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EDITOR'S NOTE

One of the aims of the SRN is to promote conservation of Santalum species worldwide and I am pleased that this edition has an article on the conservation of rare and fragmented populations of Santalum lanceolatum in Victoria, southern Australia (Trueman et al). Indeed the article highlights the importance of genetic analysis in conservation programs and also identifies a strategy for the conservation of this species in Victoria. It is interesting to note that overgrazing and fire damage—two of the most frequently cited factors affecting the survival of Santalum s species worldwide — is also noted as the major factors (with landclearing) for decline of this population in southern Australia, where population pressures are much less than other Asian and Pacific countries.

The article by Moretta et al identifies an important consideration in the analysis of oil yield and composition of Santalum species. Moretta et al has shown that Santalum spicatum oil yield and composition can change markedly from the roots to the top of the tree. This has important implications not only for research but also for the industry in comparisons of wood samples. For meaningful comparisons to be made, the section of the tree examined must be consistent.

A new publication by the Australian Quandong Industry Association is now available which details the state of knowledge on Quandong (Santalum acuminatum) and the Quandong industry. Copies can be obtained from the author, Dr Elizabeth Gordon-Mills or AQIA, details on page 8.

Articles for publication for the next edition should be received by 30th November. Questions, comments and feedback are also welcome at any time.

Tanya Vernes

Clonality in remnant populations of *Santalum lanceolatum*

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Santalum lanceolatum, the northern sandalwood or plumbush, was very heavily harvested in Victoria and New South Wales in the late 1800s. Clearing, fire and grazing have also contributed to the species' decline. Only seven populations remain in Victoria, where we studied the five southernmost populations of the species. Since exclusion of grazing animals, the remnant populations have been reproducing asexually by root suckers. However, we observed little or no fruit production in the populations, and allozyme and RAPD analyses suggested that sexual reproduction had not been contributing to recruitment. Each population appeared to exist as a unique single clone composed of numerous ramets of a single genet. Therefore, conservation of the species in Victoria may require protection of all remnant populations, and possibly the establishment of new populations.

Introduction

The northern sandalwood or plumbush, *Santalum lanceolatum*, is widespread but not abundant in arid and semi-arid parts of Australia. The species was harvested very heavily for its fragrant oil and timber in Victoria and New South Wales following the gold rushes of the mid- to late-1800s. Clearing, fire and grazing have also contributed to the species' decline. At its southern limit in Victoria, only seven widely separated populations remain, with a total of approximately 258 trees (Johnson 1996). The species has been listed as a threatened taxon under Schedule II of the Victorian Flora & Fauna Guarantee Act 1988.

The restriction and isolation of the remnant *S. lanceolatum* populations has led to the development of a conservation strategy for the species in Victoria. In addition to protection of the remaining populations, particularly from grazing by rabbits and sheep, establishment of new populations has been proposed as a conservation objective. Recruitment has occurred in the populations since the construction of protective enclosures to exclude grazers, but asexual reproduction by root suckering alone has been presumed responsible for the increase in population sizes.

Flowering occurs annually in all populations but fruit production has rarely been observed (Johnson, 1996).

We assessed the genetic structure and levels of clonality within the five southernmost populations of *S. lanceolatum* using allozyme and RAPD analyses. We also examined pollination requirements and natural levels of fruit production in the remnant populations. The combined results of the genetic and reproductive biology investigations are being used to determine priorities for the conservation of the species in Victoria.

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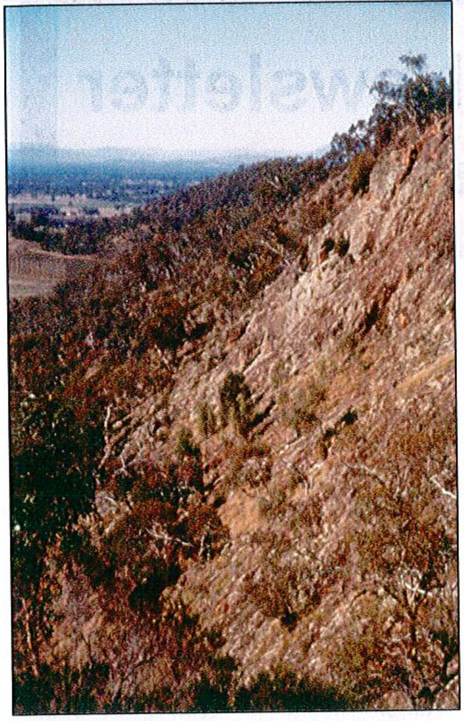

