time on Extract Composition

The gas chromatographic analyses of essential oil samples produced peak area data which were converted to percentage of the total peak area. These percentages were then used in the

principal co-ordinate and cluster analyses. Absolute values of concentration were not determined, since it is the relative concentrations that are of interest.

The level of monoterpenes present in the extracts was determined by summing the percentage concentrations of all components which eluted before α–copaene (retention time 10.5 min). The percentage sesquiterpenes is equivalent to {100 - (% monoterpenes)}, and analyses were performed only on the monoterpene data, since the inverse would be reflected in the analysis of sesquiterpene results. Table 6 shows the % monoterpenes and % polygodial for each plant. The variation in % polygodial for each plant during the trial is shown in Figure 10. The results of the cluster analysis are given in Table 7 and Figure 11. Figure 12 shows the biosynthetic pathways to calamenene.

Table 6

Mean Over Seven Samples of Total Percentage Polygodial and Monoterpenes in Extracts From Each Plant

Plant No.	% Polygodial	% Monoterpenes
1	25.241^{a*}	2.321^{b*}
2	3.242^{b}	2.797^{ab}
3	1.005^{b}	1.954^{b}
4	1.321^{b}	1.821^{b}
5	37.507^{a}	3.569^{a}
6	40.977^{a}	3.749^{a}
LSD	7.917	1.164

* Note: Means with the same letter are not significantly different (p=0.05)

Table 7

Result of Cluster Analysis

The four group solution is shown by the outlines.

The numbers 1-42 represent the plant × time (day) combinations as shown.

Time (Days) \ Plant	1	2	3	4	5	6
1 (0)	1	8	15	22	29	36
2 (91)	2	9	16	23	30	37
3 (111)	3	10	17	24	31	38
4 (137)	4	11	18	25	32	39
5 (154)	5	12	19	26	33	40
6 (189)	6	13	20	27	34	41
7 (230)	7	14	21	28	35	42

Figure 10

Percentage Polygodial Over Time

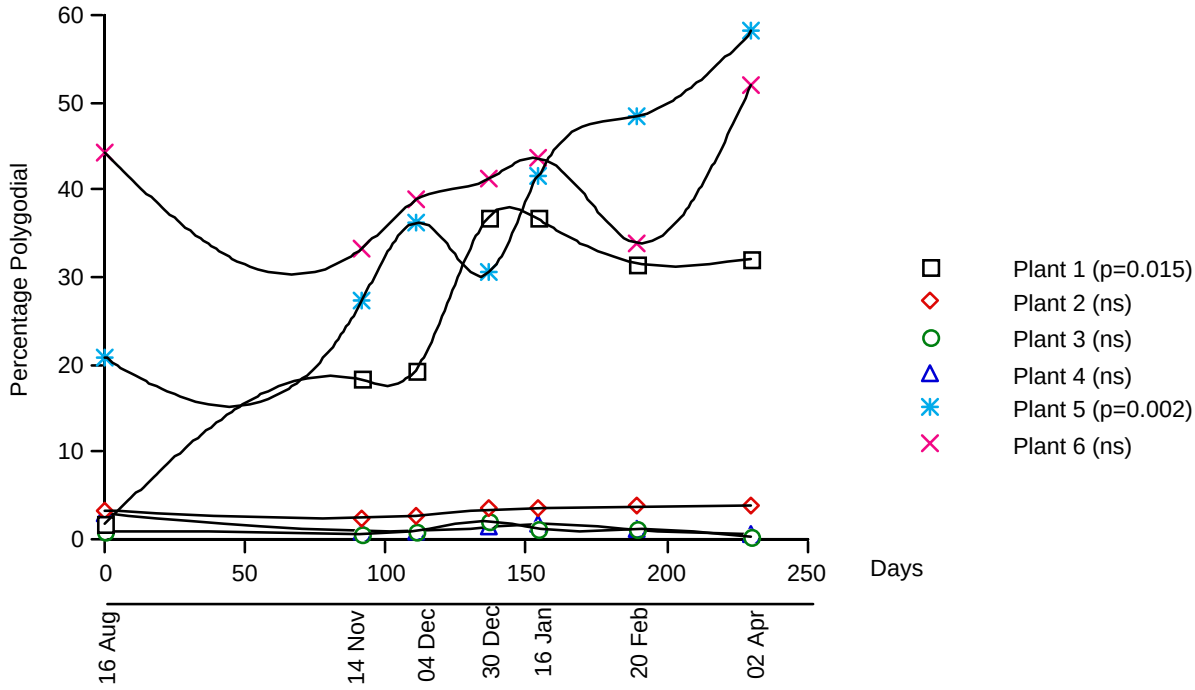


Figure 11

Graphic Illustration Of The Cluster Analysis Results

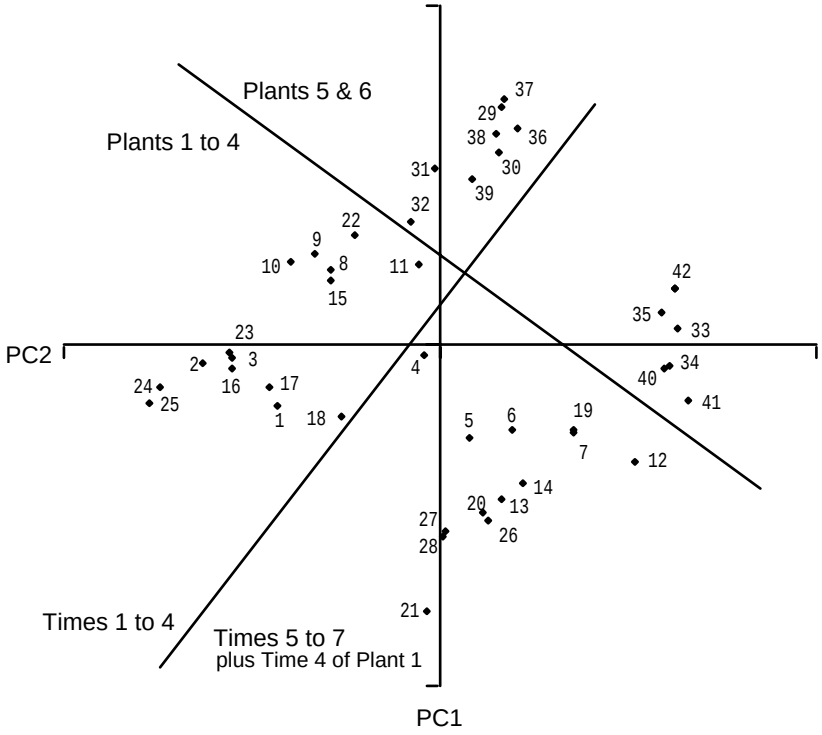
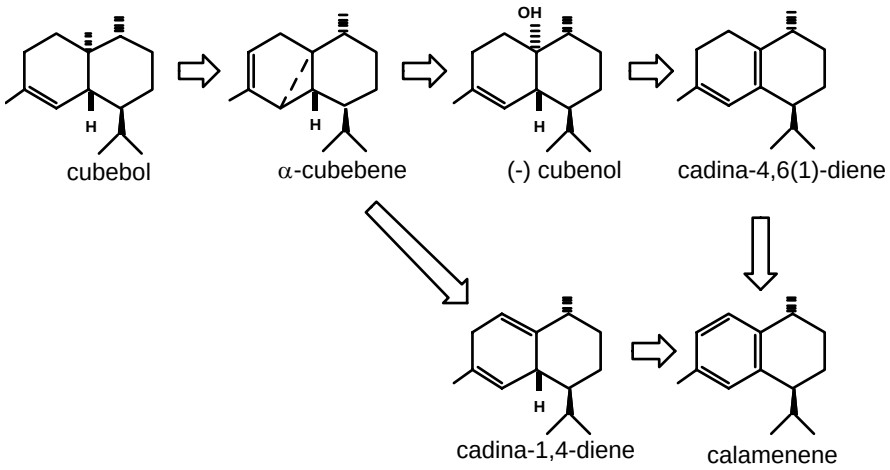


Figure 12

Biosynthetic Pathways To Calamenene



Seasonal Variation in Clones

Analyses Method Development

Method development associated with super critical fluid extraction investigated the effect of increasing the percentage of methanol introduced into the extraction solvent (carbon dioxide) under super critical conditions of temperature and pressure.

The recoveries of components of the leaves was assessed by grouping chemicals in the FID spectrum by chemical types and summing the areas of the peaks eluting within the associated retention time periods .

Chemical type	Retention time range
monoterpenes	4 to 6.12 minutes
oxygenated monoterpenes	6.12 to 9.60 minutes
sesquiterpenes	9.60 to 12.84 minutes
oxygenated sesquiterpenes	12.84 to 20.89 minutes
diterpenes	20.89 to 22.61 minutes
waxes etc.	22.61 to 30 minutes

Results were expressed as the % recovery of components from the dry weight of the leaf extracted. Tables 8 and 9 show the effect of changing the parameters on the recovery volatile components.

Table 8

Effect of Increasing the % Methanol Included as Modifier on Components

%modifier	5	8	10	12	15
% yield	1.7	1.8	2.3	2.2	1.7
% volatiles	3.6	4.1	2.8	2.1	2.2
% monoterpenes	0.02	0.02	0.026	0.03	0.027
% oxy.monoterpenes	0.013	0.022	0.026	0.031	0.027
% sesquiterpenes	0.06	0.081	0.081	0.082	0.063
% oxy. sesquiterpenes	1.29	1.29	0.75	0.68	0.58
% diterpenes	0.18	0.17	0.25	0.26	0.18
% waxes	0.21	0.23	0.36	0.43	0.24

Table 9

Effect of Increasing the SFE Pressure on Components

Pressure (atms)	**100**	**200**	**320**	**400**	**500**
% yield	1.5	2.7	1.9	1.1	0.9
% volatiles	1.3	2.4	1.6	0.9	0.8
% monoterpenes	0.005	0.046	0.014	0.016	0.022
% oxy. monoterpenes	0.043	0.105	0.079	0.045	0.032
% sesquiterpenes	1.1	2.0	1.3	0.7	0.6
% oxy. sesquiterpenes	0.004	0.011	0.017	0.009	0.006
% diterpenes	0.15	0.27	0.18	0.11	0.09
% waxes	0.18	0.36	0.29	0.16	0.11

All the samples from the experiment were analysed by GC MSD for safrole to determine the recoveries of the analyte under a range of extract conditions. The effect of change in the parameters of pressure and modifier on the recovery of safrole are shown in table 10 and 11.

Table 10

Effect of Pressure on Safrole Recoveries

Pressure (atm)	**100**	**200**	**320**	**400**	**500**
% recovery	24.5	70.8	56.3	57.9	51.2

Table 11

Effect of % Modifiers on Safrole Recoveries

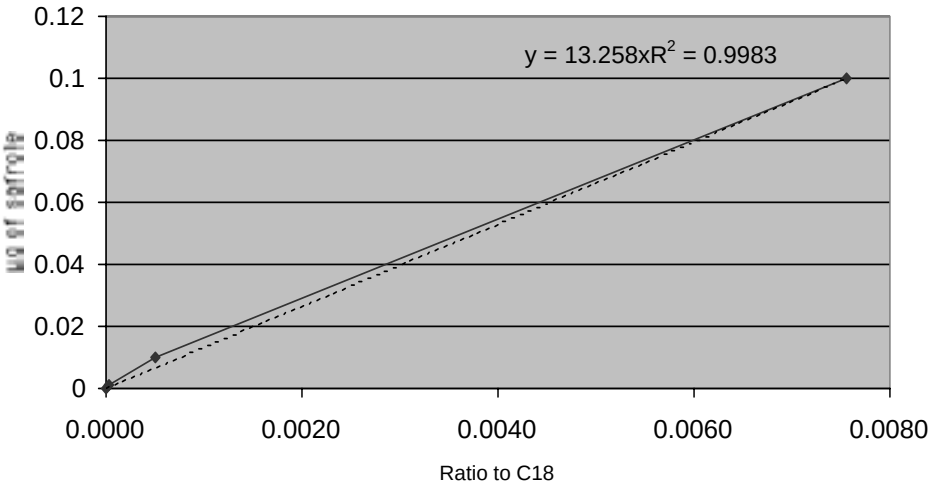
Modifier	**5%**	**8%**	**10%**	**12%**	**15%**
% recovery	49.8	48.42	70.05	46.18	48.97

Analyses of Samples

The standard curve was constructed, relating the weight of safrole to the ratio of safrole to C_{18}, as shown in Figure 13.

Figure 13

Standard Curve Relating Weight Of Safrole to Safrole:C_{18} Ratio



Sample 2 of the precision experiment was rejected since a leak was detected during the extraction cycle. Results are given in Table 12.

Table 12

Precision Experiment Results

	% volatiles rel. C18	safrole ppm	%volatiles rel. to trace	% yield rel. C18	% PG rel. to C18
repeat 1	1.15	0.28	20.24	5.68	3.57
repeat 3	1.00	0.40	21.68	4.61	2.94
repeat 4	1.16	0.40	20.78	5.56	3.66
repeat 5	1.05	0.34	20.86	5.01	3.26
average	1.09	0.36	20.89	5.21	3.36
st. dev.	0.08	0.06	0.60	0.50	0.33
% covariance	7.1	16.2	2.9	9.5	9.7

Sample Results

Table 13 lists the results for safrole content over the period of the experiment and those results are illustrated in Figure 14.

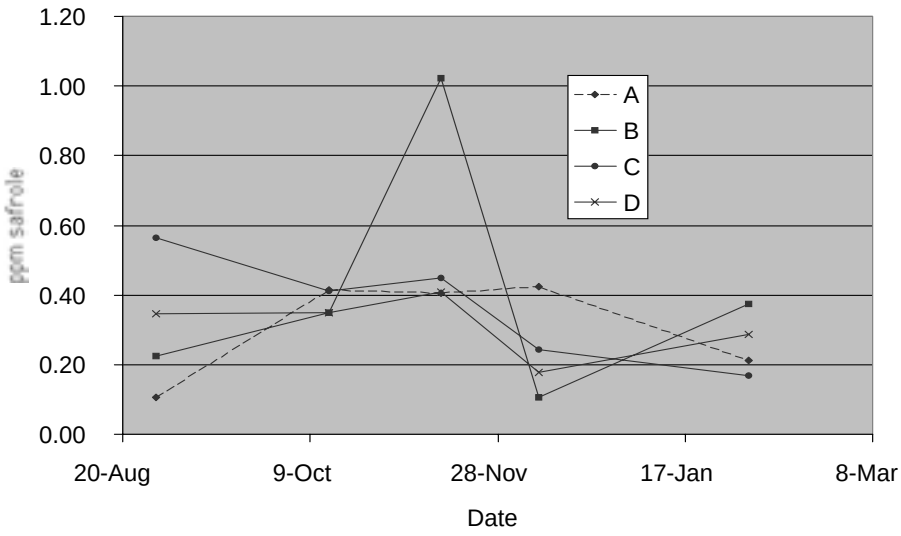
Table 13

Safrole Over Time

replicate	A	B	C	D
29-Aug	0.107	0.225	0.564	0.346
14-Oct	0.415	0.350	0.413	0.349
13-Nov	0.405	1.022	0.448	0.409
9-Dec	0.423	0.105	0.242	0.178
3-Feb	0.211	0.373	0.167	0.287

Figure 14

Safrole vs Time



Yield

Table 13 and Figure 15 show the % yield of the extraction based in the response of all the peaks relative to the internal standard. The following equations were used to calculate % yield and % volatiles.

$$\% \text{ Yield} = \frac{\text{total area} - \text{area } C_{18}}{\text{area } C_{18}} * \frac{\text{mg } C_{18}}{\text{mg Sample}} * 100$$

$$\% \text{ Volatiles} = \frac{\sum \text{area peaks} < C_{18}}{\text{Total area} - C_{18}} * 100$$

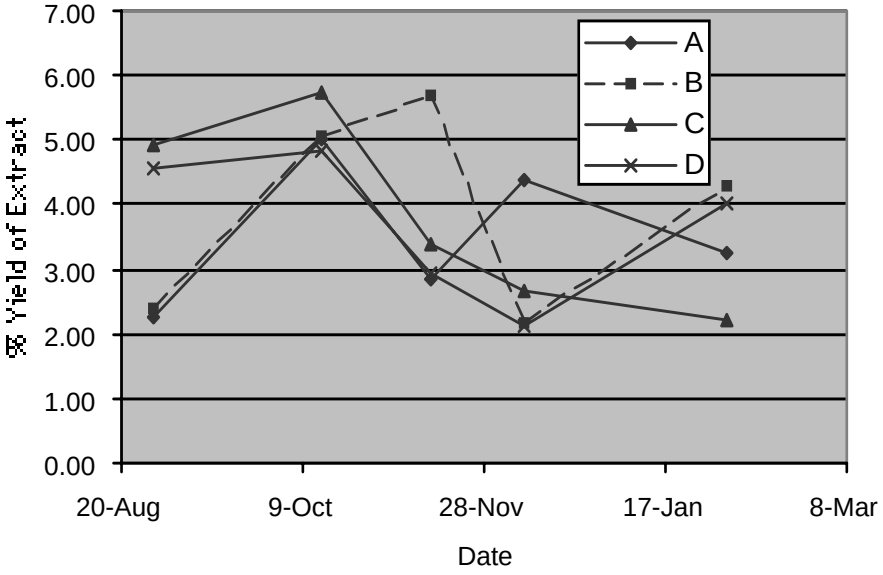
Table 13

Percentage Yield of Extract Over Time

replicate	A	B	C	D
29-Aug	2.24	2.38	4.94	4.55
14-Oct	4.99	5.04	5.72	4.84
13-Nov	2.85	5.67	3.41	2.95
9-Dec	4.40	2.17	2.68	2.14
3-Feb	3.27	4.30	2.20	4.04

