

Sandalwood Research Newsletter

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EDITOR'S NOTE

The sandalwood research newsletter has been a valued resource for many researchers, growers, and processors of sandalwood for 13 years and since the first issue the newsletter has published 58 articles from 81 different authors. I, like most, was disappointed to hear of its demise, and therefore it is my pleasure to publish issue 21 by James Cook University and hopefully continue the fine work of the previous 3 editors (Radomiljac, Vernes and Brand) and the Forest Products Commission (FPC) and the Department of Conservation and Land Management (CALM)

In the changing of the editors it is appropriate that this newsletter include contributions from both the previous and the current editors. Both studies examine the variation in heartwood and oil characteristics, with plantation Indian sandalwood (Brand et al.) and wild Vanuatu sandalwood (Page et al.). We also gain some insight into the grafting of sandalwood in Vanuatu and how it is being used within the Department of Forests sandalwood initiatives. Danica Harbaugh provides an outline of a very valuable examination of the sandalwood phylogeny, the results of which are eagerly anticipated.

Preliminary analysis of Indian sandalwood (*Santalum album* L.) oil from a 14-year-old plantation at Kununurra, Western Australia.

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Abstract

Twenty Indian sandalwood (*Santalum album* L.) trees planted at Kununurra, Western Australia, were sampled for total oil yield and santalol content, at age 14 years. "Chips" (heartwood only) and "cores" (heartwood plus sapwood) were taken from each sandalwood stem at 30 cm and 100 cm from the base, and were processed using solvent extraction. The mean total extractable oil yields were 2.9-3.4 % from chips, and 1.8-2.0 % from cores. The oil extracted from the chips contained 44.7-46.7 % α -santalol and 20.8-22.2 % β -santalol. The mean percentage of heartwood was 28.9 - 33.8 %.

Introduction

The scented heartwood from Indian sandalwood (*Santalum album*) is a much sought after commodity throughout the world. This species is a root hemi-parasite that can grow to 20 m in height, and occurs naturally within semi-tropical regions of India, Indonesia and the Northern Territory, Australia (Hewson and George 1984).

In the mid 1980's, the Department of Conservation and Land Management (CALM), identified the potential to grow Indian sandalwood for commer-

cial purposes on the Ord River Irrigation Area (ORIA), Kununurra, Western Australia. Over a 20 year period, collaborative research by CALM, FPC, Murdoch University, Curtin University of Technology, the University of Western Australia and private growers lead to the development of a successful establishment method to grow Indian sandalwood in this region. Today, over 1,000 ha of land has been planted into sandalwood on the ORIA by private companies.

Although sandalwood plantations have been successfully established

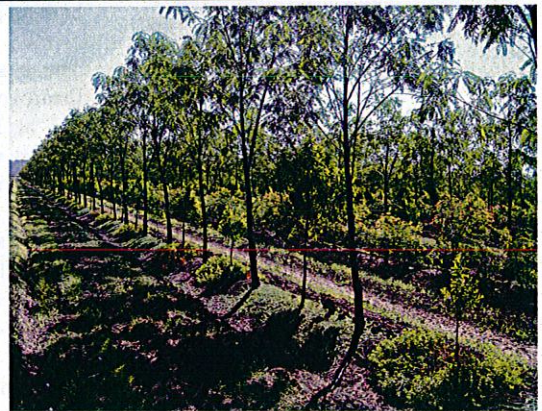


Figure 1. One-year-old Indian sandalwood seedlings beneath host plants, within an FPC plantation (Photo: G. Pronk).

and growth rates have been encouraging at Kununurra (Figures 1-3), more information is required on oil production. The value of sandalwood is determined by the volume of heartwood, oil percentage and oil quality within the tree. Indian sandalwood heartwood from mature trees contains on average 5-7 % oil (McKinnell, 1990), with over 85 % santalols (Rai, 1990). Santalols are very important because they are the main compounds that give sandalwood oil its distinctive fragrance (Adams, 1975).