Figure 1. The Warby Range sandalwoods (centre) grow across a steep, rocky northerly slope.
Photo: S. Trueman

Materials and methods

Five of the seven Victorian populations of *S. lanceolatum* are found in the north-east region of the state, in the vicinity of 36°S 146°E, and these represent the five southernmost populations of the species. The populations occur in low open woodland on rocky northern slopes with well-drained granitic soils. Three populations occur on Mt Meg, no more than 2 km apart. These populations were designated 'Mt Meg 1' (MM1), 'Mt Meg 2' (MM2) and 'Canaan College' (CAC). Another population occurs 13 km away at the Warby Range (WBR) (Figs. 1 and 2), and the fifth population is a further 30 km distant at Springhurst (SPR). All populations cover only small areas, ranging from 0.1 to 1.5 ha. Two of the populations exist on private land in small, protectively fenced enclosures.

Counts were made in 1996 and 1997 to determine population sizes. Stems separated by less than 10 cm were regarded as part of the same tree but still measured separately. Leaf material for allozyme analyses was collected from most trees from all five populations. DNA was extracted from a subset of samples for each population.

Pollen viability was assessed in all five populations, and pollen tube growth following natural or hand-pollinations was assessed in two populations. The pollination treatments were (1) open pollination (i.e. natural pollination), (2) crossed within the population, and (3) crossed between populations. Flowers of treatments (2) and (3) were bagged to exclude insects. All flowers were collected and fixed, and pollen tubes were stained for viewing under a fluorescence microscope. Natural levels of fruit set were also determined in four populations.

Full details of the allozyme, RAPD and pollination methods, and the data analyses, are provided by Warburton *et al.* (2000).

Results

The number of trees and stems per population ranged from 3 to 88 and from 5 to 116, respectively (Table 1). Most stems were greater than 2 m tall in four of the populations but at Springhurst most stems were less than 2 m tall. All populations had some flowering stems.

Within each population, all individuals were of the same genotype (Table 2). Of particular importance was that all individuals assayed from the large Warby Range population were the same heterozygous genotype (12 13) for the MDR and SDH loci. Four different multi-locus genotypes were found for these five populations. The Canaan College and Mt Meg 1 populations were indistinguishable genetically using allozymes.

A total of 30 reproducible RAPD bands was scored from six primers. Each primer generated between two and eight RAPD bands and polymorphisms were found in 63% of bands scored. No variation within populations was detected by any of the six primers but differences were detected between populations. Importantly, RAPD established Mt Meg 1 and Canaan College as different genotypes whereas they were indistinguishable using allozymes.

Viable pollen was observed in four of the populations (SPR: 52.7±4.6%; MM1: 66.6±5.1%; CAC: 68.7±4.5%; WBR: 76.6±4.2%; n = 3-4 trees), but no viable pollen was observed in samples collected from Mt Meg 2. Despite pollen deposition on the stigma, no pollen tube penetration was detected at Springhurst (Table 3). Most flowers at Springhurst abscised within 5-6 days after pollination, and so sample sizes were small. Pollen tube penetration was observed following all treatments at the Warby Range, and penetration to the ovary following deposition of pollen collected from the other population was greater than following deposition of pollen from within the same population.

Fruit production was not observed at Canaan College, Mt Meg 1 or Springhurst. However, at the Warby Range population, 1.2±0.4% (n = 5 trees) of tagged flowers produced fruit, and mature fruit were observed on other trees in the population.

	SPR	WBR	CAC	MM1	MM2
Total no. trees per population	52	50	13	88	3
Total no. stems per population	99	58	28	116	5
Percentage of stems > 2 m tall	21	90	71	78	80
Percentage of stems flowering	16	31	25	21	20

Table 1. Structure of the five southernmost *S. lanceolatum* populations. Trees include only individuals more than 10cm from each other (trees may comprise more than one stem).

Site	Multilocus allozyme genotype (Mdh2,Mdr,Sdh)	Multilocus RAPD genotype
SPR	11, 22, 33 (n = 41-45)	A (n = 4)
WBR	11, 12, 13 (n = 44-46)	B (n = 5)
CAC	11, 11, 22 (n = 13)	C (n = 5)
MM1	11, 11, 22 (n = 72-80)	D (n = 5)
MM2	12, 22, 33 (n = 3)	E (n = 3)

Table 2. Four genetic individuals were identified with the three polymorphic allozyme loci, but five genetic individuals were identified from the 30 reproducible RAPD bands scored from six primers. (n = no. of stems sampled).