Figure 15

Percentage Yield of Extract vs Time



% Volatiles in GC Profile

Table 14 and Figure 16 illustrate the % volatiles in the total amount of peaks detected by GC FID.

$$\% \text{ Volatiles} = \frac{\sum \text{area peaks} < C_{18}}{\text{Total area} - C_{18}} * 100$$

Table 14

Percentage Volatiles Over Time

replicate	A	B	C	D
29-Aug	19.76	15.22	21.96	21.19
14-Oct	20.72	21.23	18.78	19.96
13-Nov	15.63	18.82	16.77	17.51
9-Dec	18.88	16.77	19.01	17.64
3-Feb	18.07	21.29	19.91	19.96

Figure 16

Percentage Volatiles vs Time

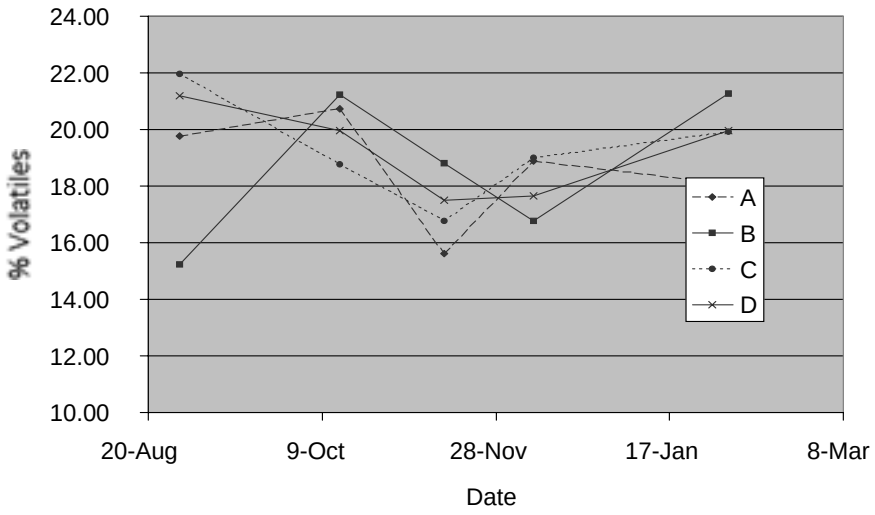


Table 15 and Figure 17 show the results for the polygodial (PG) calculated as a percentage of the total area of the GC trace.

$$\% \text{ PG} = \frac{\text{area PG}}{\text{Total area - } C_{18}} * 100$$

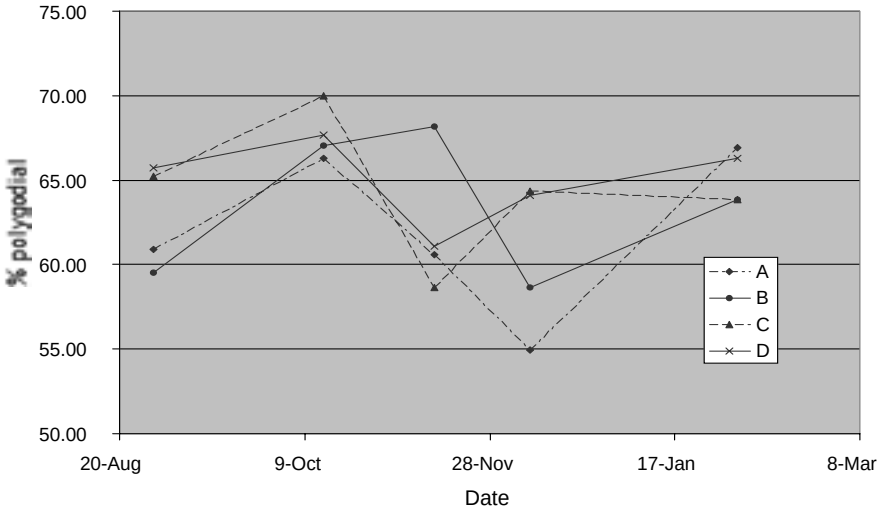
Table 15

Percentage Polygodial (%PG) Over Time

replicate	A	B	C	D
29-Aug	60.88	59.50	65.23	65.72
14-Oct	66.30	67.06	70.02	67.70
13-Nov	60.56	68.18	58.62	61.09
9-Dec	54.96	58.62	64.36	64.08
3-Feb	66.89	63.83	63.84	66.26

Figure 17

Percentage Polygodial vs Time



The average of each parameter over the 4 replicates is listed in Table 16

Table 16

Mean Values For Each Parameter Calculated

Date	% yield	safrole	% volatiles	% PG
29-Aug	3.53	0.31	19.5	62.8
14-Oct	5.15	0.38	20.2	67.8
13-Nov	3.72	0.44	17.2	62.1
9-Dec	2.85	0.19	18.1	60.5
3-Feb	3.45	0.32	19.8	65.2

Of the factors analysed, the % polygodial was marginally significant, and the others were not significantly different over the course of the trial. The % polygodial peaked at the October sample.

Chemical Characterisation and Product Development

Column Chromatography and Extract Fractionation

The fractions obtained from the three column runs were organoleptically assessed.
The results are shown in Table 17.

Table 17

Organoleptic Impressions of Fractions from Methanol Partition

Fraction/Run	Organoleptic Impressions
A1	Pine like with slight sweetness, fresh, green
B1	Menthol/Eucalyptus, floral
C1	Spicy, nutmeg and eucalyptus
D1	Sweet, woody, warm, slight spice
E1	Sweeter, caramel, slight spice
F1	Very sweet, almost vanilla, unpleasant, coffee liqueur
A2	As A1, (not much odour)
B2	Like C1 more than B1 (eucalyptus)
C2	Like D1, with E1 (sweet)
D2	Simpler than D1, E1 or F1, slightly sweet, almost like varnish
E2	Sweet, caramel, vanilla, slight spice, coffee liqueur
F2	Very little odour
A3	As A1 and A2
B3	As B2
C3	As C2 (eucalyptus, lantana like)
D3	Similar to C3 but sweeter
E3	Similar to D3 but much sweeter and heavier, caramel
F3	Sickly sweet to floral, vanilla
G3	Coconut, sweet
H3	Coconut, sweet, lime

The fractions which contained polygodial were D1, E1, C2, D2, C3 and D3, as assessed by TCL and GC analysis. These were re-run on the silica column to give several fractions containing polygodial, from which fine white crystals were isolated, as detailed in the Methods section.

HPLC Fractions

Hexane Partition
Fraction 7
Odour impressions:

1. mossy, undergrowth, woody
2. sharp, antiseptic, woody
3. sharp, woody
4. fruity, woody, lantana?
5. sweet caramel
6. spicy, fruity, woody, green colour
7. capsicum
8. tomato, spicy, rounded, woody

Fraction 8

Odour impressions:

None were lantana-like

Methanol fractions

This fractions contains most of the odour contributors, and many unknown compounds.

Subfraction
1. lantana
2. spicy
3. fruity, floral
4. woody, disturbed leaves, becoming pine-like
5. like 4
6. cloves
7. cloves
8. musty and cloves

Identification of Components

Figure 20 shows the total ion trace of an on column injection of a commercial extract of *T. lanceolata*. This is the compilation of peaks identified by their mass spectra acquired from less complex fractions prepared by column chromatography and fractional distillation of the oil. Table 18 correlates peak number with the peak identity.

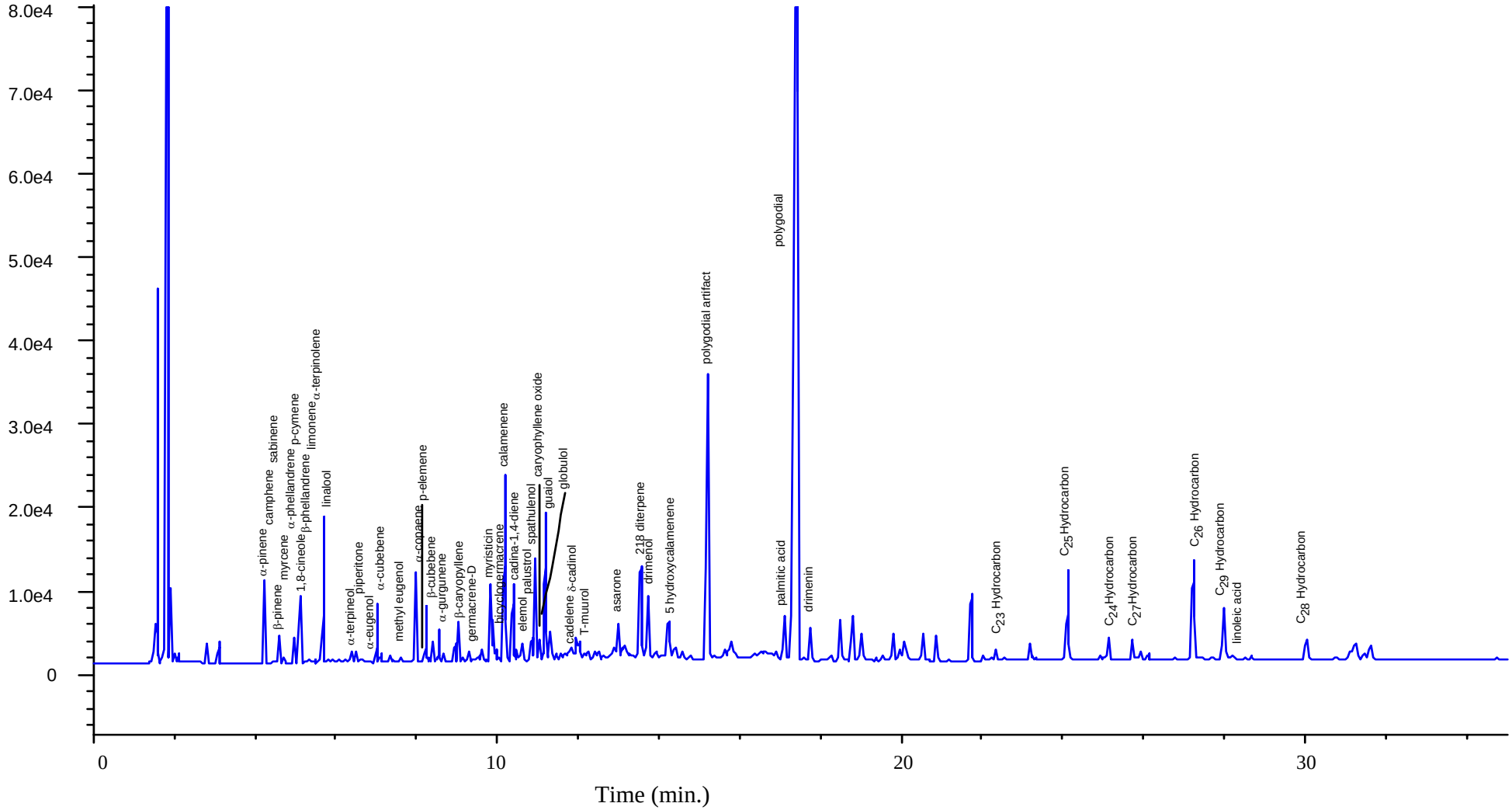


Figure 18

Gas Chromatogram Identifying Components of *T. lanceolata* extract

The Stability of Polygodial in Ethanol

When polygodial is analysed by gas chromatography, the high temperatures within the injection chamber cause polygodial to degrade such that 2 peaks are recorded. Table 19 lists the peak areas obtained for the internal standard, the two peaks recorded for polygodial and the percentage of polygodial detected in a 100% hexane solution and a 70 % ethanol solution.

Table 19

Breakdown of Pure Polygodial in Ethanol.

100%Hexane					
Time	C18 Area	polygodial degrad. Product area	polygodial area	Sum polygodial :C18 Ratio	%PG
3mins					
2hrs34mins	247432	51856	55784	0.44	54.56
30hrs16mins	242055	62550	46321	0.45	56.41
46hrs44mins	266795	60352	39741	0.38	47.06
70%EtOH					
3mins	237571	57249	44281	0.43	53.60
36mins	235781	58365	46788	0.45	55.94
1hr11mins	235993	57843	47988	0.45	56.25
24hrs44mins	248724	60738	41888	0.41	51.75
113hrs11min	248402	64768	39815	0.42	52.81

Table 20 reports the results for a similar experiment conducted for polygodial breakdown in the matrix of the 3 different standard oil extracts, when solvated in hexane and an ethanol solution.

Table 20

Time Series Expt. of Polygodial in *T. lanceolata* Extract.

Time	Area internal standard	Polygodial product Area	Polygodial Area	Sum Polygodial Ratio	Percentage Polygodial
STD 1 hex					
4mins	259391	339976	384112	2.79	19.23
8hrs21min	279983	449434	293652	2.65	18.28
96hrs49mins	296018	496039	288352	2.65	18.25
STD 1 EtOH					
1min	248608	320020	248011	2.28	18.33
1hr43mins	248315	331966	231496	2.27	18.20
5hrs43mins	247523	385192	204330	2.38	19.10
94hrs36mins	261924	381948	180393	2.15	17.22
STD 2 EtOH					
3mins	243716	373460	267703	2.63	21.01
1hr54mins	245943	394785	242196	2.59	20.68
5hrs56mins	255783	433908	225258	2.58	20.58
94hrs25mins	256827	435880	206866	2.50	19.98
STD 3 EtOH					
2mins	236930	412108	362813	3.27	24.39
1hr49mins	239953	456805	326071	3.26	24.33
5hrs53mins	247749	525870	295161	3.31	24.71
94hrs19mins	249678	515598	268425	3.14	23.42

The Preparation of an Absolute from *T. lanceolata* Extract

The absolute produced was a dark green/black extract, less viscous than the concrete. The polygodial content in the absolute was slightly higher (27.22% volatiles) than in the concrete from which it was produced (25.4% volatiles). Many of the monoterpenes, such as α and β-pinene were absent in the absolute, leaving the oil with a stronger spicy note, which is usually associated with the domination of sesquiterpenes in the odour profile.

Extract Decolourisation

Five of the lightest coloured extracts were selected for organoleptic assessment. They were samples 1, 2, 3, 9 and 15. The analytical results are given in Table 21. All five samples retained a strong characteristic aroma. The spicy element associated with the flavour was also still present, since polygodial losses were minimal.

Overall, sample 3 appears to provide the most promising combination of good colour, sufficient polygodial and volatiles, and a solvent that is relatively simple to handle.

Table 21

Light Coloured Extracts

	1	2	3	9	15	Standard
Solvent	Hex	Hex	Hex	EtOAc	Hex/EtOAc	
Charcoal (g)	0.2	0.6	1.5	1.5	1.5	
% volatiles	53.19	53.17	57.77	50.97	51.45	49.97
% polygodial	33.96	30.79	25.95	32.19	30.65	31.95
% recovery	97.67	91.00	80.67	90.67	87.00	

Production of an Extract from T. lanceolate Berries

The berries of *T. lanceolata* yielded 8.2% of a pale yellow oil. Not all components identified in the leaf extract were present in the oil extracted from the berries. The berry oil was found to have a relatively higher proportion of monoterpenes than in the leaf extract, however it had relatively lower levels of the sesquiterpenes. Polygodial constituted 45% of the volatiles. An analysis was conducted for safrole which was found to be present at levels of 23 ppm (st.dev. 0.6)

The olfactory assessment of the oils noted the domination of the monoterpenes in the aroma profile giving an initial impact of bushy green. The background note of spice is attributed to the sesquiterpenes and a strong after note of citrus was recorded.

Product Registration

The preliminary registration application was sent to FEMA at the beginning of March 1997. The information gathered is contained in Appendix 6. The response to date has been a confirmation of receipt of the application, and suggestions that there may be a revised protocol for registration of complex extracts such as this one. Information was also required on toxicology studies.

DISCUSSION

Survey and Establishment of Database

Survey of the natural stands of *Tasmannia lanceolata* around the state has been of benefit in establishing the variable nature of the species and in gaining an understanding of how the variation is distributed. With such a large range of genetic material on hand, the possibility exists for future breeding work. A well conducted program would result in clones that are both vigorous, and exhibit the flavour and aromatic characters that are demanded by the clients.