This study provides information on heartwood percentage, oil concentration and santalol content from trees within one of the first Indian sandal-

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Figure 2. 14-year-old FPC Indian sandalwood plantation (Photo: L. Barbour).

wood plantations established at Kununurra, Western Australia.

Methods

In 1990, the Indian sandalwood seedlings were planted together with a variety of intermediate host plants in rows. *Cathormion umbellatum* was planted separately as a long-term host, on alternate rows. At age 14 years (December 2004), 20 of the sandalwood trees were selected for oil sampling.

Stem diameter (over the bark) was measured from each tree at 30 cm and 100 cm above the ground. At the same heights, core samples were taken using a power drill with a custom-built coring bit (Figure 4). The drill bit was directed horizontally through the centre of the stem from one side of the bark to the other side. The outer 5 mm of the bit consisted of cutting teeth that yielded shavings classed as “chips”. The chips were sorted into heartwood and sapwood on the basis of colour and smell. The heartwood colour was yellow to brown/red, with a distinct sandalwood aroma, while the sapwood was white and odourless.

The hollow centre of the drill bit allowed extraction of a 12 mm diameter “core” of solid wood. The cores contained both heartwood and sapwood. The depth to heartwood was calculated at both ends of each core sample. The mean depth of the bark was 5 mm, and this was removed from the core samples. Mean bark thickness was subtracted from the stem diameter to estimate under-bark diameter. Heartwood and under-bark diameters

Measurement	Stem height	
	30 cm	100 cm
Diameter (cm)		
over-bark	20.3 ± 0.8	17.6 ± 0.7
under-bark	19.3 ± 0.8	16.6 ± 0.7
heartwood	10.0 ± 1.4	7.8 ± 1.2
Area (cm ²)		
under-bark	301 ± 22	224 ± 20
heartwood	109 ± 26	69 ± 14
Heartwood (%)	33.8 ± 5.6	28.9 ± 4.6

Table 1. Mean tree dimensions (± standard error), from the stem at 30 cm and 100 cm above the ground, from 20 sandalwood trees.

were used to calculate cross-sectional areas of both heartwood and stem. Percent heartwood was calculated by dividing heartwood area by the under-bark area.

The heartwood chips and the cores (heartwood + sapwood) from each tree at 30 cm and 100 cm above the ground were processed using solvent extraction and analysed for oil percentage and composition. Oil extraction and analysis of compounds within the oil was conducted by *Australian Botanical Products*, Hallam, Victoria.

Results

Tree dimensions and heartwood contents

The mean stem diameters under the bark were 19.3 cm (at 30 cm) and

16.6 cm (at 100 cm, Table 1). Heartwood was not visible in four of the trees at 30 cm and five of the trees at 100 cm. The mean percentage of the wood that was clearly heartwood was 33.8 % at 30 cm, and 28.9 % at 100 cm.

Total extractable oil yields

The mean oil yields from heartwood chips (Figure 5) were 2.9 ± 0.4 % at 30 cm, and 3.4 ± 0.5 % at 100 cm. Heartwood chip yields were highly variable between trees ranging from 0.1 to 5.6 % at 30 cm, and 0.2 to 7.1 % at 100 cm. Oil production was very poor in three of the trees, which had heartwood chip yields of only 0.2-0.3 %, at both 30 cm and 100 cm. These three trees also had no visible heartwood.

The mean oil yields from cores (Figure 5) were 1.8 ± 0.3 % at 30 cm, and 2.0 ± 0.3 % at 100 cm. Core sample yields ranged from 0.01 to 3.8 % at both 30 cm and 100 cm. Again, the same three trees with no visible heartwood had less than 0.03 % oil within core samples at both 30 cm and 100 cm.

Santalol contents

Total santalol content was 65.5-68.9 % in the heartwood chips and 60.0-62.5 % in the cores (Table 2). Between trees, the total santalol content varied from 47.8 to 80.2 % within the heartwood chips and 16.9 to 75.7 % within the cores. Three of the sandalwood trees had total santalol contents of over 75 %, within the heartwood chips.