Discussion

Each of the remnant *S. lanceolatum* populations exists as a single unique clone, apparently recruiting individuals by vegetative reproduction. This may reflect the history of disturbance and fragmentation of the populations due to harvesting, clearing, grazing and fires, coupled with disrupted gene flow. Little or no fruit production is occurring, due to pollen sterility, pollen-pistil incompatibility or pistil dysfunction.

The majority of stems in most populations were more than 2 m tall, indicating that recent recruitment is occurring to only a limited extent. Many populations may have once survived as only a single stem, with recent expansion occurring by root suckering.

The Warby Range stand was discovered in the mid-1950s, when only two or three adult trees remained. The subsequent death of one or two of the trees resulted in just a single adult tree remaining (Garnet 1967). At that stage, *S. lanceolatum* was thought to be represented in Victoria by only a single tree at the Warby Range and by two trees at one of the two more northern populations not investigated in our study. It was considered one of the rarest trees in Victoria, despite having once been common throughout the north central and northeast regions of the state, including many of the slopes of the Warby Range.

Removal of sheep in 1976 and feral goats in 1982 from the Warby Range site, followed by rabbit control programs between 1985 and 1988, have allowed recruitment in that population. The three populations at Mt Meg were discovered and first surveyed in 1994 and 1995, and previous records of these stands are not available. The Springhurst population displays the greatest amount of recent recruitment. This stand was discovered in 1989, and protected by sheep- and rabbit-proof fencing in 1990. The population at that stage consisted of nine mature trees 3.5 - 6 m tall (Johnson, 1996). Exclusion of grazing animals has allowed extensive vegetative recruitment at the site.

The allozyme and RAPD results indicate that additional 'individuals' have been derived from asexual reproduction via root suckers (Johnson, 1996). Whether this is common across the range of the species remains unknown, although growth consistent with vegetative reproduction has been observed in other populations (John Fox, pers. comm.). Clonal patches up to 20 m across within populations of *S. acuminatum* in Western Australia have also been found using AFLP analysis (Sieggy Krauss, pers. comm.).

Flowering occurred in all five populations but mature fruits were only observed at the Warby Range (1.2% fruit set). Complete lack of viable

Treatment	*n*	Pollen grains on stigma	Pollen tubes in stigma	Pollen tubes top style	Pollen tubes bottom style	Pollen tubes in ovary
Springhurst:						
Open- pollination	5	3.20±2.06	0	0	0	0
Cross within population	2	1.00±1.00	0	0	0	0
Cross between populations	9	3.00±1.81	0	0	0	0
Warby Range:						
Open- pollination	38	12.97±3.18	3.95±0.65	2.92±0.55	0.55±0.14	0.18±0.08ab
Cross within population	30	13.90±3.52	5.13±0.96	3.30±0.69	0.47±0.14	0.06±0.11a
Cross between populations	44	12.23±1.85	5.30±0.83	3.64±0.64	0.64±0.17	0.41±0.23b

Table 3. Mean (±s.e.) number of pollen grains and pollen tubes following pollinations at Springhurst and the Warby Range. *n* = number of flowers examined. Significant differences are indicated by different letters.

pollen could not account for poor fruit set in four of the populations. However, no viable pollen was obtained in the fifth population, Mt Meg 2.

Self-incompatibility partly explains the low fruit set in the Warby Range population. Pollination using Springhurst pollen (i.e. from a different genotype) resulted in greater tube penetration to the ovary than pollination using Warby Range pollen (i.e. from the same genotype). The apparent isolation of the population ensures that all natural pollination is self-pollination, involving pollen flow only within the single genet.

No pollen tube penetration was observed at Springhurst, even following deposition of pollen from another population (the Warby Range). The Springhurst population may exhibit pistil dysfunction, previously observed in one genotype of *S. album* (Rugkhla *et al.* 1997). In any case, the isolation and single genet composition of the Springhurst population again ensures that only self-pollination is occurring.

As each population appears to exist as a unique single clone, the conservation of *S. lanceolatum* in Victoria may require protection of all remnant populations. Recruitment by root suckering is occurring to some degree in all populations following the exclusion of grazing animals. Continuing control measures to exclude grazing animals appear warranted for the protection of each remnant.