Product Specification

The issue of degradation of polygodial in ethanol has now been clarified. Since there was no appreciable change in the level of polygodial over time in extracts that had been dissolved in ethanol, there are positive implications for the processing of an absolute.

The product specification, as described in the results, is an effective guideline for the selection of clones that produce acceptable extracts.

Clonal Selection

The database and clonal holding area have become valuable tools for the selection of plants with certain characteristics. The range of extracts available is extensive and incorporates those with unusual aromas.

Propagation

It was clear from the propagation work that some clones are more difficult to strike than others. The ease of propagation is considered a secondary factor in making good selections. Nonetheless, it must be considered in extreme cases, where the strike rate is below 50%. It may be prudent to consider propagation via tissue culture in these instances, and preliminary work has shown that a procedure for tissue culture is feasible.

Plantation Establishment

Observations of the two plantation areas confirmed that growth is slow in this species, and establishment rates could be increased through judicious timing of planting and selection of plants with well developed root systems.

The Winnaleah site showed that establishment can be difficult where the competition from weeds is great, and where water is not readily available during the preliminary establishment phase. A replacement rate of 33% is clearly too high.

The replacement rate at Ridgley was similar, however, it can be attributed more to plant size than moisture availability factors.

On the basis of these types of experiments, selections can be made such that vigorous clones are used in plantation situations. For instance, from the Ridgley site data analysis, the clones W3, G6 and H1 are both vigorous and have good survival rates compared with the other clones tested. The clone Q3 had both the lowest survival rate (72%) and the lowest growth rate.

No information was gathered from the Winnaleah site.

Nutrition Trial

The observed effects of low nitrogen levels (~2.5 mM) were reduced biomass, height and number of leaves. In addition, a high shoot to root ratio due to a lack of root growth was noted.

High nitrogen levels (~20 mM) produced similar effects to low nitrogen, with the exception of the shoot to root ratio shift.

High phosphorus levels (~2.0 mM) resulted in a decrease in leaf number. In conjunction with either extreme of nitrogen level an increase incidence of apical death and no new growth were observed.

Low phosphorus (0.25 mM) gave similar symptoms to high phosphorus.

Healthy, vigorous growth occurred at the nitrogen and phosphorus levels, which coincide with full strength Hoaglands solution levels, namely 1.0 mM P and 10 mM N.

The Effect of Harvest Time on Composition

Extracts from plants 5 and 6 had significantly higher levels of monoterpenes than that from plants 1, 3 and 4. The extract from plant 2 had an intermediate percentage

monoterpenes, which was not significantly different from either of the other two groups. Over all, the percentage recovered monoterpenes is below the population average of 7.95%, derived from samples of plants within the Arve Loop, and the adjacent Hartz Mt. Road area.

The changes in percentage polygodial content for each plant are shown as averages in Table 15, and as individual values over the course of the experiment, in Figure 17. It is clear that there are two distinct groups of plants, one with a relatively higher content of polygodial than the other. Plants 2, 3 and 4 form a group with a polygodial content of less than 5%. The regression equations for these plants do not have a significant slope, so total polygodial is not dependent upon the number of days elapsed. Plants 1, 5 and 6 have a polygodial content in excess of 20%. Of these, plant 6 does not have a significant regression with time, whereas plants 1 and 5 do.

The outlines shown within Table 7 are derived from the four group solution of the 42 member plant x days matrix by the TAXON analysis. The numbers 1-42 were used to represent the plant x day combinations. The clustering broadly shows that plants 5 and 6 differ from all the other plants. In both groups, the later sampling times were significantly different to the early ones, with the exception of the fourth sample from plant 1.

The question of whether the six plants were the same, in a chemical sense, and whether the chemical composition was affected by the passage of time was thought to be answerable by the use of principal co-ordinate (PC) analysis. The results of the PC analysis are shown in Figure 11 where the first two principal co-ordinates are represented by the axes PC1 and PC2. The lines drawn on the diagram delimit the four group solution from the cluster analysis. The four groups are as follows. Times 5 to 7 plus time 4 of plant 1; times 1 to 4 of plants 1 to 4, excluding time 4 of plant 1; plants 5 and 6 at time 1 to 4; and plants 5 and 6 at times 5 to 7.

The seven most influential components contributed 20% to the separation of the early times from the later ones. Among these were calamenene and germacrene-D, with the remainder being unknown sesquiterpene components. The remaining 80% of the

separation was effected by the remaining components, with no single component contributing more than just a few percent to the separation.

Similarly, only 20% of the separation of plants 5 and 6 from plants 1 to 4, within times 1 to 4, was accounted for by the six most influential components. Among these was polygodial, the others being unknown sesquiterpenes. None of the remaining components contributed more than a few percent to the separation.

Within the later three times, plants 5 and 6 were separated using six components with a similar contribution to the separation as in the early times. Polygodial, germacrene-D and eugenol were the identified components.

If polygodial alone is used as the criterion for separation, the obvious solution would put plants 2, 3 and 4 in one group and 1, 5 and 6 in another, as shown in Figure 10 and Table 6. By including as many components as are available, the grouping is refined to plants 5 and 6 being separate from the remaining four. The analysis must include as many of the components as possible when contemplating the question of chemical similarity, in order to give a complete representation of the extract. This would be particularly true when using this sort of information for taxonomic purposes. Southwell and Brophy [10] state that *Tasmannia lanceolata extracts* are predominantly monoterpenic, with 1,8-cineole predominating. This study, and other unpublished data associated with this project, indicate that sesquiterpenes are predominant in the Tasmanian plants, with monoterpenes contributing only 5% to the total extract. In addition, the concentration of polygodial approached 40%, in some instances. This compound has been isolated and purified and was found to be a principal contributor to the peppery taste of the leaves [11]. Polygodial has also been isolated in our laboratories, confirming its hot, spicy character.

The behaviour of polygodial content over the growing period shows that:

a) there are two patterns of response. Polygodial levels may either increase or stay constant over time. Groups of plants were found which exhibited each of these trends.

b) there are plants which have levels of polygodial with a mean of around 30%, and others which have a much lower mean, 2-3%.

It should, therefore, be possible to select plants that either have a constant high level of polygodial, or in which the level increases during the season. However, the overall balance of flavour components will ultimately determine which plant types are selected for commercial purposes.

The correlation analysis of component peak areas produces an array of relationships between the components of the extract. The strongest correlations were unknown peak #38 with #84 (-0.80), #82 with #15 (0.89), δ–cadinol with #61 (0.88) and #177 with #183 (0.87). α–cubebene is negatively correlated with both calamenene and unknown peak #84 (-0.74, and -0.73, respectively). α–cubebene is the precursor for calamenene, via one of two pathways, as shown in Figure 12. One path requires the conversion of α–cubebene to (-)cubenol, to cadina-4,6(1)diene and then to calamenene. The other path involves cadina-1,4-diene as the intermediate. The negative correlation may be a result of this duality, which gives calamenene production the potential to be independent of cadina-1,4-diene synthesis. Calamenene is also negatively correlated with components #93, #80 and #85 (-0.76, -0.74 and -0.60 respectively).

Among the important positive correlations are α–cubebene with cadina-1,4-diene (0.81). From the schematic of the biosynthetic pathways, α–cubebene is the precursor for cadina-1,4-diene. The regulation of these pathways produces the situation where there is a negative correlation between the quantities of α–cubebene and calamenene and a strong positive correlation between α–cubebene and a possible precursor of calamenene, cadina-1,4-diene. The correlation between cadina-1,4-diene and calamenene is -0.55, which may point to the fact that the production of calamenene can be achieved via an alternate route. No strong correlations with the polygodial peak were observed.

There are many relationships between components which could be studied further. However, a pre-requisite for such work would be the identification of the unknown compounds in question.

In summary, the variations observed within a small population of *Tasmannia* sp. was demonstrated through the use of principal co-ordinate analysis and clustering. The polygodial content of the extract may be either high or low. At low polygodial, there is no significant change in concentrations with time. In the high polygodial group, the concentration may increase or remain constant over the course of the growing season.

Seasonal Variation in Clones

Method Development

Super Critical Fluid Extraction - 10% modifier optimised the recovery of safrole, although it reduced the extraction of oxygenated sesquiterpenes. Although the optimum pressure for all components, except the heavy end waxes, appeared to be 200 atmospheres this pressure was not used in the extraction of the samples. The experiment for which this method was developed was to look at the relative concentrations of extract components over time. The critical influence of the pressure around 200 atmospheres would have introduced error if analytical conditions had varied through the analyses.

Sample Analyses in Relation to Season

The outcomes from the safrole experiments indicate that the levels of safrole do not vary significantly during the growth cycle. This implies that clones with low levels of safrole may be harvested at any time without the risk of high levels of safrole being present.

The analysis of the 23 major components of the 182 examined, showed that only eight of these varied significantly over the experimental period. However, the times at which a maximum was reached varied from one peak to another. In general, maxima were reached in spring rather than in the summer months. Peaks at retention times 11.28 (guaiol), 11.95, 14.11 (218 diterpene), 15.26, 16.13 (polygodial artefact) and 18.87 (drimenol related unknown) minutes all fell into this category.

Chemical Characterisation

Chromatography and Extract Fractionation

The complexity of the solvent extract of *T. lanceolata* is such that isolation and characterisation of each component was not possible within the scope of this project. However, the fractionation of the oil has allowed for the identification of many components by their mass spectra. The simplification of the aroma profile in these fractions has allowed for the assessment as to which chemicals contribute most to the quality of the typical *T.lanceolata* flavour.

The Stability of Polygodial in Ethanol

Results show that there is no appreciable degradation of polygodial in ethanol at room temperature over a period of almost 4 days. From this it may be assumed that an ethanol absolute may be prepared without significant loss of polygodial

Extract Decolourisation

The results of the decolourisation trials showed that the decolourising ability of charcoal is superior to Tonsil (a bleaching earth), in combination with the solvents used.

Also, as polarity increases, the % volatiles decrease. The greatest amount of volatile material is retained with the use of hexane. Volatiles are also influenced by the quantity of charcoal.

Retention of polygodial is important since this component imparts the characteristic spicy nature to the extract. As the level of charcoal is increased, the polygodial content decreases. Ethyl acetate treatment removed the least polygodial, followed by the hexane/ethyl acetate mixture and hexane removed the most.

Overall recovery followed the same pattern as polygodial content. Recovery was greatest with the smallest addition of charcoal and hexane. Recovery fell from 98% to 80% as charcoal increased from 0.2 to 1.5 g.

Production of Extract from *T. lanceolata* Berries

The extraction of *Tasmannia lanceolata* berries produced a light yellow oil with a pleasant bushy green fragrance, reflecting the relatively high levels of monoterpenes present. A spice note was none the less quite strong in the back ground, with a citrus aroma persisting after the oil had been exposed for a few minutes. Polygodial constituted 45% of the volatile fraction and safrole was present at levels of 8ppm.

Product Registration

Application was made through the Flavour and Extract Manufacturers' Association of the United States (FEMA) for registration of the *Tasmannia* extract on the Generally Recommended As Safe (GRAS) list. The information that was collated is shown in full in the Appendix 6.

A request for further toxicological information was received and is being addressed through contacts established by EOT.

IMPLICATIONS AND RECOMMENDATIONS

This project has identified the chemical parameters of extracts of *Tasmannia lanceolata* which is most critical for acceptance of the product on the world market. These are:

Low safrole content

Low piperitone content

High polygodial content

Spicy notes reminiscent of *Lantana* spp.

Absence of sweet floral or fruity notes

Concrete yield of 6% or more

The safrole content of the extract has been monitored and safety issues have been addressed. At the envisaged levels of usage, safrole will not present a problem.

Plantation trials and establishment of a comprehensive database has indicated that a viable industry utilising *Tasmannia lanceolata* will require the propagation of clones and establishment of plantations. Markets require the regular supply of a consistent product in terms of chemical composition and physical properties. It is unlikely that these parameters will be met with extracts from wild stands.

Growers should be encouraged to use plant material from clonal origins in order to be able to tailor the extracts to comply with established quality guidelines.

In conjunction with industry it is recommended that exposure of this product to local and overseas markets be continued. The sale of the concrete and absolute should be promoted with the presentation of new products, such as a decolourised extract and the flavoured canola oil.

Future research will further the registration bid to enable sales in the international marketplace. Other benefits will include safety and quality assurance as well as potential for product diversification. The toxicological studies, in particular, will be aid in the inclusion of *T. lanceolata* extract in anti-microbial preparations, where it has shown some potential.

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APPENDIX 1

STATISTICAL ANALYSIS FOR DATA FROM RIDGLEY SITE

N P A R 1 W A Y P R O C E D U R E
Analysis of Variance for Variable HEIGHT
Classified by Variable ID

ID	N	Mean	Among MS	Within MS
			874.020732	74.6730553
3	30	6.2666667		
5	23	1.8260870	F Value	Prob > F
6	29	5.2413793	11.705	0.0001
8	30	11.6000000		
4	32	13.6875000		
1	31	15.8709677		
7	24	2.1666667		
2	24	2.3333333		

Average Scores Were Used for Ties

Wilcoxon Scores (Rank Sums) for Variable HEIGHT
Classified by Variable ID

ID	N	Sum of Scores	Expected Under H0	Std Dev Under H0	Mean Score
3	30	2819.50000	3360.0	328.513761	93.983333
5	23	1538.00000	2576.0	292.814919	66.869565
6	29	2869.00000	3248.0	323.827814	98.931034
8	30	4184.00000	3360.0	328.51376	139.466667
4	32	4931.50000	3584.0	337.525009	154.109375
1	31	5136.50000	3472.0	333.077845	165.693548
7	24	1700.50000	2688.0	298.364016	70.854167
2	24	1797.00000	2688.0	298.364016	74.875000

Kruskal-Wallis Test (Chi-Square Approximation)
CHISQ = 73.136 DF = 7 Prob > CHISQ = 0.0001

Median Scores (Number of Points Above Median)
for Variable HEIGHT
Classified by Variable ID

ID	N	Sum of Scores	Expected Under H0	Std Dev Under H0	Mean Score
3	30	10.1111111	14.9327354	2.53301770	0.337037037
5	23	3.1111111	11.4484305	2.25776044	0.135265700
6	29	13.0000000	14.4349776	2.49688654	0.448275862
8	30	20.2222222	14.9327354	2.53301770	0.674074074
4	32	26.1111111	15.9282511	2.60249927	0.815972222
1	31	27.2222222	15.4304933	2.56820924	0.878136201
7	24	6.0000000	11.9461883	2.30054696	0.250000000
2	24	5.2222222	11.9461883	2.30054696	0.217592593

Median 1-Way Analysis (Chi-Square Approximation)
CHISQ = 64.275 DF = 7 Prob > CHISQ = 0.0001
Height Differences 90

Van der Waerden Scores (Normal) for Variable HEIGHT
Classified by Variable ID

ID	Sum of N	Expected Std Dev Scores	Mean Under H0	Under H0	Score
3	30	-6.8050242	0.0	4.99726005	-.226834140
5	23	-14.7787874	0.0	4.45421919	-.642555972
6	29	-6.8686506	0.0	4.92597873	-.236850020
8	30	11.7692158	0.0	4.99726005	0.392307192
4	32	19.4519739	0.0	5.13433665	0.607874185
1	31	24.6427709	0.0	5.06668763	0.794928095
7	24	-14.0669557	0.0	4.53863050	-.586123153
2	24	-13.3445428	0.0	4.53863050	-.556022617

Average Scores Were Used for Ties

Van der Waerden 1-Way Analysis (Chi-Square Approximation)
CHISQ = 66.918 DF = 7 Prob > CHISQ = 0.0001

Savage Scores (Exponential) for Variable HEIGHT
Classified by Variable ID

ID	N	Sum of Scores	Expected Under H0	Std Dev Under H0	Mean Score
3	30	-11.2972417	0.0	5.03384405	-.376574722
5	23	-13.8991169	0.0	4.48682769	-.604309429
6	29	-1.8090292	0.0	4.96204090	-.062380318
8	30	8.6052145	0.0	5.03384405	0.286840483
4	32	15.8128824	0.0	5.17192416	0.494152576
1	31	26.4568557	0.0	5.10377989	0.853446958
7	24	-12.2823685	0.0	4.57185696	-.511765353
2	24	-11.5871964	0.0	4.57185696	-.482799850

Average Scores Were Used for Ties

Savage 1-Way Analysis (Chi-Square Approximation)
CHISQ = 58.926 DF = 7 Prob > CHISQ = 0.0001

Kolmogorov-Smirnov Test for Variable HEIGHT
Classified by Variable ID

ID	N	EDF at Maximum	Deviation from Mean at Maximum
3	30	0.633333333	0.91450837
5	23	0.826086957	1.72515289
6	29	0.551724138	0.45965842
8	30	0.266666667	-1.09380768
4	32	0.156250000	-1.75429070
1	31	0.064516129	-2.23741493
7	24	0.750000000	1.38950876
2	24	0.708333333	1.18538461
	----	----------	
	223	0.466367713	

Maximum Deviation Occurred at Observation 116
Value of HEIGHT at Maximum 7.00000000

Kolmogorov-Smirnov Statistic (Asymptotic)
KS = 0.273161 KSa = 4.07917

Cramer-von Mises Test for Variable HEIGHT
Classified by Variable ID

ID	N	Summed Deviation from Mean
3	30	0.47738874
5	23	1.22751196
6	29	0.16001906
8	30	0.59609479
4	32	1.41775824
1	31	2.41642869
7	24	1.07848720
2	24	0.80053821

Cramer-von Mises Statistic (Asymptotic)
CM = 0.036656 CMa = 8.17423

General Linear Models Procedure
Class Level Information

Class	Levels	Values
ID	8	1 2 3 4 5 6 7 8

Number of observations in data set = 256

NOTE: Due to missing values, only 223 observations can be used in this analysis.