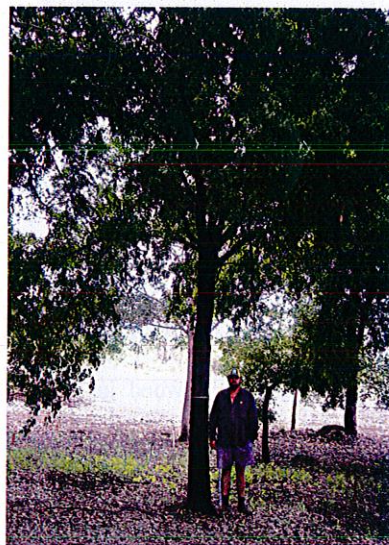


Figure 3. Large 17-year-old Indian sandalwood tree (Photo: G. Pronk).

Discussion

The *S. album* trees growing at Kununurra had a mean total extractable oil yield of 2.9-3.4 % from the heartwood, which is approximately half that of natural *S. album* trees from India (5-7 %; McKinnell, 1990). Within the oil there were 44.7-46.7 % α -santalol and 20.8-22.2 % β -santalol. These santalol levels meet the current ISO standards for *S. album* oil, which are 41-55 % α -santalol and 16-24 % β -santalol (ISO 3518:2002E). Although the heartwood oil yields were reasonable and the oil quality was high, the mean heartwood percentage was only 29-34%. This indicates that at age 14 years, the mean volume of heartwood per tree is low. However, it would be expected that both heartwood percentage and oil yields will increase with age.

Total extractable oil yield from the heartwood chips was highly variable between trees, with yields as high as 7.1 %, contrasted by three trees hav-

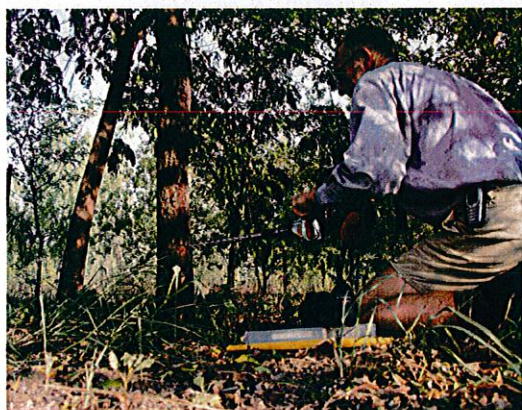


Figure 4. Core sample being taken at 30 cm above the ground from a 14-year-old FPC Indian sandalwood tree (Photo: C. Done).

ing less than 0.3 %. Mean total santalol content also varied from 47.8 % to 80.2 % within the heartwood chips and three trees had over 75 % santalol. The observed variability in heartwood percentage, oil yield and santalol content between similar aged trees growing under the same conditions indicates that heartwood initiation and oil production may be influenced by tree genotype. However, until this is better understood, future planting of sandalwood at Kununurra should aim to use seeds from superior oil producing parent trees.

Samples	Compounds (%)		
	α -santalol	β -santalol	Total santalol
Heartwood chips			
30 cm	46.7 \pm 1.0	22.2 \pm 0.5	68.9 \pm 1.4
100 cm	44.7 \pm 1.0	20.8 \pm 0.6	65.5 \pm 1.5
Cores			
30 cm	42.5 \pm 1.7	19.4 \pm 1.3	62.0 \pm 2.9
100 cm	42.9 \pm 1.9	19.6 \pm 1.2	62.5 \pm 3.1

Table 2. Mean santalol contents (\pm standard error) within the oil of chips and cores, at 30 cm and 100 cm above the ground, from 20 sandalwood trees.

These results were encouraging, providing evidence that young Indian sandalwood plantations at Kununurra are producing heartwood and good quality oil. However, the heartwood volumes appear to be low at age 14 years. To more accurately predict sandalwood yields, whole trees need to be harvested and heartwood weights and volumes measured.