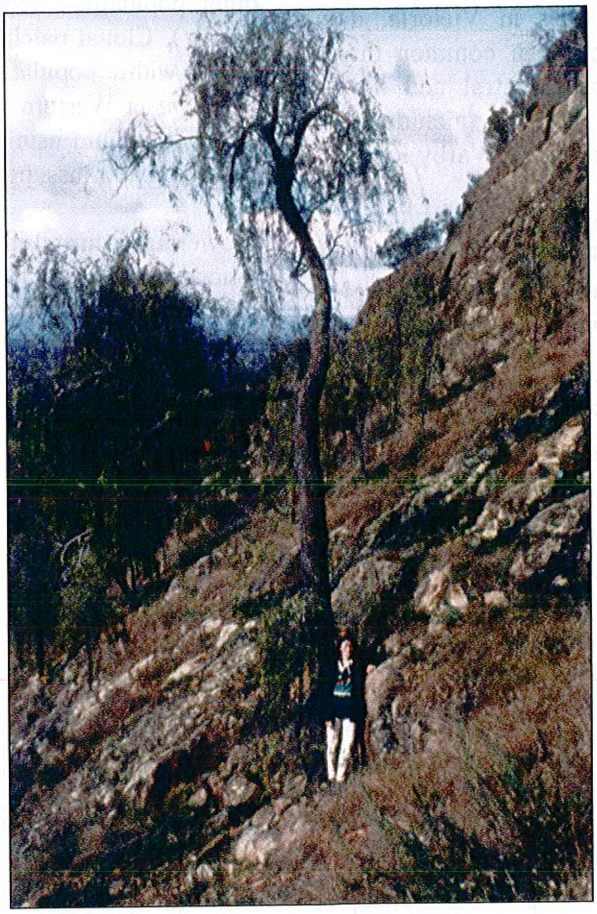

Figure 2. This tree, the sole survivor at the Warby Range in 1967, is apparently the origin of the clone. Photo: S. Trueman

The fragmentation of the populations into isolated stands of single genets suggests an added need for a broad conservation strategy. Ex-situ cultivation of the remnant genets, as proposed by Johnson (1996), remains an option. Construction of new *S. lanceolatum* stands comprising a range of genotypes may provide self-sustaining populations, capable of sexual reproduction and valuable as sources of commercial germplasm.

Acknowledgements

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Longitudinal Variation in the Yield and Composition of Sandalwood Oil From Santalum Spicatum

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The difference in oil yield and composition along the length of three West Australian sandalwood trees, Santalum spicatum, was examined. The buttwood contained a higher oil content in two out of three trees sampled. The amount of oil extracted from the wood above ground level decreased slightly along the length of each tree. Changes in levels of alpha- and beta- santalol were recorded along the length of the tree. The combined santalol content was greatest in the roots of all three trees with alpha-santalol the major constituent of volatile oil. Above ground level a dramatic decrease in the amount of alpha- and beta-santalol was observed and changes in composition of the oil in each tree is given.

Introduction

Numerous studies have shown that considerable variation occurs in both the oil yield and composition from stand to stand and tree to tree within a particular sandalwood species. Differences within a species may be attributed to environmental factors like climate, altitude, soil type and host species, but also arise due to the different methodologies used in the recovery of the oil. For example, it has been shown that variations in both yield and composition of sandalwood oil exist in the oil extracted from the same sample using different extraction procedures (Piggott *et al* 1997).

Another reasons for the variation observed is most likely due to the differences in oil yield and composition within the different sections of the trees. There is general agreement that the roots and buttwood contain more oil with a higher santalol content than the stem and branches. Work carried out on East Indian sandalwood showed that the roots contained 8.43 % oil, compared to 5.79% in the trunk and 3.52% in the branches (Jayppa *et al* 1981). Along the length of the tree, smaller differences were found in the composition of the oil, and the santalol content decreased by only 3%.

Similar studies on *S. spicatum* have shown completely different trends in the oil and santalol content from different sections of the tree. The oil content was found to decrease moving up the length of the tree, from a maximum of 8.38% in the buttwood to a minimum of 1.78% in the high branches (Piggott *et al* 1997). The santalol content of the oil in *S. spicatum* was shown to be higher in the roots and buttwood, and decline markedly moving further up the tree (Piggott *et al* 1997, Brand *et al* 1999). In contrast, the relative amounts of both *trans, trans*-farnesol and alpha- bisabolol increased. For meaningful comparisons to be made between studies, the extraction procedure and section of the tree examined must be equivalent.