General Linear Models Procedure
Dependent Variable: HEIGHT

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	7	6118.145121	874.020732	11.70	0.0001
Error	215	16054.706897	74.673055		
Corrected Total		222	22172.852018		

R-Square	C.V.	Root MSE	HEIGHT Mean
0.275930	108.9945	8.641357	7.928251

General Linear Models Procedure

Dependent Variable: HEIGHT

Source	DF	Type I SS	Mean Square	F Value	Pr > F
ID	7	6118.145121	874.020732	11.70	0.0001
Source	DF	Type III SS	Mean Square	F Value	Pr > F
ID	7	6118.145121	874.020732	11.70	0.0001

General Linear Models Procedure
T tests (LSD) for variable: HEIGHT

NOTE: This test controls the type I comparisonwise error rate not the experimentwise error rate.

Alpha= 0.05 df= 215 MSE= 74.67306
Critical Value of T= 1.97
Least Significant Difference= 4.5978
WARNING: Cell sizes are not equal.
Harmonic Mean of cell sizes= 27.44716

Means with the same letter are not significantly different.

T Grouping	Mean	N	ID
A	15.871	31	1
A			
A	13.688	32	4
A			
A	11.600	30	8
B	6.267	30	3
B			
B	5.241	29	6
B			
B	2.333	24	2
B			
B	2.167	24	7
B			
B	1.826	23	5

SURVIVAL DATA

N P A R 1 W A Y P R O C E D U R E
Analysis of Variance for Variable RESP
Classified by Variable CLONE

CLONE	N	Mean	Among MS	Within MS
			0.404604906	0.104846872
3	32	0.93750000		
5	32	0.71875000	F Value	Prob > F
6	31	0.90322581	3.859	0.0005
8	32	0.93750000		
4	32	1.00000000		
1	32	0.96875000		
7	32	0.75000000		
2	32	0.75000000		

Average Scores Were Used for Ties
Wilcoxon Scores (Rank Sums) for Variable RESP
Classified by Variable CLONE

CLONE	N	Sum of Scores	Expected Under H0	Std Dev Under H0	Mean Score
3	32	4369.00000	4096.0	226.837067	136.531250
5	32	3476.50000	4096.0	226.837067	108.640625
6	31	4097.00000	3968.0	223.764640	132.161290
8	32	4369.00000	4096.0	226.837067	136.531250
4	32	4624.00000	4096.0	226.837067	144.500000
1	32	4496.50000	4096.0	226.837067	140.515625
7	32	3604.00000	4096.0	226.837067	112.625000
2	32	3604.00000	4096.0	226.837067	112.625000

Average Scores Were Used for Ties

Kruskal-Wallis Test (Chi-Square Approximation)
CHISQ = 25.040 DF = 7 Prob > CHISQ = 0.0007

General Linear Models Procedure
Class Level Information

Class	Levels	Values
CLONE	8	1 2 3 4 5 6 7 8

Number of observations in data set = 256

General Linear Models Procedure

Dependent Variable: RESP

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	7	2.83223435	0.40460491	3.86	0.0005
Error	247	25.89717742	0.10484687		
Corrected Total	254	28.72941176			

R-Square	C.V.	Root MSE	RESP Mean
0.098583	37.19332	0.32380067	0.87058824

Source	DF	Type I SS	Mean Square	F Value	Pr > F
CLONE	7	2.83223435	0.40460491	3.86	0.0005

Source	DF	Type III SS	Mean Square	F Value	Pr > F
CLONE	7	2.83223435	0.40460491	3.86	0.0005

General Linear Models Procedure

T tests (LSD) for variable: RESP

NOTE: This test controls the type I comparisonwise error rate not the experimentwise error rate.

Alpha= 0.05 df= 247 MSE= 0.104847

Critical Value of T= 1.97

Least Significant Difference= 0.1598

WARNING: Cell sizes are not equal.

Harmonic Mean of cell sizes= 31.87149

Means with the same letter are not significantly different.

T Grouping	Mean	N	CLONE
A	1.00000	32	4
A			
A	0.96875	32	1
A			
A	0.93750	32	3
A			
A	0.93750	32	8
A			
B A	0.90323	31	6
B			
B C	0.75000	32	7
B C			
B C	0.75000	32	2
C			
C	0.71875	32	5

TABLE OF CLONES BY SURVIV

CLONES	SURVIV		
Frequency / Percent / Row Pct / Col Pct	1	2	Total
1	31 12.16 96.88 13.96	1 0.39 3.13 3.03	32 12.55
2	24 9.41 75.00 10.81	8 3.14 25.00 24.24	32 12.55
3	30 11.76 93.75 13.51	2 0.78 6.25 6.06	32 12.55
4	32 12.55 100.00 14.41	0 0.00 0.00 0.00	32 12.55
5	23 9.02 71.88 10.36	9 3.53 28.13 27.27	32 12.55
6	28 10.98 90.32 12.61	3 1.18 9.68 9.09	31 12.16
7	24 9.41 75.00 10.81	8 3.14 25.00 24.24	32 12.55
8	30 11.76 93.75 13.51	2 0.78 6.25 6.06	32 12.55
Total	222 87.06	33 12.94	255 100.00

STATISTICS FOR TABLE OF CLONES BY SURVIV

Statistic	DF	Value	Prob
Chi-Square	7	25.139	0.001
Likelihood Ratio Chi-Square	7	27.946	0.001
Mantel-Haenszel Chi-Square	1	0.619	0.431
Phi Coefficient		0.314	
Contingency Coefficient		0.300	
Cramer's V		0.314	

Sample Size = 255

69

APPENDIX 2

METEROLOGICAL DATA

Clone	Easting	Northing	Altitude	Annual Rain	Rain Wet M	Rain Dry M	Var M	Rain Wet Q	Rain Dry Q	Rain Cold Q	Rain Hot Q	An Mean Temp	Min Cold M	Max Hot M	Mean Hot M	An Temp Range	Mean T Hot Q	Mean T Cold Q	Mean T Dry Q	Mean T Wet Q
AL1	480600	5226500	259	1714	198	85	24.1	536	279	518	279	10.3	2.2	21	15	18.7	6.3	14.3	6.9	14.3
AL2	480600	5226600	259	1714	198	85	24.1	536	279	518	279	10.3	2.2	21	15	18.7	6.3	14.3	6.9	14.3
AL3	479700	5224900	335	2017	225	98	24	628	328	599	328	9.8	2	20.1	15.6	18	5.9	13.8	6.5	13.8
BR1	556900	5421600	642	1481	197	64	35.9	541	215	541	215	9	0.8	19.8	14.7	19	4.8	13.3	5	13.3
BR2	552800	5421300	642	1481	197	64	35.9	541	215	541	215	9	0.8	19.8	14.7	19	4.8	13.3	5	13.3
BR3	552500	5420800	642	1481	197	64	35.9	541	215	541	215	9	0.8	19.8	14.7	19	4.8	13.3	5	13.3
DB1	475900	5379400	1120	1655	219	73	34.2	599	246	599	246	5.5	-1.8	16.1	11.5	18	1.3	10.1	1.3	10.1
DB2	475700	5374900	1120	1655	219	73	34.2	599	246	599	246	5.5	-1.8	16.1	11.5	18	1.3	10.1	1.3	10.1
FG1	477900	5386400	751	1382	189	62	34.7	507	211	507	211	7.9	0.4	18.5	13.6	18.1	3.9	12.3	3.9	12.3
FG2	477800	5386400	751	1382	189	62	34.7	507	211	507	211	7.9	0.4	18.5	13.6	18.1	3.9	12.3	3.9	12.3
FG3	477800	5386400	751	1382	189	62	34.7	507	211	507	211	7.9	0.4	18.5	13.6	18.1	3.9	12.3	3.9	12.3
FG4	476900	5383200	751	1382	189	62	34.7	507	211	507	211	7.9	0.4	18.5	13.6	18.1	3.9	12.3	3.9	12.3
FG5	476900	5383200	751	1382	189	62	34.7	507	211	507	211	7.9	0.4	18.5	13.6	18.1	3.9	12.3	3.9	12.3
FG6	476600	5384600	751	1382	189	62	34.7	507	211	507	211	7.9	0.4	18.5	13.6	18.1	3.9	12.3	3.9	12.3
FG7	476600	5384600	751	1382	189	62	34.7	507	211	507	211	7.9	0.4	18.5	13.6	18.1	3.9	12.3	3.9	12.3
FG8	477400	5385600	751	1382	189	62	34.7	507	211	507	211	7.9	0.4	18.5	13.6	18.1	3.9	12.3	3.9	12.3
GL1	474300	5364700	1072	820	102	39	30.2	283	129	283	129	5.8	-1.6	16.3	11.7	18	1.6	10.3	1.6	10.3
GL2	474500	5366400	1072	820	102	39	30.2	283	129	283	129	5.8	-1.6	16.3	11.7	18	1.6	10.3	1.6	10.3
GL3	474500	5366400	1072	820	102	39	30.2	283	129	283	129	5.8	-1.6	16.3	11.7	18	1.6	10.3	1.6	10.3
GL4	474100	5367200	1072	820	102	39	30.2	283	129	283	129	5.8	-1.6	16.3	11.7	18	1.6	10.3	1.6	10.3
GL5	474000	5367300	1072	820	102	39	30.2	283	129	283	129	5.8	-1.6	16.3	11.7	18	1.6	10.3	1.6	10.3
GL6	473900	5367300	1072	820	102	39	30.2	283	129	283	129	5.8	-1.6	16.3	11.7	18	1.6	10.3	1.6	10.3
GL7	473900	5367200	1072	820	102	39	30.2	283	129	283	129	5.8	-1.6	16.3	11.7	18	1.6	10.3	1.6	10.3
GL8	473900	5367200	1072	820	102	39	30.2	283	129	283	129	5.8	-1.6	16.3	11.7	18	1.6	10.3	1.6	10.3

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GL9	473400	5371600	1120	1655	219	73	34.2	599	246	599	246	5.5	-1.8	16.1	11.5	18	1.3	10.1	1.3	10.1
GL10	473400	5371600	1120	1655	219	73	34.2	599	246	599	246	5.5	-1.8	16.1	11.5	18	1.3	10.1	1.3	10.1
GL11	473400	5371600	1120	1655	219	73	34.2	599	246	599	246	5.5	-1.8	16.1	11.5	18	1.3	10.1	1.3	10.1
GL12	473400	5371600	1120	1655	219	73	34.2	599	246	599	246	5.5	-1.8	16.1	11.5	18	1.3	10.1	1.3	10.1
HY1	382100	5426900	505	1861	241	81	35.2	669	264	669	264	9.5	2.2	20	14.3	17.7	5.6	13.6	5.8	13.6
HY2	382100	5426900	505	1861	241	81	35.2	669	264	669	264	9.5	2.2	20	14.3	17.7	5.6	13.6	5.8	13.6
HY3	382100	5426600	505	1861	241	81	35.2	669	264	669	264	9.5	2.2	20	14.3	17.7	5.6	13.6	5.8	13.6
HY4	382100	5426500	505	1861	241	81	35.2	669	264	669	264	9.5	2.2	20	14.3	17.7	5.6	13.6	5.8	13.6
HY5	381500	5416300	602	2031	254	93	33.4	712	295	712	295	8.8	1.7	19.3	13.8	17.7	5	13	5	13
HY6	381500	5416300	602	2031	254	93	33.4	712	295	712	295	8.8	1.7	19.3	13.8	17.7	5	13	5	13
HY7	382600	5414800	602	2031	254	93	33.4	712	295	712	295	8.8	1.7	19.3	13.8	17.7	5	13	5	13
HY8	382900	5411300	602	2031	254	93	33.4	712	295	712	295	8.8	1.7	19.3	13.8	17.7	5	13	5	13
HY9	380100	5410300	602	2031	254	93	33.4	712	295	712	295	8.8	1.7	19.3	13.8	17.7	5	13	5	13
HY10	380100	5410300	602	2031	254	93	33.4	712	295	712	295	8.8	1.7	19.3	13.8	17.7	5	13	5	13
HY11	380100	5410300	602	2031	254	93	33.4	712	295	712	295	8.8	1.7	19.3	13.8	17.7	5	13	5	13
HY12	380100	5410300	602	2031	254	93	33.4	712	295	712	295	8.8	1.7	19.3	13.8	17.7	5	13	5	13
HY13	381800	5415900	602	2031	254	93	33.4	712	295	712	295	8.8	1.7	19.3	13.8	17.7	5	13	5	13
HY14	381400	5420700	505	1861	241	81	35.2	669	264	669	264	9.5	2.2	20	14.3	17.7	5.6	13.6	5.8	13.6
HZ1	482400	5216900	515	2235	258	118	23.2	686	379	656	379	8.6	1.3	18.7	12.4	17.3	4.9	12.5	5.4	12.5
HZ2	481700	5216500	515	2235	258	118	23.2	686	379	656	379	8.6	1.3	18.7	12.4	17.3	4.9	12.5	5.4	12.5
HZ3	481500	5217000	515	2235	258	118	23.2	686	379	656	379	8.6	1.3	18.7	12.4	17.3	4.9	12.5	5.4	12.5
HZ4	481300	5217000	515	2235	258	118	23.2	686	379	656	379	8.6	1.3	18.7	12.4	17.3	4.9	12.5	5.4	12.5
HZ5	481300	5217000	515	2235	258	118	23.2	686	379	656	379	8.6	1.3	18.7	12.4	17.3	4.9	12.5	5.4	12.5
HZ6	481100	5217200	515	2235	258	118	23.2	686	379	656	379	8.6	1.3	18.7	12.4	17.3	4.9	12.5	5.4	12.5
LL1	475400	5374400	1120	1655	219	73	34.2	599	246	599	246	5.5	-1.8	16.1	11.5	18	1.3	10.1	1.3	10.1
LL2	475500	5374400	1120	1655	219	73	34.2	599	246	599	246	5.5	-1.8	16.1	11.5	18	1.3	10.1	1.3	10.1
LR1	477000	5381200	751	1382	189	62	34.7	507	211	507	211	7.9	0.4	18.5	13.6	18.1	3.9	12.3	3.9	12.3
LR2	477000	5381400	751	1382	189	62	34.7	507	211	507	211	7.9	0.4	18.5	13.6	18.1	3.9	12.3	3.9	12.3
LR3	477200	5381700	751	1382	189	62	34.7	507	211	507	211	7.9	0.4	18.5	13.6	18.1	3.9	12.3	3.9	12.3