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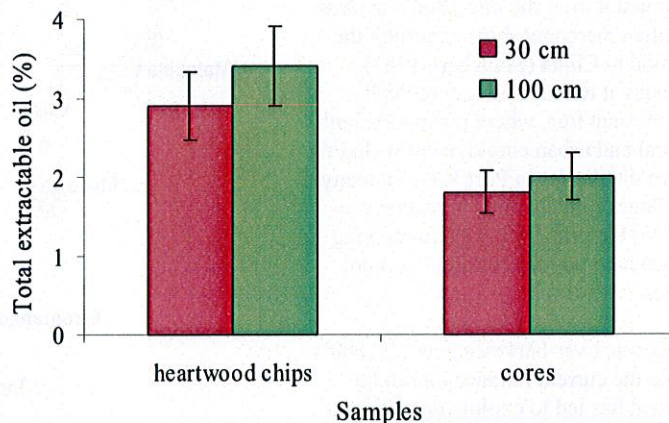


Figure 5. Mean per cent oil yields (\pm standard error) from heartwood chips and solid core samples, at 30 cm and 100 cm above the ground, from 20 sandalwood trees.

Acknowledgements

Tropical Forestry Services (TFS) and the FPC provided funding for the coring of the trees and oil analysis. Chris Done is thanked for helping to core the sandalwood trees. Charles Cornwell from Australian Botanical Products is thanked for extracting and analysing the sandalwood oil.

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Evaluation of heartwood and oil characters in nine populations of *Santalum austrocaledonicum* from Vanuatu*.

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*extract from work presented at the Regional Workshop on Sandalwood Research, Nadi Fiji 28th Nov- 1st Dec 2005

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Abstract

Heartwood cores were collected from 222 trees across nine populations on six different islands from Vanuatu. Oil was ethanol extracted and oil concentration and the main constituents were determined for each of the cores sampled and analysed on a tree-to-tree and site-by-site basis.

Heartwood oil concentration and all major oil constituents exhibited significant tree-to-tree variation, within and between all populations. Each population had a range of trees with high and low concentrations of α - and β -santalol. The populations from the two northern islands had a greater proportion of trees with high santalol content than the populations sampled from the southern islands.

Introduction

Sandalwood in Vanuatu (*S. austrocaledonicum*) was commercially exploited during the mid 1800's by Australian merchant ships exporting the wood to China (Shineberg 1967). Today it remains an economically important tree, where it supports both rural and urban employment including two distilleries in Port Vila. In many villages sandalwood harvesting provides the primary means for earning money to pay for children's school fees.

Historic over-harvesting in the 1800's and the current reliance on sandalwood has led to exploitation of natural sources of sandalwood and in many areas the tree has become rare in the wild. The establishment of sandalwood agroforests is actively encouraged by the Vanuatu Department of Forests as a means for alleviating pressure on natural sources and improving village income potential.

Sandalwood-oil is graded and priced according to levels of α - and β -santalol using a standard derived from *Santalum album*, and invariably the market prices reflect the relative species average quality. However the likely existence of tree-to tree variation in oil quality within *S. austrocaledonicum* offers the opportunity to improve oil-quality through selection and domestication

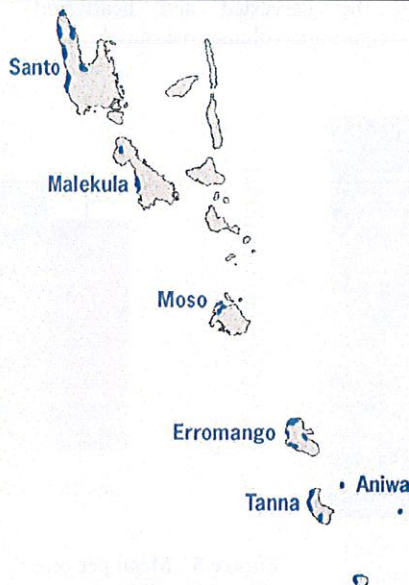


Figure 1. Sandalwood distribution in Vanuatu and the islands sampled in the present study.