> *"..variations in both yield and composition of sandalwood oil exist in the oil extracted from the same sample using different extraction procedures.."*

The purpose of this study was to examine in detail the differences arising in the oil yield and composition along the length of three Western Australian sandalwood trees (*Santalum spicatum*) extracted using Supercritical carbon dioxide.

Experimental Method

Three sandalwood trees were harvested from Lakeside Reserve approximately 20 km east of Kalgoorlie. The trees harvested were situated on the edge of washed out river beds and were chosen for ease of removal.

The roots were exposed by washing away the soil on the edge of the river bank with water from a fire-fighting water hose supplied by a water tanker. The tree was loosened from the soil by a combination of digging and washing the soil from the tree with water.

Core samples were taken along a length of each tree from the roots to the branches at 5cm intervals using a ½ inch auger drill bit attached to a electric hammer drill. The drill bit was rotated slowly during core sampling to limit the heat generated and minimise volatilisation of components of the oil within the wood. In between taking core samples, the auger drill bit was cooled in a dry ice/acetone mixture. The shavings collected were powdered in a stainless steel Waring commercial blender in the presence of solid carbon dioxide. The powder was removed from the blender and stored in labelled glass jars. The ground samples were dried for 48 hours in a desiccator prior to extraction.

Extractions of core sample were performed in triplicate using a HP7680T SFE module equipped with a solid bed trap for the collection of the oil. Supercritical carbon dioxide was used as the extraction fluid. The extracted oil was analysed by GC-FID using a HP 5890 Series II Gas Chromatograph.