LR4	477200	5381700	751	1382	189	62	34.7	507	211	507	211	7.9	0.4	18.5	13.6	18.1	3.9	12.3	3.9	12.3
LR5	477200	5381700	751	1382	189	62	34.7	507	211	507	211	7.9	0.4	18.5	13.6	18.1	3.9	12.3	3.9	12.3
MB1	535700	5420600	596	1469	203	68	36.9	552	214	552	214	9.3	1.2	20	14.7	18.7	5.1	13.5	5.1	13.5
MB2	536000	5420600	596	1469	203	68	36.9	552	214	552	214	9.3	1.2	20	14.7	18.7	5.1	13.5	5.1	13.5
MB3	536000	5420600	596	1469	203	68	36.9	552	214	552	214	9.3	1.2	20	14.7	18.7	5.1	13.5	5.1	13.5
MB4	535700	5419400	780	1409	189	61	35	511	207	511	207	8	0.1	18.7	13.6	18.6	3.9	12.3	3.9	12.3
MB5	535300	5419400	780	1409	189	61	35	511	207	511	207	8	0.1	18.7	13.6	18.6	3.9	12.3	3.9	12.3
MB6	535400	5419200	780	1409	189	61	35	511	207	511	207	8	0.1	18.7	13.6	18.6	3.9	12.3	3.9	12.3
MB7	535400	5419600	780	1409	189	61	35	511	207	511	207	8	0.1	18.7	13.6	18.6	3.9	12.3	3.9	12.3
MB8	534700	5419700	780	1409	189	61	35	511	207	511	207	8	0.1	18.7	13.6	18.6	3.9	12.3	3.9	12.3
MB9	534700	5419800	780	1409	189	61	35	511	207	511	207	8	0.1	18.7	13.6	18.6	3.9	12.3	3.9	12.3
MB10	534700	5420400	596	1469	203	68	36.9	552	214	552	214	9.3	1.2	20	14.7	18.7	5.1	13.5	5.1	13.5
NW1	389400	5442400	304	1541	208	63	37	567	214	567	214	10.6	3.4	21	15.6	17.6	7	14.8	7	14.8
NW2	384600	5436800	410	1717	229	71	36.5	629	241	628	241	10	2.7	20.5	15.1	17.7	6.3	14.1	6.3	14.1
PP1	559700	5421700	642	1481	197	64	35.9	541	215	541	215	9	0.8	19.8	14.7	19	4.8	13.3	5	13.3
PP2	559700	5421700	642	1481	197	64	35.9	541	215	541	215	9	0.8	19.8	14.7	19	4.8	13.3	5	13.3
PP3	559700	5421700	642	1481	197	64	35.9	541	215	541	215	9	0.8	19.8	14.7	19	4.8	13.3	5	13.3
RR1	524700	5433300	407	1266	171	61	35	468	190	468	190	10.3	1.8	21.6	15.7	19.8	6.1	14.8	6.1	14.8
RR2	525100	5432000	407	1266	171	61	35	468	190	468	190	10.3	1.8	21.6	15.7	19.8	6.1	14.8	6.1	14.8
SR (a)	498500	5232900	269	1270	140	71	20.1	380	224	369	224	10.3	2.4	20.7	15.5	18.3	6.4	14.3	7.1	14.3
SR (b)	498500	5232900	269	1270	140	71	20.1	380	224	369	224	10.3	2.4	20.7	15.5	18.3	6.4	14.3	7.1	14.3
SR2	498500	5232900	269	1270	140	71	20.1	380	224	369	224	10.3	2.4	20.7	15.5	18.3	6.4	14.3	7.1	14.3
SR3	498500	5232900	269	1270	140	71	20.1	380	224	369	224	10.3	2.4	20.7	15.5	18.3	6.4	14.3	7.1	14.3
TR1	552300	5420600	642	1481	197	64	35.9	541	215	541	215	9	0.8	19.8	14.7	19	4.8	13.3	5	13.3
TR2	553000	5420100	642	1481	197	64	35.9	541	215	541	215	9	0.8	19.8	14.7	19	4.8	13.3	5	13.3
TR3	553300	5421000	642	1481	197	64	35.9	541	215	541	215	9	0.8	19.8	14.7	19	4.8	13.3	5	13.3
TR4	553700	5418700	719	1401	182	59	34.2	497	203	494	203	8.5	0.4	19.2	14.2	18.8	4.3	12.8	4.5	12.8
TR5	554200	5417100	719	1401	182	59	34.2	497	203	494	203	8.5	0.4	19.2	14.2	18.8	4.3	12.8	4.5	12.8
TR6	554200	5414200	719	1401	182	59	34.2	497	203	494	203	8.5	0.4	19.2	14.2	18.8	4.3	12.8	4.5	12.8

UB1	552600	5412900	719	1401	182	59	34.2	497	203	494	203	8.5	0.4	19.2	14.2	18.8	4.3	12.8	4.5	12.8
UB2	552100	5413200	719	1401	182	59	34.2	497	203	494	203	8.5	0.4	19.2	14.2	18.8	4.3	12.8	4.5	12.8
UB3	553700	5413300	719	1401	182	59	34.2	497	203	494	203	8.5	0.4	19.2	14.2	18.8	4.3	12.8	4.5	12.8
UB4	553700	5413300	719	1401	182	59	34.2	497	203	494	203	8.5	0.4	19.2	14.2	18.8	4.3	12.8	4.5	12.8
UB5	551800	5412500	719	1401	182	59	34.2	497	203	494	203	8.5	0.4	19.2	14.2	18.8	4.3	12.8	4.5	12.8
WH1	375900	5408200	604	2331	265	105	28.7	766	356	765	356	8.8	1.7	19.2	13.7	17.6	5	13	5	13
WH2	375600	5407900	604	2331	265	105	28.7	766	356	765	356	8.8	1.7	19.2	13.7	17.6	5	13	5	13
WH3	373900	5405800	604	2331	265	105	28.7	766	356	765	356	8.8	1.7	19.2	13.7	17.6	5	13	5	13
WH4	367700	5408200	503	2356	268	103	29.5	777	353	769	353	9.3	2.2	19.7	14.2	17.3	5.5	13.3	5.8	13.3
WH5	370500	5406900	604	2331	265	105	28.7	766	356	765	356	8.8	1.7	19.2	13.7	17.6	5	13	5	13
WH6	374000	5406100	604	2331	265	105	28.7	766	356	765	356	8.8	1.7	19.2	13.7	17.6	5	13	5	13
WH7	378300	5410800	511	2092	249	92	32	714	304	711	304	9.3	2.2	19.8	14.4	17.5	5.6	13.5	5.8	13.5

APPENDIX 3

SITE DATA

ID	GRID REF	SEX	DATE	ASPECT	BEDROCK	SOIL
AL1	DN806265	M	23/11/95	NW	permian mudstone	mottled yellow/brown clay soils under wet forest
AL2	DN806266	?	3/6/96	E	permian mudstone	mottled yellow/brown cla soils under wet forest
AL3	DN797249	M	3/6/96	N	permian mudstone	mottled yellow/brown clay soils under wet forest
BR1	EQ569216	M	31/11/95	NE	granite	dark brown
BR2	EQ528213	F	31/11/95	E	granite	dark brown
BR3	EQ525208	M	31/11/95	S/SE	granite	yellow/brown clay
DB1	DP759749	M	12/8/95	NW	dolerite	unknown
DB2	DP757749	F	12/8/95	E	dolerite	unknown
FG1	DP779864	F	12/8/95	SW	sandstone/mudstone	orange chocolate brown sandy loam
FG2	DP778864	F	12/8/95	SE	sandstone/mudstone	orange chocolate brown sandy loam
FG3	DP778864	F	12/8/95	NNE	sandstone/mudstone	orange chocolate brown sandy loam
FG4	DP769832	F	12/8/95	W	sandstone/mudstone shale	orange sandy clay
FG5	DP769832	?	12/8/95	SW	sandstone/mudstone shale	orange sandy clay
FG6	DP766846	F	12/8/95	S	sandstone/granite	orange chocolate brown sandy loam
FG7	DP766846	M	12/8/95	E	sandstone/granite	orange chocolate brown sandy loam
FG8	DP774856	M	12/8/95	N	sandstone/granite	orange chocolate brown sandy loam
GL1	DP743647	M	12/8/95	E	dolerite	sandy clay
GL2	DP745664	F	12/8/95	SW	dolerite	sandy clay
GL3	DP745664	M	12/8/95	E	dolerite	sandy clay
GL4	DP741672	?	12/8/95	E	dolerite	sandy clay
GL5	DP741672	M	12/8/95	NE	dolerite	sandy clay
GL6	DP741672	F	12/8/95	NE	dolerite	sandy clay
GL7	DP741672	F	12/8/95	NE	dolerite	sandy clay
GL8	DP741672	F	12/8/95	NE	dolerite	sandy clay
GL9	DP734716	F	12/8/95	NE	dolerite	unknown
GL10	DP734716	M	12/8/95	E	dolerite	sandy clay
GL11	DP734716	F	12/8/95	E	dolerite	sandy clay
GL12	DP734716	F	12/8/95	E	dolerite	sandy clay
HY1	CQ821269	F	19/12/95	SW	basalt	deep red brown self cracking clay
HY2	CQ821269	F	19/12/95	SW	basalt	deep red brown self cracking clay
HY3	CQ821266	F	19/12/95	S	basalt	deep red brown self cracking clay
HY4	CQ821265	?	19/12/95	NW	basalt	deep red brown self cracking clay
HY5	CQ809172	?	19/12/95	SW	basalt	dark brown loamy clay
HY6	CQ809172	?	19/12/95	SW	basalt	dark brown loamy clay
HY7	CQ826148	M	19/12/95	NE	basalt	dark brown loamy clay
HY8	CQ829113	F	19/12/95	SE	basalt	dark brown loamy clay
HY9	CQ801103	?	19/12/95	NE	basalt	peaty
HY10	CQ801103	?	19/12/95	Open	basalt	dark brown sandy clay
HY11	CQ801103	?	19/12/95	Open	basalt	dark brown sandy clay
HY12	CQ801103	F	19/12/95	NW	basalt	dark brown sandy clay
HY13	CQ818159	F	19/12/95	E	basalt	dark brown sandy clay
HY14	CQ814207	?	19/12/95	Open	basalt	dark brown loamy clay
HZ1	DN824169	?	23/11/95	NE	permian mudstone	mottled yellow/brown clay soils under wet forest
HZ2	DN817165	?	23/11/95	SW	permian mudstone	mottled yellow/brown clay soils under wet forest
HZ3	DN815170	?	23/11/95	SSW	permian mudstone	mottled yellow/brown clay soils under wet forest
HZ4	DN813170	M	23/11/95	NW	permian mudstone	mottled yellow/brown clay soils under wet forest
HZ5	DN813170	F	23/11/95	NE	permian mudstone	mottled yellow/brown clay soils under wet forest
HZ6	DN811172	?	23/11/95	SW	permian mudstone	unknown
LL1	DP754744	M	12/8/95	SE	sandstone	sandy loam
LL2	DP755744	F	12/8/95	SW	sandstone	sandy loam
LR1	DP770812	F	12/8/95	W	dolerite/alluvium	dark orange brown clay
LR2	DP770814	F	12/8/95	SW	dolerite/alluvium	dark orange brown clay
LR3	DP772817	M	12/8/95	SSE	dolerite/alluvium	dark orange brown clay
LR4	DP772817	M	12/8/95	N	dolerite/alluvium	dark orange brown clay
LR5	DP772817	M	12/8/95	NNW	dolerite/alluvium	dark orange brown clay
MB1	EQ357206	?	11/1/95	N	dolerite	red/brown clayey under wet forest
MB2	EQ360206	M	11/1/95	SE	dolerite	red/brown clayey under wet forest
MB3	EQ260206	M	11/1/95	E	dolerite	red/brown clayey under wet forest

ID	GRID REF	SEX	DATE	ASPECT	BEDROCK	SOIL
MB4	EQ357194	M	11/1/95	SSW	dolerite	red/brown clayey under wet forest
MB5	EQ353149	M	11/1/95	NNW	dolerite	red/brown clayey under wet forest
MB6	EQ354192	?	11/1/95	NW	dolerite	red/brown clayey under wet forest
MB7	EQ354196	?	11/1/95	S	dolerite	red/brown clayey under wet forest
MB8	EQ347197	F	11/1/95	SW	dolerite	red/brown clayey under wet forest
MB9	EQ347198	F	11/1/95	NW	dolerite	red/brown clayey under wet forest
MB10	EQ347204	F	11/1/95	S	dolerite	red/brown clayey under wet forest
NW1	CQ894424	?	19/12/95	Open	basalt	brown clayey
NW2	CQ846368	?	19/12/95	NW	basalt	brown clayey
PP1	EQ597217	?	31/11/95	E/W	granite	red/brown clay
PP2	EQ597217	?	31/11/95	SE	granite	red/brown clay
PP3	EQ597217	?	31/11/95	Open	granite	red/brown clay
RR1	EQ247333	F	11/1/95	S	dolerite	unknown
RR2	EQ251320	F	11/1/95	Open	dolerite	unknown
SR1 (a)	Swamp Rd	?	17/11/95	Open	dolerite	unknown
SR1 (b)	Franklin	?	17/11/95	Open	dolerite	unknown
SR2	Franklin	?	17/11/95	Open	dolerite	unknown
SR3	Franklin	?	17/11/95	Open	dolerite	unknown
TR1	EQ523206	F	31/11/95	S	granite	yellow brown clay
TR2	EQ530201	F	31/11/95	NE	granite	yellow brown clay
TR3	EQ533210	F	31/11/95	S	granite	yellow brown clay
TR4	EQ537187	F	31/11/95	SW	granite	yellow brown clay
TR5	EQ542171	F	31/11/95	SW	granite	yellow brown clay
TR6	EQ542142	F	31/11/95	W	granite	yellow brown clay
UB1	EQ526129	F	31/11/95	NE	granite	loamy clayey brown
UB2	EQ532132	M	31/11/95	N	granite	loamy clayey brown
UB3	EQ537133	F	31/11/95	SSW	granite	loamy clayey brown
UB4	EQ537133	F	31/11/95	SE	granite	loamy clayey brown
UB5	EQ518125	F	31/11/95	SE	granite	loamy clayey brown
WH1	CQ759082	F	19/12/95	SW	basalt	dark brown sandy clay
WH2	CQ756079	F	19/12/95	NW	basalt	dark brown sandy clay
WH3	CQ739058	?	19/12/95	SW	basalt	dark brown sandy clay
WH4	CQ705069	?	19/12/95	SE	mudstone/dolerite	orange brown clay
WH5	CQ705069	F	19/12/95	Open	basalt	dark brown sandy clay
WH6	CQ740061	?	19/12/95	SSE	basalt	dark brown sandy clay
WH7	CQ783108	F	19/12/95	Open	basalt	dark brown sandy clay

APPENDIX 4

SUMMARY CHEMICAL DATA AND ORGANOLEPTIC ASSESSMENT OF CLONES

ID	ORGANOLEPTIC ASSESSMENT	% mono-terpenes	% sesqui-terpenes	safrole ppm	Total % Polygodial
AL1	Strong lantana spicey, woody lantana balanced, powerful	6.632	93.368	0.7	20.1
AL2					0.0
AL3					0.0
BR1	spicey nutmeg; medium lantana and spice	8.377	91.623	49.3	41.9
BR2	citrus fresh lantana; strong lantana and spice	5.176	94.824	5.5	54.4
BR3	lantana sharp; spicey weak lantana	2.465	97.535	50.5	55.1
DB1	dry lantana faint citrus; green citrus; citrus	4.144	95.856	4.4	3.8
DB2	lantana; much lighter faint spice; citrus fruit	4.784	95.216	339.1	53.0
FG1	mint; very strong Tasmannia spice; spice lantana	5.456	94.544	50.7	0.2
FG2	strong lantana; limey green note; lanata	5.631	94.369	5.2	0.0
FG3	Tasmannia; nutmeg, green minty; lantana spice	2.036	97.964	11.3	0.0
FG4	sweet; piney apple sweet; fruity slight lantana	3.454	96.546	36.4	0.0
FG5	sweet; coarse terpy with spice; strong lantana spice	2.258	97.742	44.0	0.0
FG6	sharp sweet raspberry; dusty cinnamon, floral; strong lantana spice woody	4.020	95.980	18.1	0.0
FG7	faint sweet spicey; faint floral; fruity med/strong lantana	2.938	97.062	13.6	0.0
FG8	faint sweet floral rose; spice floral; woody lantana	3.051	96.949	9.0	23.1
GL1	lemon; citrus, ripe; sharp, tobacco	3.124	96.876	6.9	38.4
GL2	spicy, lantana; stronger tobacco; lighter, still fruity	11.840	88.160	180.1	7.0
GL3	spicey cinnamon, lantana; 'old spice'; peppery very lanceolata, spice	11.252	88.748	635.2	43.9
GL4	spicey citrus; sweeter spicey, floral	8.358	91.642	7.9	14.3
GL5	spicey, steely; light spicey/hot; unpleasant, terpentine	9.105	90.895	229.7	37.4
GL6	spicey, citrus; similar to 5; clear green note, lime	2.652	97.348	3.4	49.6
GL7	spicey, citrus, slight lantana; faint lantana; clear fragrant	6.142	93.858	7.5	0.5
GL8	cinnamon; spicey, faint lantana, sharp; flat	5.897	94.103	12.1	7.5
GL9	similar to 6; greener, rounded, warm spicey; lime floral	5.720	94.280	14.7	4.1
GL10	sweet, citrus; sharp, coarser, peppery; harsh, fiaint spice note	6.892	93.108	53.7	4.2
GL11	lantana; peppery, dusty, lantana; floral sweet	2.842	97.158	17.2	2.5
GL12	spicey, lantana; spicey, sharp, lantana; very citrusy	6.146	93.854	133.3	12.9
HY1	clear sweet Tasmannia; lovely lemony fresh; lantana strong	5.377	94.623	538.9	16.9
HY2	lavender strong spicey; rose, more spice than HY1 lavender; sweet menthol	13.710	86.290	19.6	44.4
HY3	lime; flatter greener note with citrus; fruity sweet	3.363	96.637	76.8	57.0
HY4	very persistent; sharp spice; sweet floral; spicey lantana	5.005	94.995	64.8	11.4
HY5	woody spice sassafrass; floral spice sweet; spicey, persistent	4.422	95.578	6.6	25.8
HY6	sharp similar to HY5; harsh citrus earthy woody; spicey lantana, persistent	8.143	91.857	12.1	53.7
HY7	spicey woody slight lantana; lemony cinnamon; spicey	1.717	98.283	27.4	55.9
HY8	almost minty slight lantana; spicey fragrant; herby and spice	6.700	93.300	40.6	33.1
HY9	fruity sweet persistent; floral; herby	4.238	95.762	39.0	47.8
HY10	fruity sweet persistent; spice; fruit	6.372	93.628	52.0	55.4
HY11	sharp onion; persistent; faint pine harsh solventy ; citrus lantana	6.223	93.777	40.5	37.5
HY12	spicey persistent; lime sweet green, lantana	5.753	94.247	74.3	22.3
HY13	woody Tasmannia lantana; high light and spicey; lantana	5.428	94.572	164.1	20.9
HY14	spicey; herbaceous low; lantana fruity	5.283	94.717	37.5	28.3
HZ1	lantana, woody sassafrass, strong sweet lantana pepper	8.880	91.120	1.7	32.7
HZ2	lantana, similar to 1, weaker, bitter solventy	6.676	93.324	3.0	43.2
HZ3	spicey, sweet woody spicey	13.175	86.825	4.3	44.4
HZ4	sweet, floral more balanced than 3	8.630	91.370	353.9	43.0
HZ5	sweet spicey, spicey pungent, feel it in sinuses pleasant	6.646	93.354	6.2	44.1
HZ6	spicey, lantana sweet woody	5.013	94.987	1.9	39.1
LL1	woody sassafrass lantana; weak; citrus slight lantana	4.301	95.699	32.0	67.3
LL2	lantana; flat faint floral; spice fruity	2.498	97.502	154.5	33.2
LR1	lantana sweet; dusky green harsh; citrus and spice	4.786	95.214	5.9	8.7
LR2	sharp lantana; lantana and spice	1.662	98.338	12.5	1.7
LR3	spicey Tasmannia; orange blossom sweet; lantana and spice	2.108	97.892	177.2	12.7
LR4	mellow spicey Tasmannia; fragrant spicey; fruity and fresh	1.553	98.447	10.8	35.2
LR5	pepper spicey; green limey; good lantana	2.651	97.349	9.3	20.7
MB1	burnt sharp, slight lantana balanced	4.338	95.662	7.5	48.3
MB2	tropical fruit spicey,strong lantana (high volatives) very fruity	8.797	91.203	27.1	23.7