The aim of this project, supported by ACIAR (Australian Centre for International Agricultural Research), was to survey the wild stands of the species and identify a resource that would support an improvement programme to increase the availability of planting material with superior oil characteristics. These initiatives can help to ensure that future sandalwood plantations in Vanuatu are established with trees that produce high concentrations of superior quality sandal-

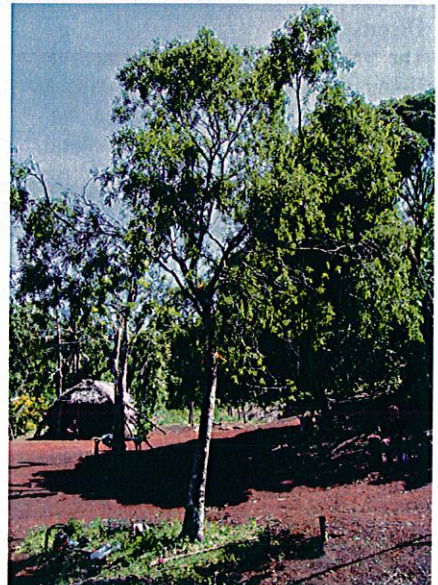


Figure 2. Sandalwood tree sampled in the village of Tamsel (Erromango).

wood oil. This should help to have this species recognized in the international marketplace.

Materials & Methods

Wood core samples were collected from 222 trees from nine populations on six islands across Vanuatu (Santo, Malekula, Moso, Erromango, Tanna and Aniwa). Trees were sampled based on a minimum basal trunk diameter of 100mm (Figure 2) to ensure adequate heartwood development and allow sampling of a heartwood core. Heartwood samples were extracted from living trees using the CSIRO 'Treecor'. Bark-to-bark samples were taken and the remaining hole was sterilized with 70% ethanol, filled with sterilized doweling (22mm) and sealed with bitumen. Cores were sampled at an average of 30cm from the base to account for variation in oil concentration and quality within each tree. Heartwood colour was evaluated parallel to the heartwood fibres using a Methuen Colour Chart (Komerup 1978). Heartwood oil was ethanol extracted and constituents were identified through analytical gas chromatography mass spectrometry (GCMS). Percentage heartwood calculations were based on the depths of heart and sapwood and calculated according to the area of a circle.

Results and Discussion

Percentage Heartwood

The percentage heartwood varied significantly between different popula-

tions, the trees in Tanna and Erromango were found to have the greatest percentage (32%) of heartwood (Figure 3). Aniwa produced trees of comparatively large basal diameter (22cm), but they produced a comparatively small percentage of heartwood (19%). Conversely trees from Malekula had a relatively small basal diameter (16cm) but high heartwood percentage (27%). Both cases highlight that the size of the tree is not

Heartwood-oil concentration

Significant variation was found for heartwood oil concentration within and between populations with a mean of 2.2% across all trees sampled. Trees from Malekula had a significantly greater mean essential oil concentration (3.5%) than the other populations (Figure 5). Overall, approximately 12% of trees were found to have an oil concentration greater than 4% and individual trees from all is-

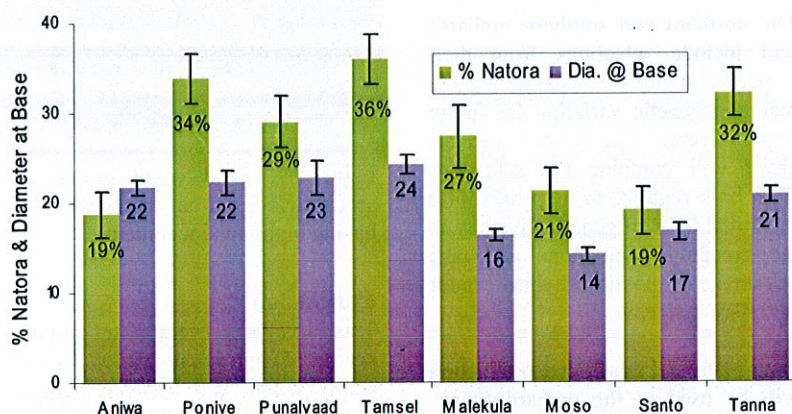


Figure 3. Variation in percentage heartwood (natora) and stem diameter at base in *S. austrocaledonicum*, across 6 islands of Vanuatu (Ponive, Punalvaad and Tamsel are discrete populations of Erromango). Vertical bars represent standard errors.