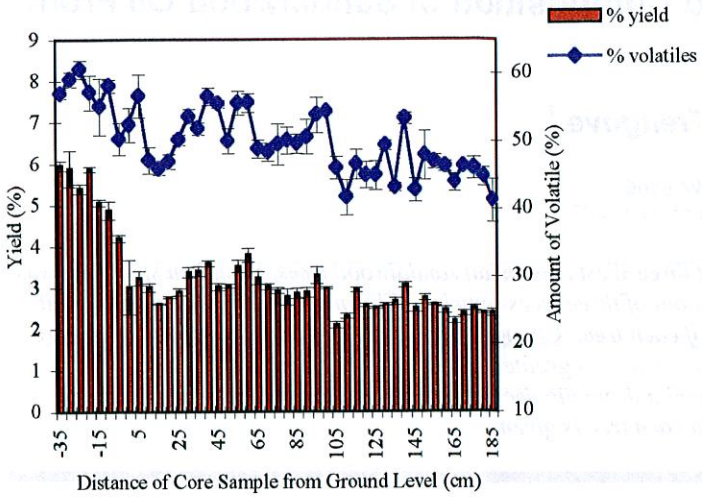

Figure 1.1. Oil yield and percentage volatiles of sandalwood oil along Tree 1

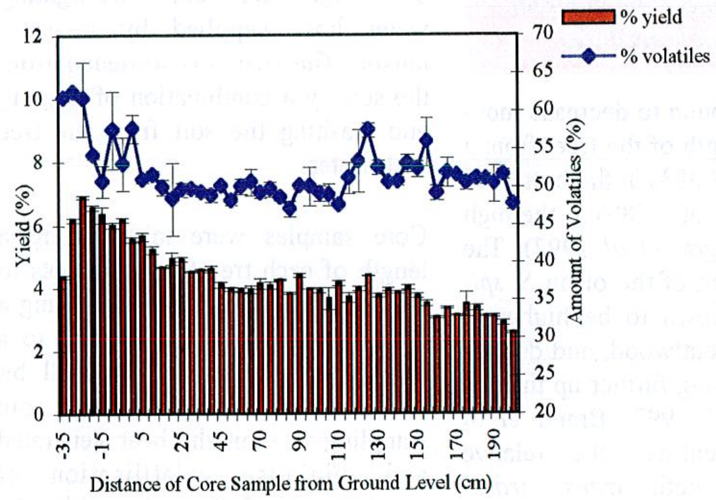

Figure 1.2. Oil yield and percentage volatiles of sandalwood oil along Tree 2

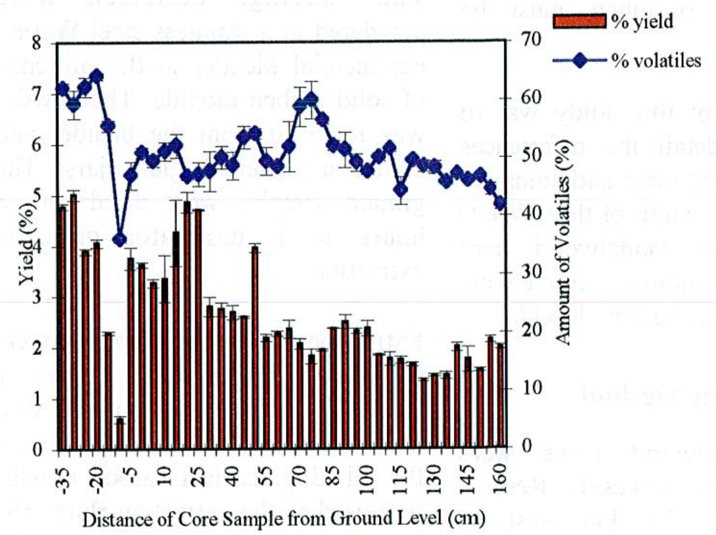

Figure 1.3. Oil yield and percentage volatiles of sandalwood oil along Tree 3

Results and Discussion

The percentage yield and volatiles of the oil extracted from samples taken along the length of three trees is shown in Figures 1.1 –1.3. The percentage yield relates to the percentage of oil extracted from the wood expressed as dry weight. The percentage volatiles represents the percentage of the total oil which elutes from the Gas Chromatograph during analysis. This value indicates the relative amount of the compounds that are responsible for the aroma of the oil.

The results varied from tree to tree, even though the three trees were situated within a 50 m radius of each other. Similar trends in oil yield were observed for the length of the trees. In each case, the highest amount of oil was found in the roots below ground level (Tree 1, 5.9%; Tree 2, 6.9%; Tree 3, 5.0%). Tree 3 showed an exceptionally low percentage yield between 15 and 10 cm below ground level, decreasing to a minimum of 0.59. The reason for this is that a grub, which infects the heartwood had infected part of the root system.

The amount of oil extracted from the wood above ground level decreased slightly along the length of each tree. The buttwood, which is the region of the tree from just below ground level to 20 cm above ground level, contained a slightly higher oil content in Trees 2 and 3, than the upper sections of the tree.

Figures 2.1- 2.3 show the major compositional changes in the oil extracted from the core samples taken along the length of the three trees. Although the composition of the oils varied between the trees, similar trends in the changes of compounds along the trees were observed.

The most noticeable trend was the change in the levels of alpha- and beta- santalol. In all cases the combined santalol content was greatest in the roots, with alpha- santalol being the major constituent of the volatile oil. Moving above ground level from the roots, a dramatic decrease in the amount of alpha- and beta- santalol was observed. The amount of each compound decreased by more than five fold to below 5% over a distance of 25 cm, from the roots (-15 cm) to the buttwood (10 cm) in all three trees. This sharp decrease in the amount of alpha- and beta- santalol was accompanied by increases in the levels of alpha bisabolol and/or *t,t*-farnesol. The relative increase in these compounds was dependent on the tree examined, and for this reason, the results for each tree will be discussed separately.