ID	ORGANOLEPTIC ASSESSMENT	% mono-terpenes	% sesqui-terpenes	safrole ppm	Total % Polygodial
MB3	like 4, medium lantana (good)	4.208	95.792	108.6	40.4
MB4	strong, weaker lantana	5.106	94.894	39.8	18.0
MB5	sweet	2.699	97.301	69.2	62.3
MB6	fewer top notes, strong lantana, medicinal	3.672	96.328	2.3	21.1
MB7	strong heavy sharp notes	8.158	91.842	636.4	48.8
MB8	lantana sweet	2.727	97.273	29.1	25.5
MB9	similar to 10	2.506	97.494	56.0	28.3
MB10	rounded sweet fruity spicey, medium lantana (good)	4.796	95.204	46.6	50.6
NW1	lemon/lime; floral; lemon; soapy spicey	11.196	88.804	11.7	10.0
NW2	woody sassafrass pine; pineapple; fishy oily slight lantana	6.505	93.495	207.9	54.9
PP1	strong lantana; very fruity (tropical); cloves	6.129	93.871	84.3	59.2
PP2	floral (lavender) lantana; strong lantana and spice	4.193	95.807	190.4	16.9
PP3	Tasmannia; strong to medium lantana and spice	5.271	94.729	7.2	50.6
RR1	aromatic spicey lantana, (good)	4.720	95.280	2.9	37.8
RR2	spicey	2.893	97.107	5.5	63.6
SR1 (a)	lantana, strong spicey	8.680	91.320	2.5	33.3
SR1 (b)	lantana, woody strong lantana	12.774	87.226	1.3	7.8
SR2	lantana - less, glue spicey typical T.lanceolata	7.414	92.586	2.4	37.3
SR3	sweet/lantana, floral sweet	7.788	92.212	0.9	13.0
TR1	light fresh sharp fruity sweet; citrus fruity	7.697	92.303	64.4	25.9
TR2	lantana; fruity lantana; bushy fruity tending to lantana	4.355	95.645	131.2	63.2
TR3	sharp spicey; stronger lantana fruity; like TR2 stronger lantana	3.958	96.042	53.9	44.8
TR4	lantana; spice less fruit and good lantana	3.674	96.326	3.6	49.9
TR5	lantana; spice and lantana	8.024	91.976	7.1	60.6
TR6	astringent sweet; menthol type lacks spice and lantana	4.730	95.270	6.6	43.7
UB1	citrus peppery; citrus strong; good lantana	5.192	94.808	113.9	47.6
UB2	pepper strong Lantana; pepper strong coarse note; spicey some lantana	5.344	94.656	662.5	62.6
UB3	solvent, glue, lantana complex; green bitter; fruity spice light lantana	3.098	96.902	144.0	65.9
UB4	sweet fruity spicey; bitter strong; woody/spice some lantana	6.028	93.972	7.0	64.0
UB5	pepper lantana; unpleasant terpentine; lantana/spice/herb	4.755	95.245	5.4	48.7
WH1	nutmeg strong; very spicey nutmeg; floral spicey citrus	6.577	93.423	215.4	51.9
WH2	spicey Tasmannia, sharp, green tomato leaf , lantana; yeasty sweet	8.550	91.450	128.4	41.0
WH3	swet fruity; lemon spice cinnamon sweet; green spicey	6.312	93.688	110.0	25.0
WH4	fruity sweet; weak; spicey woody	6.487	93.513	86.6	16.4
WH5	faint citrus; musty faint; fruit spicey	5.548	94.452	58.4	16.3
WH6	spicey sweet; faint citrus; spice lantana	5.608	94.392	55.5	13.8
WH7	sharp lantana; husky camphor like; fruity	6.609	93.391	448.1	41.6

APPENDIX 5

Chemical Data

Clone	α-pinene	β-pinene	1,8-cineole	β-phellandrene+ limonene	linalool	piperitone	eugenol	α-cubebene	α-copanene	caryophyllene	germacrene-D	calamenone	cadina-1,4-diene	spathulenol	guaiol	δ-cadinol	218 diterpene	drimenol	polygodial artifactct	drimenol	polygodial	272 diterpene	drimenol
AL1	0.22	0.29	0.09	0.28	0.20	0.31	0.00	0.06	1.22	2.19	0.21	17.19	6.45	2.50	3.48	0.43		1.17	15.09	0.37	5.05	1.05	0.00
BR1	0.21	0.00	0.44	0.78	1.88	2.41	1.89	0.41	0.15	1.53	0.00	0.30	0.22	1.91	6.61	0.46	2.85	2.01	38.55	1.28	3.39	0.76	1.12
BR2	0.24	0.28	0.16	0.30	1.27	0.81	1.96	0.16	0.11	0.60	0.27	0.53	0.16	0.91	6.39	0.37	2.27	1.32	34.06	1.06	20.36	0.55	0.87
BR3	0.11	0.07	0.13	0.25	0.78	0.73	0.30	0.09	0.09	0.49	0.41	0.33	0.15	1.01	4.38	0.16	1.88	1.35	35.13	1.15	19.99	1.61	1.34
DB1	0.00	0.00	0.00	0.45	1.69	0.24	1.05	0.21	0.00	0.79	0.88	0.35	0.00	1.78	0.73	0.00	0.63	3.12	0.00	0.36	3.80	1.47	4.53
DB2	0.13	0.00	0.15	0.48	0.85	0.81	1.91	0.45	0.00	0.92	0.47	0.58	0.15	1.59	2.43	0.17	0.11	1.22	39.13	0.81	13.88	1.18	1.62
FG1	0.19	0.08	0.24	0.54	1.46	1.09	0.59	1.10	0.38	0.87	2.64	0.75	0.24	2.09	2.08	0.31	0.74	0.72	0.25	0.17	0.00	0.21	1.24
FG2	0.20	0.00	0.21	0.31	1.48	0.65	0.09	0.08	0.71	1.24	1.13	6.98	7.88	2.16	4.38	0.31	0.23	0.76	0.00	0.59	0.00	0.63	1.39
FG3	0.17	0.13	0.09	0.41	0.45	0.51	0.20	0.07	0.38	0.86	2.24	0.80	0.27	1.86	2.86	0.28	0.65	0.74	0.00	0.72	0.00	0.59	1.33
FG4	0.00	0.00	0.00	0.34	1.92	0.11	0.43	0.07	0.15	1.00	2.62	0.00	0.32	1.79	0.32	0.23	0.28	1.57	0.00	0.12	0.00	0.11	0.80
FG5	0.13	0.00	0.00	0.00	1.07	0.31	0.56	0.20	0.23	0.58	2.16	0.73	0.20	1.87	2.37	0.14	0.62	0.79	0.00	0.88	0.00	0.83	1.25
FG6	0.19	0.29	0.15	0.56	1.05	0.67	0.28	0.11	0.27	1.18	2.65	7.85	0.20	2.77	0.87	0.30	0.48	1.03	0.00	0.00	0.00	0.72	1.93
FG7	0.19	0.22	0.19	0.25	0.72	1.03	0.25	0.08	0.25	1.19	3.00	0.00	0.19	2.41	1.13	0.55	0.19	0.91	0.00	0.16	0.00	1.05	1.85
FG8	0.14	0.09	0.00	0.68	1.19	0.37	0.26	0.00	0.25	0.57	2.16	0.76	0.16	1.58	0.76	0.00	0.24	0.55	17.35	0.32	5.74	0.24	0.72
GL1	0.00	0.13	0.00	0.46	1.47	0.27	0.16	0.13	0.13	0.24	0.92	0.14	0.19	1.25	1.57	0.53	0.59	2.18	28.75	0.51	9.61	2.42	1.35
GL2	0.00	0.00	0.11	1.30	5.75	0.58	2.07	0.44	0.00	2.28	0.00	0.40	0.76	2.96	4.26	0.45	0.42	1.73	4.83	0.30	2.17	1.09	1.35
GL3	0.11	0.26	0.07	3.23	0.95	0.34	3.47	0.49	0.16	1.68	0.15	0.15	1.02	3.88	4.78	0.49	0.00	0.23	30.76	0.81	13.17	0.73	1.73
GL4	0.00	0.00	0.00	0.57	1.30	0.76	1.15	3.41	0.00	0.98	0.00	0.00	0.25	1.84	1.67	0.00	1.63	5.18	9.87	0.52	4.43	3.92	2.63
GL5	0.00	0.06	0.11	0.76	0.42	1.49	0.31	0.70	0.15	1.14	0.09	0.43	0.25	2.36	4.21	0.55	0.30	0.31	26.54	0.64	10.83	1.44	0.54

Clone	α-pinene	β-pinene	1,8-cineole	β-phellandrene+ limonene	linalool	piperitone	eugenol	α-cubebene	α-copanene	caryophyllene	germacrene-D	calamenone	cadina-1,4-diene	spathulenol	guaiol	δ-cadinol	218 diterpene	drimenol	polygodial artifactct	drimenol	polygodial	272 diterpene	drimenol
GL6	0.00	0.00	0.00	0.17	0.87	0.74	0.16	0.72	0.00	1.29	1.10	0.00	0.48	1.60	3.03	0.90	0.18	0.00	36.04	0.98	13.61	0.60	1.08
GL7	0.00	0.00	0.00	0.36	2.23	0.82	1.43	0.31	0.10	2.15	0.00	0.00	0.33	1.02	1.85	0.23	0.38	1.97	0.27	0.23	0.23	0.85	0.96
GL8	0.00	0.11	0.11	0.59	0.22	1.30	0.93	0.46	0.11	2.23	0.71	0.00	0.35	1.40	1.73	0.00	0.21	3.73	5.28	0.28	2.23	2.19	2.39
GL9	0.00	0.12	0.16	1.13	0.35	0.83	0.80	0.17	0.00	0.56	1.29	0.35	0.24	1.50	1.05	0.00	0.45	3.97	2.85	0.00	1.28	0.96	2.68
GL10	0.26	0.72	0.00	0.86	2.44	0.46	0.83	0.17	0.16	1.24	1.96	1.69	0.58	2.78	5.73	0.42	1.32	2.78	2.91	0.17	1.26	1.96	2.74
GL11	0.26	0.73	0.00	0.26	1.05	0.37	0.25	0.11	0.17	0.64	1.76	0.36	0.40	1.58	0.19	0.27	0.00	0.00	1.53	0.20	1.00	1.81	0.86
GL12	0.00	0.00	0.00	0.38	2.60	0.42	1.45	0.08	0.00	2.22	0.55	0.36	0.45	1.82	3.27	0.34	0.24	3.26	9.44	0.41	3.46	2.12	2.63

Clone	α-pinene	β-pinene	1,8-cineole	β-phellandrene+ limonene	linalool	piperitone	eugenol	α-cubebene	α-copanene	caryophyllene	germacrene-D	calamenone	cadina-1,4-diene	spathulenol	guaiol	δ-cadinol	218 diterpene	drimenol	polygodial artifactct	drimenol	polygodial	272 diterpene	drimenol
HY1	0.39	0.22	0.00	0.54	3.10	0.28	0.68	0.20	0.98	0.97	0.30	13.22	13.59	2.01	7.21	0.47	1.04	1.03	16.65	0.26	0.29	1.71	0.51
HY2	0.56	0.34	0.00	1.42	6.92	0.30	2.45	0.48	0.17	0.55	0.30	0.48	0.34	0.96	5.38	0.00	4.09	1.39	32.87	1.80	11.57	1.98	1.25
HY3	0.13	0.11	0.06	0.36	0.93	0.29	0.60	0.18	0.23	1.38	0.27	2.47	2.83	0.73	1.34	0.14	2.77	1.23	42.04	0.83	14.97	1.70	1.04
HY4	0.26	0.20	0.00	0.50	2.48	0.10	1.06	0.21	1.06	0.85	0.41	0.18	13.12	2.15	6.91	0.58	1.30	0.96	8.52	0.21	2.89	0.43	1.68
HY5	0.63	0.31	0.17	0.51	1.95	0.54	0.43	0.15	0.25	1.16	1.81	0.55	0.28	1.60	4.59	0.18	0.93	1.48	19.41	0.48	6.34	0.79	0.63
HY6	0.31	0.21	0.23	0.82	0.50	0.77	4.46	0.46	0.10	0.52	1.29	0.49	1.52	1.24	5.66	0.39	0.45	0.22	41.00	0.80	12.75	1.06	0.84
HY7	0.45	0.32	0.00	0.49	0.13	0.33	0.25	0.00	0.18	2.05	0.49	0.37	1.74	0.58	6.87	0.35	4.38	1.56	42.74	0.71	13.16	0.88	0.30
HY8	0.61	0.33	0.11	0.26	2.83	0.41	1.32	0.36	0.19	0.84	1.56	0.47	0.43	1.67	3.59	0.22	10.59	1.69	25.34	0.39	7.75	4.52	1.43
HY9	0.25	0.21	0.00	0.72	2.03	0.45	1.68	0.26	0.11	1.18	0.65	0.00	0.29	1.25	3.99	0.37	0.24	1.13	36.50	0.71	11.29	1.91	0.80
HY10	0.43	0.28	0.00	0.74	2.77	0.62	0.92	0.16	0.00	0.68	1.16	0.26	0.22	0.54	3.60	0.62	2.61	1.11	42.42	0.72	13.00	0.53	0.66
HY11	0.74	0.35	0.17	0.48	2.35	0.53	1.14	0.18	0.19	0.71	1.45	0.57	0.32	1.13	5.66	0.19	0.41	0.94	28.62	0.50	8.90	1.20	0.77
HY12	0.88	0.38	0.11	0.69	2.13	0.35	0.87	0.15	0.21	0.85	2.15	0.28	0.23	1.32	1.55	0.48	0.17	0.95	16.98	0.25	5.28	1.39	0.84
HY13	0.27	0.16	0.09	0.38	0.15	0.36	0.79	0.52	0.20	0.53	2.34	0.70	0.34	2.34	0.91	0.43	3.22	0.86	16.03	0.35	4.89	0.72	1.06
HY14	0.40	0.25	0.08	0.93	1.81	0.39	0.61	0.23	0.16	0.90	1.93	0.71	0.22	1.32	2.53	0.53	1.22	0.76	21.67	0.40	6.68	0.80	0.59
HZ1	0.39	0.09	0.43	3.38	0.41	1.67	0.13	0.11	0.34	3.09	0.09	0.00	0.18	2.83	4.75	0.59	2.49	1.59	23.48	0.81	9.25	2.03	0.23
HZ2	0.00	0.00	0.00	0.40	2.41	0.87	1.39	0.53	0.13	1.57	0.77	0.15	0.22	3.60	0.28	0.34	1.29	1.60	32.23	0.63	10.98	0.27	3.09
HZ3	0.00	0.00	0.00	0.61	5.22	1.17	3.80	0.11	0.15	2.85	0.35	0.08	0.15	2.32	4.41	0.38	0.67	1.96	33.10	0.60	11.27	1.03	0.57
HZ4	0.00	0.00	0.00	0.64	3.94	0.55	0.98	0.35	0.11	1.88	1.28	0.00	0.16	0.48	3.62	0.37	0.25	0.42	32.19	0.52	10.83	0.35	3.61
HZ5	0.00	0.00	0.00	0.16	3.96	0.45	0.52	0.35	0.00	0.52	0.00	0.00	0.31	0.34	0.44	0.38	4.04	0.41	33.06	0.46	11.05	1.13	3.07
HZ6	0.00	0.07	0.00	0.37	0.21	0.72	2.67	0.10	0.12	0.88	0.00	0.06	0.12	0.83	3.00	0.31	0.40	1.90	29.57	0.42	9.51	0.98	0.77
LL1	0.00	0.00	0.00	0.74	1.24	0.21	1.18	0.38	0.00	1.02	0.77	0.24	0.33	1.29	2.73	0.00	0.18	1.55	49.49	1.15	17.84	1.23	3.19
LL2	0.00	0.00	0.00	0.53	0.64	0.00	0.72	0.00	0.00	0.82	0.67	0.00	0.35	1.26	0.93	0.00	0.15	1.39	23.91	0.76	9.27	1.02	2.13
LR1	0.00	0.00	0.00	0.00	3.96	0.83	0.00	0.00	0.00	2.89	4.88	4.19	1.03	3.82	1.95	0.42	0.00	4.53	6.12	0.00	2.60	7.03	12.71
LR2	0.00	0.00	0.00	0.00	0.18	0.53	0.10	0.10	0.20	0.87	2.46	0.52	0.21	1.98	0.22	0.24	0.22	1.02	1.22	0.00	0.43	0.98	2.31
LR3	0.00	0.00	0.00	0.00	0.98	0.34	0.79	0.00	0.15	0.25	2.53	0.55	0.24	1.98	1.36	0.22	0.29	1.87	9.27	0.32	3.48	2.32	0.96
LR4	0.00	0.00	0.00	0.00	0.74	0.43	0.38	0.00	0.00	0.36	0.94	0.20	0.27	1.11	1.32	0.36	0.17	1.46	25.53	0.63	9.64	1.32	0.96