directly indicative of the heartwood percentage, which is confirmed by the lack of correlation between them ($r^2=0.15$). The age of onset for heartwood formation has been reported to be variable in *S. album* ranging from 14 to 46 years in Timor (Haffner 1993), while Doran (2002) indicates approximately 10 years for plantation grown *S. album* in north-western Australia.

Heartwood colour

Variation in heartwood colour was exhibited between trees (Figure 4), but was found to have no predictable effect on the relative levels of oil constituents, and therefore is not an indicator of oil quality. The colour saturation of the heartwood (*i.e.* how dark or light) gave a broad indication of oil yield, but saturation categories used in the current study could only discriminate between very low (light) and very high (dark) oil concentrations. The majority (80%) of the heartwood samples conformed to more moderate saturations and the scale could not sufficiently differentiate these according to concentration.



Figure 4. Heartwood colour varied between trees but was not related to any oil quality characteristics.

tional standard (>41% α -santalol and >16% β -santalol). The selected trees from the remaining southern populations had a mean of 31% α - and 17% β -santalol.

Continuous variation was found in all the major essential oil constituents across the samples collected. The continuous nature of the santalol:non-santalol ratio indicates there is no evidence to support previous reports of different chemotypes in *S. austrocaledonicum* (Ehrhart and Raharivelomanana 1998). Chemotype variation misleadingly appears when examining the relative proportions of major constituents on a site-by-site basis (Figure 6), but the greater number of samples in this study reveal a continuous variation in each constituent across the species.

lands, except Tanna, were represented. Significant tree-to-tree variation in oil concentration was found within each population ranging from 0.05% to 8% over all trees sampled, indicating that this character could be improved through clonal and recurrent selection.

Heartwood-oil constituents

A total of 28% of the trees sampled in the two northern islands produced heartwood-oil meeting the interna-

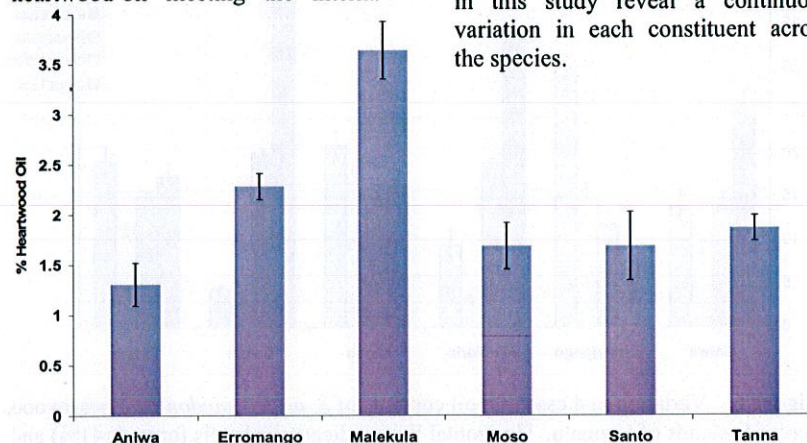


Figure 5. Variation in heartwood oil concentration (%) in *S. austrocaledonicum*, across 6 islands of Vanuatu. Vertical bars represent standard errors.