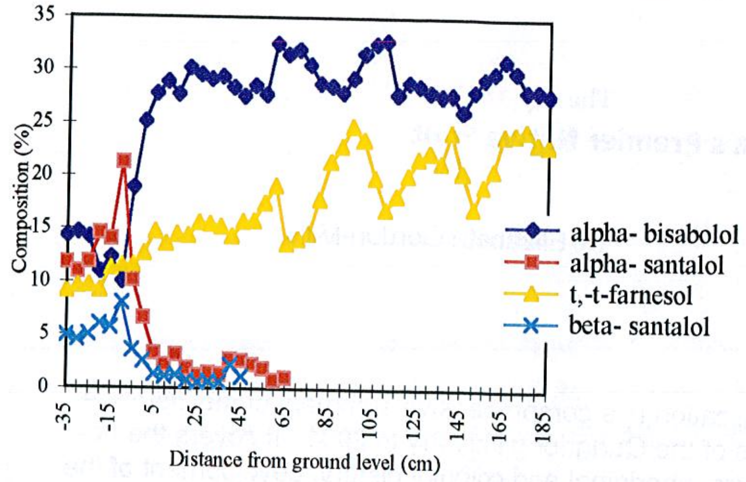

Figure 2.1. Major compositional changes along Tree 1

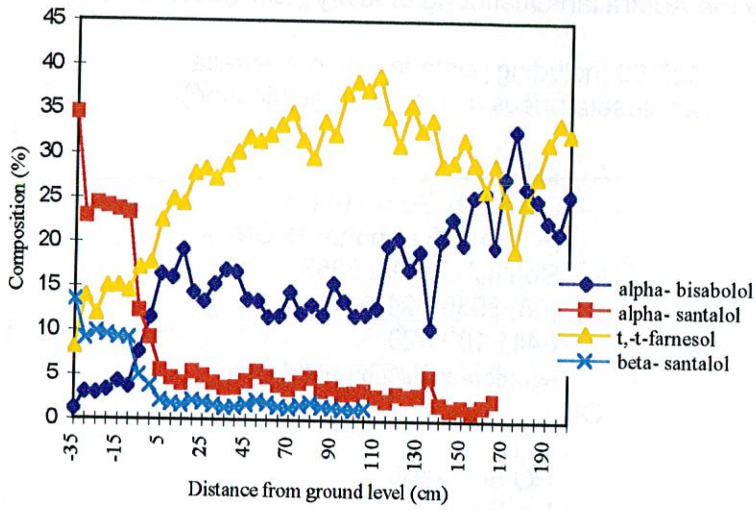

Figure 2.2. Major compositional changes along Tree 2

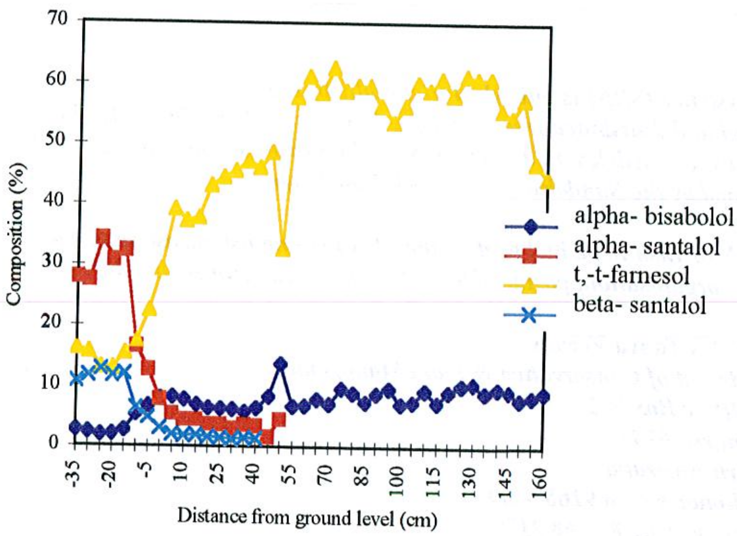

Figure 2.3. Major compositional changes along Tree 3

For Tree 1 (Figure 2.1), the decrease (26.1%) in santalol content (alpha- and beta- santalol) between -10 and 10cm was accompanied by an increase in the amount of alpha- bisabolol (18.7%) and *t,t*- farnesol (2.0%). Above ground level, alpha- bisabolol was the major component (~30%) of the extracted volatile oil. This value remained unaffected by further small decreases in alpha- and beta- santalol, however the amount of *t,t*- farnesol increased.

Tree 2 (Figure 2.2) showed similar changes in the composition of the oil, however *t,t*- farnesol was the major component of the oil above ground level (~35%). The decrease (14.7%) in santalol content between -10 and 10 cm was accompanied by an increase of *t,t*- farnesol (8.3%) and alpha bisabolol (10.6%). As seen in Tree 1 these compounds fluctuated along the length of the tree.

Tree 3 (Figure 2.3) had lower levels of alpha bisabolol compared to the other two trees and, consequently, there was a smaller increase in the percentage of alpha- bisabolol moving from the roots to above ground level where the santalol content decreased. The major constituent of the oil above ground level, *t,t*-farnesol, increased by 21.8%, between -15 and 10 cm, whereas the percentage of santalols decreased by 37.1%. As the santalol content declined below 1%, the amount of *t,t*- farnesol increased further and levels fluctuated along the length of the tree.

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