Clone	α-pinene	β-pinene	1,8-cineole	β-phellandrene+ limonene	linalool	piperitone	eugenol	α-cubebene	α-copanene	caryophyllene	germacrene-D	calamenone	cadina-1,4-diene	spathulenol	guaiol	δ-cadinol	218 diterpene	drimenol	polygodial artifactct	drimenol	polygodial	272 diterpene	drimenol
LR5	0.00	0.00	0.00	0.13	2.05	0.24	0.22	0.16	0.28	0.86	1.99	0.92	0.32	1.81	2.64	0.19	1.74	1.51	15.08	0.49	5.62	2.16	1.04
MB1	0.00	0.00	0.00	0.00	0.19	1.08	2.66	0.00	0.00	0.17	0.00	0.30	0.00	0.95	1.49	0.30	0.97	1.34	35.72	1.04	12.59	1.12	2.28
MB2	0.14	0.09	0.00	0.59	2.72	0.36	0.76	0.36	0.74	0.88	0.79	0.00	11.75	1.93	6.03	0.40	1.40	1.17	17.56	0.41	6.12	1.41	1.32
MB3	0.00	0.00	0.09	0.14	1.37	0.80	1.37	0.17	0.12	1.64	0.10	0.80	0.20	2.16	8.39	0.00	0.31	1.52	30.06	0.67	10.32	2.69	7.39
MB4	0.00	0.19	0.00	1.34	1.68	0.69	0.21	0.00	0.19	0.65	3.21	0.53	0.21	3.09	4.15	0.00	0.00	0.47	13.55	0.36	4.45	0.82	3.42
MB5	0.00	0.00	0.00	0.14	1.36	0.52	0.43	0.26	0.00	0.53	0.82	0.20	0.25	0.46	2.92	0.00	0.28	1.52	46.59	0.82	15.67	1.80	2.71
MB6	0.00	0.00	0.00	0.15	2.95	0.46	0.00	0.00	0.22	1.20	2.57	1.47	0.28	1.97	3.10	0.00	0.00	1.84	15.79	0.36	5.35	2.58	2.06
MB7	0.00	0.14	0.14	2.02	1.42	0.81	2.46	0.34	0.00	1.63	1.67	0.39	0.25	0.71	6.42	0.00	1.82	0.36	36.34	0.73	12.44	1.86	2.39
MB8	0.00	0.00	0.00	0.24	1.24	0.58	0.55	0.12	0.23	0.70	1.57	0.77	0.25	1.23	1.12	0.00	0.22	2.05	19.00	0.38	6.45	3.41	1.35
MB9	0.00	0.00	0.00	0.13	1.34	0.29	0.29	0.19	0.13	0.68	2.55	0.61	0.29	1.39	0.46	0.00	1.12	1.60	21.11	0.43	7.23	1.98	1.13
MB10	0.00	0.00	0.09	0.11	1.45	0.94	1.89	0.31	0.00	0.44	0.54	0.29	0.23	0.87	3.70	0.00	0.72	2.70	37.51	0.81	13.12	4.86	2.57
NW1	0.70	0.35	0.00	0.72	1.97	0.16	0.58	0.16	1.23	0.92	0.14	0.00	7.46	2.30	4.65	0.56	1.08	0.15	7.44	0.20	2.57	0.85	0.93
NW2	0.86	0.75	0.30	0.79	1.07	1.06	0.75	0.29	0.15	0.25	0.13	0.26	0.27	1.15	3.75	0.11	0.00	0.00	40.35	0.87	14.52	0.91	1.09
PP1	0.14	0.13	0.08	1.55	1.28	0.42	1.69	0.13	0.11	1.13	0.16	0.23	0.16	0.54	5.21	0.34	0.79	1.65	38.46	1.06	20.78	0.76	0.49
PP2	0.10	0.00	0.11	0.55	2.09	0.47	0.55	0.10	0.11	1.64	0.17	0.19	0.20	1.22	7.59	0.48	0.29	1.85	0.28	0.77	16.59	0.56	1.19
PP3	0.13	0.00	0.20	0.61	1.69	0.82	1.83	0.00	0.09	0.55	0.41	0.18	0.23	1.19	3.06	0.31	3.36	1.42	34.45	0.88	16.18	0.93	0.90
RR1	0.14	0.10	0.07	0.57	0.28	0.24	0.69	0.18	0.53	0.42	0.51	0.00	5.23	1.34	3.83	0.28	1.33	1.40	28.02	0.64	9.75	2.53	1.52
RR2	0.00	0.00	0.00	0.00	0.21	0.40	1.83	0.20	0.00	0.00	0.00	0.15	0.17	0.98	0.76	0.00	0.20	2.85	47.18	1.01	16.39	1.10	2.25
SR1 (a)	0.00	0.09	0.00	0.71	0.22	0.46	1.65	0.57	0.88	2.02	0.32	0.19	9.10	2.11	3.90	0.21	0.42	1.71	24.56	0.50	8.76	1.37	0.49
SR1 (b)	0.00	0.14	0.00	1.26	3.84	0.07	0.89	0.14	1.23	1.35	0.16	0.24	13.07	2.96	6.25	0.19	0.74	0.80	5.06	0.37	2.73	0.91	0.50
SR2	0.00	0.00	0.00	0.29	0.83	0.08	2.19	0.47	0.62	1.63	0.18	0.18	2.47	1.61	5.03	0.37	0.51	2.43	27.42	0.70	9.89	2.01	1.77
SR3	0.32	0.66	0.00	0.89	0.18	0.15	0.14	0.00	1.14	1.14	0.21	0.21	11.70	2.39	4.90	0.15	1.40	0.99	9.37	0.32	3.60	1.22	2.56
TR1	0.96	0.45	0.14	0.77	1.62	0.60	0.57	1.67	0.12	0.55	1.04	0.45	0.22	1.26	10.05	0.43	3.86	1.78	0.90	1.26	24.97	1.30	1.26
TR2	0.09	0.00	0.16	0.40	1.75	0.71	0.99	0.26	0.08	0.31	0.08	0.14	0.14	0.46	4.27	0.25	0.10	1.05	44.18	0.95	18.98	0.52	1.37
TR3	0.13	0.00	0.20	0.72	0.35	1.05	0.83	0.09	0.09	0.62	0.61	0.28	0.17	1.03	6.35	0.37	2.03	1.63	31.11	0.82	13.71	0.75	0.99
TR4	0.00	0.00	0.00	0.00	2.40	0.63	0.64	0.00	0.00	0.53	0.35	0.00	0.00	0.76	6.51	1.12	0.30	1.49	35.85	0.79	14.04	0.83	1.67
TR5	0.22	0.00	0.20	0.64	3.38	1.24	2.07	0.27	0.00	0.37	0.13	0.25	0.00	1.12	6.92	0.38	2.06	1.50	43.98	0.93	16.58	0.59	1.21
TR6	0.00	0.00	0.08	0.19	3.06	0.42	0.80	0.00	0.12	1.22	3.05	0.74	1.87	0.97	6.60	0.36	1.32	1.41	31.78	0.71	11.90	0.50	0.50
UB1	0.00	0.00	0.11	0.55	3.06	0.50	0.97	0.00	0.10	1.72	0.50	0.25	2.00	1.06	6.97	0.39	1.06	0.84	34.83	0.86	12.73	0.83	0.85

Clone	α-pinene	β-pinene	1,8-cineole	β-phellandrene+ limonene	linalool	piperitone	eugenol	α-cubebene	α-copanene	caryophyllene	germacrene-D	calamenone	cadina-1,4-diene	spathulenol	guaiol	δ-cadinol	218 diterpene	drimenol	polygodial artifactct	drimenol	polygodial	272 diterpene	drimenol
UB2	0.00	0.00	0.24	0.49	0.98	1.13	1.30	0.57	0.10	0.24	0.00	0.20	0.19	1.03	3.33	0.45	0.12	1.13	45.77	1.04	16.80	0.73	0.88
UB3	0.00	0.00	0.00	0.10	1.14	0.31	1.17	0.37	0.00	0.21	0.00	0.12	0.22	0.86	2.64	0.68	2.18	1.59	48.19	1.21	17.72	0.90	1.78
UB4	0.14	0.40	0.22	0.82	1.91	1.37	0.74	0.43	0.13	0.95	1.20	0.57	0.18	1.01	6.43	0.33	1.99	1.13	47.30	0.99	16.69	0.73	2.26
UB5	0.00	0.00	0.20	0.25	2.32	1.47	0.29	0.00	0.00	0.63	0.00	0.15	0.24	1.30	4.99	0.00	0.64	1.85	45.58	1.21	3.14	1.53	1.42
WH1	0.44	0.31	0.12	0.34	0.91	0.55	3.10	0.08	0.15	1.39	0.36	0.37	0.18	1.17	5.86	0.00	1.60	1.65	38.54	0.80	13.40	3.22	0.79
WH2	0.31	0.24	0.07	0.21	4.70	0.31	1.69	0.14	0.18	1.02	1.53	0.49	0.29	1.62	4.98	0.12	5.55	0.81	30.16	0.62	10.87	1.48	0.80
WH3	0.25	0.20	0.10	0.23	2.61	0.67	1.71	0.19	0.40	1.29	2.19	3.94	0.29	1.52	3.31	0.16	1.36	0.77	18.55	0.41	6.45	0.60	0.29
WH4	0.19	0.15	0.00	0.43	3.37	0.17	1.74	0.00	0.20	1.00	2.40	9.23	0.28	2.00	0.31	0.11	1.53	0.62	12.29	0.25	4.14	0.42	2.11
WH5	0.16	0.13	0.00	0.29	2.87	0.27	1.61	0.12	0.25	1.26	2.31	9.81	0.27	1.68	0.27	0.17	0.65	0.51	12.20	0.24	4.06	0.22	0.73
WH6	0.16	0.17	0.10	0.23	2.16	0.60	1.80	0.08	0.31	1.33	2.31	1.21	0.29	2.21	0.44	0.27	0.76	1.10	10.43	0.27	3.33	0.99	1.11
WH7	0.17	0.12	0.10	0.26	3.23	0.57	1.52	0.16	0.15	0.70	1.20	0.33	0.28	1.38	3.17	0.09	5.57	1.34	30.78	0.20	10.79	2.33	1.06

APPENDIX 6

FEMA-GRASS APPLICATION

I. Physical-Chemical Data

A. Chemical Data

1. Nomenclature

a. Preferred Name(s)
***Tasmannia lanceolata* concrete**
***Tasmannia lanceolata* absolute**
Tasmanian Mountain Pepper Concrete
Tasmanian Mountain Pepper Absolute

b. Chemical Abstracts Name and Registry Number
CAS No: 183815-52-3

c. IUPAC Name
NA

d. Botanical Name
***Tasmannia lanceolata* (Poiret) A C Smith**

e. Other Names
Common name: Mountain Pepper
Base name: *Winterana lanceolata* Poiret
Drimys lanceolata
Note: The taxonomical history shows that *Drimys aromatica* and *Drimys lanceolata* are synonyms.

f. Other Designations/Identity Numbers
NA

2. Formula

a. Chemical
NA

b. Molecular Weight
NA

C. Structure
NA

B. Analytical Data

1. Composition

Component	*% Volatiles*
α-pinene	1.056
camphene	0.007
sabinene	0.193
β-pinene	0.363
myrcene	0.104
α-terpinene	0.167
α-phellandrene	0.068
p-cymene	0.050
1,8 cineole	0.319
limonene + β-phellandrene	0.840
γ-terpinene	0.038
unknown	0.039
terpinolene	0.000
linalool	2.101
α-terpineol	0.140
piperitone	0.690
eugenol	1.138
α-cubebene	1.075
methyl eugenol	0.302
α-copanene	0.530
β-cubebene	0.270
α-gurjunene	0.787
β-caryophyllene	1.078
germacrene-D	0.541
myristicin	1.256
iso-eugenol	0.953
calamenene	4.169
cadina-1,4-diene	1.790
α-amorphene	0.725
palustrol	0.504
spathulenol	2.082
guaiol	5.677
δ-cadinol	0.748
218 oxygenated sesquiterpene	2.146
drimenol	1.334
cadenol related unknown	2.437
polygodial	45.902
drimenol related unknown	0.882
unknown diterpene	1.287
C_{24} aldehyde unknown	1.307
Total	85.095

2. Purity and Identity
   The product is 100% natural, without any additions of either artificial or nature identical substances. A gas chromatogram run on a BP1 column is shown in Appendix II.
3. Methods of Detection (other than above)
4. Comments

C. Physical Data

1. Stability

The product is stable at storage temperatures of 2°C or less for at least ten months. There is no advantage in storage under nitrogen. The test is described in Attachment 1.

2. Solubility

a. Water

Not Soluble

b. Fat (designate what non-polar solvent was used .e.g. heptane)

Partially soluble in hexane

c. Octanol / Water partition coefficient

0

d. Other

Soluble in ethanol

3. Miscellaneous Physical Data

a. Appearance

The extract is very dark citron green/twice grade, code #000763 (Wilson Horticultural Colour Chart). It is viscous at room temperature and has a distinctive and exotic aroma, possessing fresh, spicy top notes overlying a peppery background. It has a hot and spicy flavour with the heat being conferred by the primary pungent principle, polygodial.

b. Melting Point

Liquifies above 40°C

c. Boiling Point

156°C

d. Specific Gravity / Density

0.98 at 25°C

e. Refractive Index

1.64 at 20°C

f. Flash Point

52°C (Pensky-martens closed cup)

g. Optical Rotation

h. Acid Value

11.4

D. How Obtained

1. Purchased (if so, give manufacturer's specifications)
2. Natural Product (if so, give source and method of preparation)

Petroleum ether extract of dried leaf material from *Tasmannia lanceolata*.

3. Synthesized (if so, complete the following)
   a. Methodology
   b. Indicate all solvents and reagents employed

II. Biological Data

A. Literature Citations

1. Data Sources: List databases queried, e.g. Medline, Toxline, Toxback, Agricola, Toxnet, etc. and append copies of the searches with a brief narrative summarizing the relevant data.

Search for *Tasmannia lanceolata*.

Data Source	Number of Postings
Medline Express 1983-89	0
Life Sciences 1990-92	0
Life Sciences 1993-95	0
ASFA1988-9/96	0
CAB Abstracts 1/96-10/96	0
Analytical Abstracts 1/80-12/96	0
Serline	0
Healthstar	0

The following postings were returned when a search was conducted for polygodial, the main constituent of the extract.

Data Source	Number of Postings
Medline Express 1983-89	2
Life Sciences 1990-92	12
Life Sciences 1993-95	6
ASFA1988-9/96	2
CAB Abstracts 1/96-10/96	26
Analytical Abstracts 1/80-12/96	3

Polygodial was found to be an antifungal potentiator, by increasing cell membrane permeability. It also has some antibiotic effects. It was shown by many studies to be an antifeedant, interfering with chemoreceptors in insects.
Polygodial occurs in other species which are used as foods or spices (*Warburgia ugandensis*, *W. stuhlmannii* and *Polygonum hydropiper*).