The sandalwood populations of the northern islands (Santo and Malekula) had a greater proportion of trees with high values for the commercially important α - and β -santalol than the populations sampled from the southern islands. A total of 28% of the trees in each northern island have oil quality that exceeds the International Standard (ISO 2002). The high quality of the *S. austrocaledonicum* from the northern islands indicates that if the homozygous state of these populations (unpublished data) can be ameliorated, the sandalwood industry of Vanuatu could compete on the international market with Indian sandal-

Grafted Seed Orchard		Santalol	
		α -	β -
Northern	(n=16)	38%	20%
Southern	(n=26)	31%	17%
Central	(n=42)	33%	18%

Table 1. Mean percentage of α - and β -santalol in the trees selected for the three grafted seed orchards

wood on the basis of high oil quality. This possibility raises the urgency of making seed collections and cross-pollinations, particularly given that the value of the existing high quality wild trees have increased as a result of this study.

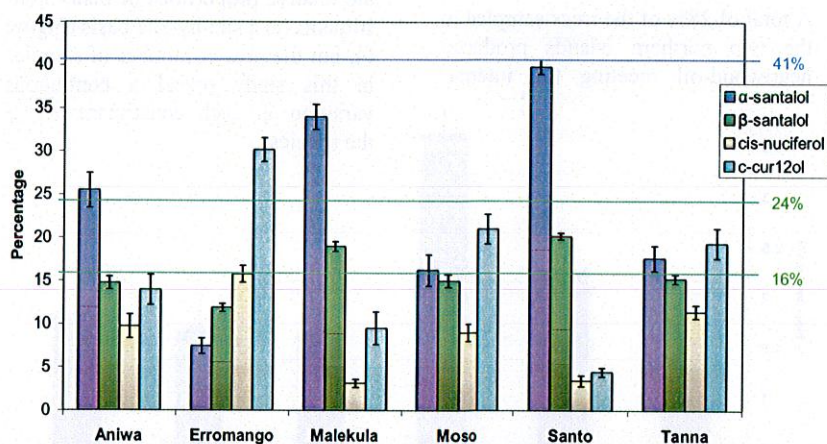


Figure 6. Variation in 4 essential oil contents of *S. austrocaledonicum* heartwood, across 6 islands of Vanuatu. Horizontal lines indicate the levels for α - (>41%) and β -santalol (16-24%) to meet the international standard (ISO 2002). Vertical bars represent standard errors

Domestication

Sandalwood trees selected for elevated levels of α - and β -santalol will be established in replicated grafted seed orchards on three sites (northern, southern and central) in Vanuatu (Table 1). Given the heritability of oil characteristics is unknown in sandalwood, clonal material (scions) will be collected from the selected trees to ensure that the desirable oil characters are secured in the breeding populations.

The northern and southern orchards will include selections from their respective regions as separate lines to maintain genetic variation for future improvement. The central seed orchard will combine the selections from both regions, to help maximise the initial level of genetic diversity in the breeding populations and take advantage of possible hybrid vigour in 'cross' progeny.

Both open and controlled pollination will be used in the orchard to enhance the level of diversity in the seedling progeny and in the long-term determine the genetic control and heritability of oil characteristics. Work on the mass propagation of sandalwood using leafy stem cuttings is currently being undertaken with the aim of developing clonal cultivars to rapidly improve the planted sandalwood resource in Vanuatu.



Figure 7. Sandalwood grown from seeds collected from tree identified as having high santalol content.

Conclusion

This survey has revealed that oil concentration and quality varies considerably within and between populations. A domestication programme has been initiated to diversify the genetic base of these populations and through genetic selection enhance the yield and quality of *S. austrocaledonicum* heartwood oil.

The wider planting of improved seed (Figure 7) in garden, forestry and agroforestry plantings may help to: i) reduce the current pressures on already depleted natural resources, ii) allow local communities to enhance their livelihoods through the planting of superior varieties in agroforestry systems and (iii) increase the future international competitiveness of the Vanuatu sandalwood industry through a consistent supply of trees with high quality oil.

Acknowledgements

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Grafting sandalwood in Vanuatu

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Abstract

Historically sandalwood plantings in Vanuatu have been established mainly by seed propagation and transplanted wildings. This method continues to be very important for village communities to grow sandalwood collected from their natural sources. With increasing interest across the country in planting sandalwood the Department of Forests (DoF) is actively encouraging improved clonal seed orchards to keep up with demand.

Clonal propagation of mature trees by cuttings has been difficult to achieve by conventional methods, but grafting has proven a viable alternative method. The superior individuals identified within the current ACIAR sandalwood project are now being grafted using the methods developed in conjunction with SPRIG.

Introduction

Grafting has been used widely in many horticultural crops for clonal propagation of difficult to root species and also to combine the desirable features of the tree crown (scion) and rootstocks.

Grafting of sandalwood is now being used in Vanuatu to develop sexually mature clones. Grafted clones can potentially set seed after 8 months and produce viable seed. These plants are valuable for the establishment of seed orchards as they can begin to produce large volumes of seed very quickly.

Establishment of seed orchards is promoted by the Vanuatu DoF because of the following benefits:

- higher seed production and seed viability resulting from improved pollination between closely spaced trees.



Figure 1: Villager training in Vanuatu.

- Ease of monitoring seed maturation and seed collection from sites established close to the village.
- Improved oil quality of seedlings established from orchards with parents of high oil quality,
- *Ex-situ* conservation of genetic material.

Training

The Department of Forests is now implementing villager training in grafting which includes a recent sandalwood propagation workshop in Vila, jointly conducted by DoF, JCU, SPRIG and DPI.

One component of the training aimed to assist village communities to establish their own grafted seed orchards, and encouraged propagation from the trees identified as having high quality oil.

Acknowledged by village participants the propagation and nursery training has also been important for the propagation of other timber and nut species.

Methods

Grafting success depends on a lot of factors including compatibility between rootstock and scion, rootstock management, selection and matching of both cambiums; and post-management of grafts. The condition of the rootstock

and scion is critical and the best success has been achieved with actively growing plants. While there are many methods for grafting woody perennial trees good success in sandalwood has been achieved using the wedge graft (Fig 1).

Grafting success varies considerably between people, based on their level of experience in grafting. Success is maximised when the diameter of the scion and the rootstock are equal and both the cambium tissues are aligned. Once grafted the plant is grown under shade and the scion covered with clear plastic film. Experience has shown that inexperienced grafters may achieve up to 60 % success, while experienced grafters can achieve 80 to 90 % success rates. Scions collected from sexually mature plants can flower from eight months after grafting while immature scion material will delay flowering.



Figure 1: Wedge graft technique used to successfully graft sandalwood.

Conclusion

The Department of Forests sees the significance of grafting as important for enhancing its sandalwood domestication strategies. Grafted seed orchards are the most rapid way of increasing the availability of improved seed throughout Vanuatu.

The current high value of sandalwood has generated interest in planting sandalwood in non-traditional sandalwood growing areas. Consequently the demand for sandalwood seed is expected to increase. Therefore training village communities to establish their own grafted seed orchards is seen to be the most efficient way of achieving their goals of establishing commercial plantings of high quality sandalwood.

Molecular and morphological phylogeny of sandalwoods: Insights for biogeography and taxonomy

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The main objective of this doctoral dissertation research is to reconstruct the evolutionary history of the genus *Santalum*. A phylogeny will be reconstructed by the combination of morphological characters, as well as sequence data from nuclear ribosomal (ITS, ETS) (Baldwin & Markos 1998; Baldwin *et al.* 1995), low-copy nuclear (*waxy*) (Mason-Gamer 1998) and chloroplast (*3'trnK*) (Johnson & Soltis 1995) gene regions. This will elucidate ancient dispersal patterns and demonstrate patterns of relationship among taxa. Re-circumscription of taxa based on the phylogenetic hypotheses will aid in the recognition and conservation of rare and cryptic lineages.

A number of regional treatments have been produced within *Santalum*, however no comprehensive monograph of the genus has yet been completed. Sandalwoods have considerable morphological variation within a single taxon, which has led to taxonomic confusion. The use of advanced phylogenetic methods, using both molecular and morphological data, is essential to properly revise the genus. Taxonomic revisions will be published in a monograph, both printed and web-based, at the end of the dissertation research (projected Spring 2007).

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