2. Publications: List author, title and source below. Append abstracts and copies of original publications (or English translantions of the abstracts of foreign language publications).
   Abstracts are shown in Attachment III

TI: Polygodial, an antifungal potentiator.
AU: Kubo-I; Taniguchi-M
SO: J-Nat-Prod. 1988 Jan-Feb; 51(1): 22-9

TI: Insect antifeedant activity and hot taste for humans of selected natural and synthetic 1,4-dialdehydes.
AU: Caprioli-V; Cimino-G; Colle-R; Gavagnin-M; Sodano-G; Spinella-A
SO: J-Nat-Prod. 1987 Mar-Apr; 50(2): 146-51

TI: Interference with normal chemoreceptor activity by some sesquiterpenoid antifeedants in an herbivorous insect Pieris brassicae .
AU: Schoonhoven,-L.M.; Fu-Shun,-Yan
SO: J.-INSECT-PHYSIOL. 1989. vol. 35, no. 9, pp. 725-728

TI: Activity of drimane antifeedants and related compounds against aphids, and comparative biological effects and chemical reactivity of (-)- and (+)-polygodial.
AU: Asakawa,-Y.; Dawson,-G.W.; Griffiths,-D.C.; Lallemand,-J.-Y.; Ley,-S.V.; Mori,-K.; Mudd,-A.; Pezechk-Leclaire,-M.; Pickett,-J.A.; et-al.
SO: J.-CHEM.-ECOL. 1988. vol. 14, no. 10, pp. 1845-1856

TI: Polygodial-induced sensitivity to rifampicin and actinomycin D of Saccharomyces cerevisiae .
AU: Taniguchi,-M.; Yano,-Y.; Motoba,-K.; Tanaka,-T.; Oi,-S.; Haraguchi,-H.; Hashimoto,-K.; Kubo,-I.
SO: AGRIC.-BIOL.-CHEM. 1988. vol. 52, no. 7, pp. 1881-1883

TI: Observations on the toxicity and metabolic relationships of polygodial, the chemical defense of the nudibranch Dendrodoris limbata .
AU: Cimino,-G.; De-Rosa,-S.; De-Stefano,-S.; Sodano,-G.
SO: EXPERIENTIA. 1985. vol. 41, no. 10, pp. 1335-1336

TI: Dorid nudibranch elaborates its own chemical defense.
AU: Cimino,-G.; De-Rosa,-S.; De-Stefano,-S.; Sodano,-G.; Villani,-G.
SO: SCIENCE-WASH.. 1983. vol. 219, no. 4589, pp. 1237-1238

TI: The effects of the repellents dodecanoic acid and polygodial on the acquisition of non-, semi- and persistent plant viruses by the aphid Myzus persicae .
AU: Gibson,-R.W.; Rice,-A.D.; Pickett,-J.A.; Smith,-M.C.; Sawicki,-R.M.
SO: ANN.-APPL.-BIOL. 1982. vol. 100, no. 1, pp. 55-59

TI: Antibiotic substances from New Zealand plants. II. Polygodial, an anti-Candida agent from Pseudowintera colorata .
AU: McCallion,-R.F.; Cole,-A.L.J.; Walker,-J.R.L.; Blunt,-J.W.; Munro,-M.H.G.
SO: PLANT.-MED. 1982. vol. 44, no. 3, pp. 134-138

TI: Responses of Myzus persicae to the repellent polygodial in choice and no-choice video assays with young and mature leaf tissue
AU: Powell,-G.; Hardie,-J.; Pickett,-J.A.
SO: ENTOMOL.-EXP.-APPL. 1995 vol. 74, no. 1, pp. 91-94

TI: Sesquiterpenoid unsaturated dialdehydes increase the concentration of intracellular free Ca super(2+) in human neuroblastoma SH-SY5Y cells
AU: Forsby,-A.; Witt,-R.; Walum,-E.*
SO: NAT.-TOXINS 1994 vol. 2, no. 2, pp. 89-95

TI: Antimicrobial activity of the volatile constituents of Perilla frutescens and its synergistic effects with polygodial
AU: Kang,-R.; Helms,-R.; Stout,-M.J.; Jaber,-H.; Chen,-Zhengqing; Nakatsu,-T.*
SO: J.-AGRIC.-FOOD-CHEM. 1992 vol. 40, no. 11, pp. 2328-2330

TI: Effects of the antifeedant polygodial on plant penetration by aphids, assessed by video and electrical recording
AU: Powell,-G.; Hardie,-J.; Pickett,-J.A.
SO: ENTOMOL.-EXP.-APPL. 1993 vol. 68, no. 2, pp. 193-200

TI: Sesquiterpenoids from a cell suspension culture of the liverwort Porella vernicosa Lindb.
AU: Ono-K; Sakamoto-T; Tanaka-H; Asakawa-Y
SO: Flavour-and-Fragrance-Journal. 1996, 11: 1, 53-56; 8 ref.

TI: Antifeedant and toxic effects of drimanes on Colorado potato beetle larvae.
AU: Gols-GJZ; Loon-JJA-van; Messchendorp-L; Van-Loon-JJA
SO: Entomologia-Experimentalis-et-Applicata. 1996, 79: 1, 69-76; 26 ref.

TI: Use of insect antifeedants against aphid vectors of plant virus disease.
AU: Griffiths-DC; Pickett-JA; Smart-LE; Woodcock-CM
SO: Pesticide-Science. 1989, 27: 3, 269-276; 22 ref.

TI: Secondary plant metabolites as targets for genetic modification of crop plants for pest resistance.
AU: Dawson-GW; Hallahan-DL; Mudd-A; Patel-MM; Pickett-JA; Wadhams-LJ; Wallsgrove-RM
SO: Pesticide-Science. 1989, 27: 2, 191-201; Paper presented at the symposium 'Natural Products as a Source for New Agricultural Chemicals', held in London, UK, 21-22 February 1989 ; 28 ref.

TI: The combination of electronic monitoring and video-assisted observations of plant penetration by aphids and behavioral effects of polygodial.
AU: Hardie-J; Holyoak-M; Taylor-NJ; Griffiths-DC
SO: Entomologia-Experimentalis-et-Applicata. 1992, 62: 3, 233-239; 26 ref.

TI: Anethole, a synergist of polygodial against filamentous microorganisms.
AU: Kubo-I; Himejima-M
SO: Journal-of-Agricultural-and-Food-Chemistry. 1991, 39: 12, 2290-2292; 19 ref.

TI: Potential of secondary metabolites in genetic engineering of crops for resistance.
AU: Hallahan-DL; Pickett-JA; Wadhams-LJ; Wallsgrove-RM; Woodcock-CM; Gatehouse-AMR (ed.); Hilder-VA (ed.); Boulter-D (ed.)
SO: Plant-genetic-manipulation-for-crop-protection. 1992, 215-248; Biotechnology in Agriculture No. 7 ; 115 ref.

PB: CAB International; Wallingford; UK

TI: Antimicrobial agents from Licaria puchuri-major and their synergistic effect with polygodial.
AU: Himejima-M; Kubo-I
SO: Journal-of-Natural-Products. 1992, 55: 5, 620-625; 16 ref.

TI: Insecticidal properties of the terpenoids polygodial, 9-deoxymuzigadial and azadirachtin.
AU: Gerard-PJ; Ruf-LD; Perry-NB; Foster-LB; Popay-AJ
SO: Proceedings of the Forty Fifth New Zealand Plant Protection Conference, Wellington, New Zealand, 11-13 August 1992. 1992, 239-242; 13 ref.

TI: Antifeedant and insecticidal activity of compounds from Pseudowintera colorata (Winteraceae) on the webbing clothes moth, Tineola bisselliella (Lepidoptera: Tineidae) and the Australian carpet beetle, Anthrenocerus australis (Coleoptera: Dermestidae).
AU: Gerard-PJ; Perry-NB; Ruf-LD; Foster-LM
SO: Bulletin-of-Entomological-Research. 1993, 83: 4, 547-552; 17 ref.

TI: Ethnobotanical drug discovery based on medicine men's trials in the African savanna: screening of East African plants for antimicrobial activity II.
AU: Taniguchi-M; Kubo-I
SO: Journal-of-Natural-Products. 1993, 56: 9, 1539-1546; 35 ref.

TI: Fungicidal activity of polygodial in combination with anethole and indole against Candida albicans.
AU: Himejima-M; Kubo-I
SO: Journal-of-Agricultural-and-Food-Chemistry. 1993, 41: 10, 1776-1779; 13 ref.

TI: Potentiation of antifungal activity of sesquiterpene dialdehydes against Candida albicans and two other fungi.
AU: Kubo-I; Himejima-M
SO: Experientia. 1992, 48: 11-12, 1162-1164; 14 ref.

TI: Comparison of the antimicrobial and cytotoxic activities of twenty unsaturated sesquiterpene dialdehydes from plants and mushrooms.
AU: Anke-H; Sterner-O
SO: Planta-Medica. 1991, 57: 4, 344-346; 16 ref.

TI: The antifeedant activiity of polygodial against aphids.
AU: Zhang-ZN; Liu-X; Lou-ZX; Li-HW; Zhu-SX; Zou-F
SO: Acta-Entomologica-Sinica. 1993, 36: 2, 172-176; 7 ref.

TI: Anethole, a synergist of polygodial and warburganal against Candida albicans.
AU: Kubo-I; Schilcher-H (ed.); Phillipson-JD (ed.); Loew-D
SO: First world congress on medicinal and aromatic plants for human welfare (WOCMAP), Maastricht, Netherlands, 19-25 July 1992. Acta-Horticulturae. 1993, No. 332, 191-197; 14 ref.

TI: The effect of six sesquiterpenoid unsaturated dialdehydes on cell membrane permeability in human neuroblastoma SH-SY5Y cells [published erratum appears in Chem Biol Interact 1993 Feb;86(2):183]
AU: Forsby-A; Walum-E; Sterner-O
SO: Chem-Biol-Interact. 1992 Sep 14; 84(1): 85-95

TI: Antimicrobial agents from Licaria puchuri-major and their synergistic effect with polygodial.
AU: Himejima-M; Kubo-I
SO: J-Nat-Prod. 1992 May; 55(5): 620-5

TI: Synthesis of antifeedants for insects: novel behaviour-modifying chemicals from plants.
AU: Ley-SV
SO: Ciba-Found-Symp. 1990; 154: 80-7; discussion 87-98

TI: Stereospecificity of allergic contact dermatitis (ACD) to enantiomers. Part III. experimentally induced ACD to a natural sesquiterpene dialdehyde, polygodial in guinea pigs.
AU: Stampf-JL; Benezra-C; Asakawa-Y
SO: Arch-Dermatol-Res. 1982; 274(3-4): 277-81

TI: Determination of the sesquiterpene dialdehyde polygodial by high-pressure liquid chromatography.
AU: Van-Beek,-T.-A.; Van-Dam,-N.; De-Groot,-A.; Geelen,-T.-A.-M.; Van-der-Plas,-L.-H.-W.
SO: Phytochem.-Anal., Jan-Feb 1994, 5 (1), 19-23

B. Test Data: Append copies of reports (including protocol) of any toxicity, metabolism, pharmacological or other studies that you are aware of and are not available in the publicly accessible literature.

Nil

C. Other Data

1. Are there other relevant data (occupational, epidemiological, microbiological, etc.) of which you are aware?
   No
2. Does this compound have any known pharmacologic or physiologic activity? If so, describe the effect(s), the system in which the effect(s) is/are elicited, and the dose of the substance required to elicit the effect(s).
3. Provide an MSDS , if available.
   See Attachment IV
4. Sensory Tolerance test: 83 Percent (%) of the test panel found the substance intolerable at 295 PPM. (Include the number of subjects in the test panel and generally describe the protocol used.)

   The evaluation medium was water. The test solution was prepared by dissolving the extract in alcohol (1% solution). The final tasting solutions were prepared by adding different volumes of the test solution to 100mL aliquots of water. A panel of six was asked to taste the product, leaving it in the mouth long enough for the full impact to be assessed.

D. Related Substances: Summarize and append relevant data on structurally related substances (both FEMA and non-FEMA substances as well as any naturally occurring synthetic substances: do not confine your search to food and flavor substances). Use the general format designated below:

Extract components registered with FEMA are shown in the table below:

Component	FEMA No	Comments
α-pinene	2902	fresh piney
camphene	2229	camphoraceous oily
β-pinene	2903	resinous piney
myrcene	2762	sweet
α-terpinene	3558	lemon like
α-phellandrene	2856	mint like background
d-limonene	2633	mildly citrus no camphor or terpene
γ-terpinene	3559	herbaceous citrusy
linalool	2635	pleasant floral odour
α-terpineol	3045	lilac like
eugenol	2467	pungent spicey taste, cloves
methyl eugenol	2475	delicate clove carnation
β-caryophyllene	2252	clove like

1. Structurally related GRAS substances
   a. Chemical **NA**
   b. Structure **NA**
   c. Food use categores (e.g. baked goods, soups, etc.) and use levels **NA**

2. Structurally related (non-GRAS) synthetic or naturally occurring substances (be sure to include references)
   a. Chemical **NA**
   b. Structure **NA**
   c. Food **NA**
   d. Quantity **NA**

III. USAGE AND OCCURRENCE IN FOODS

A. History

1. First known date of use:
   European use of the family began in 1597, when *Drimys wintera* was used to relieve scurvy. When this species became hard to obtain, it was replaced by *T. lanceolata.* (LeStrange, 1977)

2. First known date of common use:
   There are references as early as 1866, which cite *D. aromatica* as being a substitute for pepper. (Tanaka, 1976) This use has been confirmed by more recent references (Maiden, 1889 and Cribb, 1974).

B. Flavor Type
Spice, herb

C. Annual volume anticipated (lb)
Since 1994, 30kg (66 lb) of extract has been used in Japan annually.
This has been used in limited confectionery lines.
The anticipated volumes following the introduction to broader food categories could be in the order of 250kg (550 lb) of extract in Japan alone.

D. Usage

Please estimate as closely as possible the usual and maximum levels of usage anticipated in each of the following food categories (next page). Figures should reflect concentration in final food product as eaten.

References :

***1. Cribb, A.B. and J.W. Cribb, *Wild Food in Australia*. 1974, Sydney: William Collins Publishers Pty. Ltd.**

***2. Maiden, J.H., *The useful native plants of Australia, (including Tasmania)*. 1889, Sydney: Turner and Henderson.**

3. LeStrange, R., *A history of herbal plants.* 1977, London: Angus and Robertson.

***4. Tanaka, T., *Tanaka's cyclopedia of edible plants of the world*. ed. S. Nakao. 1976, Yokyo, Japan: Keigaku Publishing Co.**

***Excerpts are provided in Attachment V.**

Daily Consumption of Regular Foods by Males & Females,
2-65+ Years, Total Sample‡

NO.	Food Category	Mean consumption gms/day‡	*Anticipated Usual Usage PPM*	*Anticipated Maximum Usage PPM*
01	Baked Goods	137.2		
02	Breakfast Cereals	20.0		
03	Other Grains	27.8		
04	Fats & Oils	17.5		
05	Milk Products	39.5		
06	Cheese	9.4		
07	Frozen Dairy	25.6		
08	Processed Fruits	118.3		
09	Fruit Ices	0.7		
10	Meat Products	78.4		
11	Poultry	12.9		
12	Egg Products	1.9		
13	Fish Products	12.4		
14	Processed Vegetables	85.0		
15	Condiments & Relishes	8.8		
16	Soft Candy	5.8		
17	Confectionery & Frosting	0.3		
18	Jams & Jellies	5.7		
19	Sweet Sauce	6.8		
20	Gelatins & Puddings	20.4		
21	Soups	31.7		
22	Snack Foods	1.3	**0.5**	
23	Beverages Type I - Non alcoholic	104.0	**0.2**	
24	Beverages Type II- Alcoholic	32.5		
25	Nut Products	5.2		
26	Reconstituted Vegetables	0.2		
27	Gravies	8.3		
28	Imitation Dairy Products	0.9		
29	*Fresh Milk	238.3		
30	Hard Candy	0.6	**6**	**10**
31	Chewing Gum	0.2	**50**	
32	Granulated Sugar	8.6		
33	Sugar Substitutes	0.08		
34	Instant Coffee & Tea	121.1		
37	*Fresh Fruits	70.8		
38	*Fresh Meat	72.1		
39	*Fresh Poultry	13.3		
40	*Fresh Eggs	26.9		
41	*Fresh Fish	2.6		
42	*Fresh Vegetables	77.4		
43	*Homemade Jams	2.9		
44	*Homemade Soup	9.0		
45	Seasonings & Flavours	0.01		

‡ Based on MRCA mean frequency of eating and USDA mean portion size. Consumption not used in FRAS daily intake calculations

E. Natural Occurrence in Food

List at least one reference (preferably the most recent) for each food source where occurrence has been reported and include any available quantitative data. Quantitative data should be presented in PPM. Please include one copy of each referenced article (and the English translation, if necessary).

Food	PPM	Reference