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EDITOR'S NOTE

This is the final issue of the SRN by the Forest Products Commission (FPC). The SRN, which started in 1993 under the Department of Conservation Land Management as a forum for information exchange on Santalum species, no longer fits within the FPC's charter. However, further information on the FPC's tropical and arid sandalwood establishment and management can be sought through our website: www.fpc.wa.gov.au

In this issue of the SRN, Ben Lethbridge from South Australia describes the grafting of Santalum acuminatum (quandong) onto both S. spicatum (WA sandalwood) and S. acuminatum. Quandong grafted onto sandalwood could provide an annual return from the edible fruit, while waiting for the root stock to produce valuable oils?

John Doran, Lex Thomson, Joe Brophy, Bob Goldsack, Ponijesi Bulai, Tevita Faka'osi and Terry Mokoia discuss sandalwood oil production within S. yasi, S. album and hybrids between the two species, growing in the South Pacific. Wood cores from young sandalwood trees of age 5-25 years were analysed for oil content and quality. This information is of high commercial importance to sandalwood growers, especially at Kununuura, Western Australia. Oil yields at different ages will help determine tree values and the optimum time to harvest plantations.

*Please note that the **Asia-Pacific Regional Sandalwood Workshop** will be held in Suva, Fiji in October, 2005. For further details contact Mr Sairusi Bulai, SPC Regional Forestry Adviser, SPC Private Mail Bag, Suva, Fiji, Sairusib@spc.int, Fax: 679 3305212, Ph: 679 3300432*

Finally, I would like to thank everyone for their contributions over the years, and wish everyone the very best with their sandalwood endeavours.

Regards

Jon Brand

Field Grafting of Quandong (*Santalum acuminatum*)

Ben Lethbridge.
Lobopogon Research, 62 Grants Gully Rd. Clarendon SA

Introduction

As an alternative to nursery production, field grafting of quandong could be used to transfer selected scions producing good quality fruit onto seedling trees with less than satisfactory fruit (Possingham 1986). With the development of direct seeding of quandong (Lethbridge 2003) and sandalwood (Brand and Jones 1999, Woodall 2003, Jones 2004) next to appropriate host plants proving to be a simple and efficient technique, field grafting would nicely complement the development of uniform orchards. There also exists the potential to graft quandong onto established sandalwood, thereby allowing harvesting of trunks and roots of sandalwood, when the quandongs orchard usefulness has passed (Lethbridge 2001, 2004b).

Field grafting of quandongs has so far met with limited success (Bentley 1997) and involves a complex multi-layered technique to protect the scion material from the elements. Simplification of this technique with the mummy graft (Moody 1999) is starting to show promise (Lethbridge 2004a). An updated version is described in this report for the field grafting of non irrigated sandal-

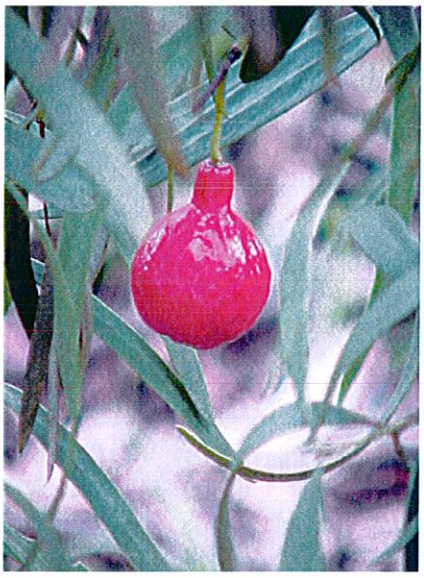

Photo 1. A fruit (2.5 cm diam.) of the selected quandong scion source tree.

inside...

wood with selected quandong scions.

Methods

Selected non irrigated quandong and sandalwood trees, 1-2 metres tall growing near various hosts but predominantly acacia species on a south facing slope at Clarendon S.A. (Adelaide Hills), were field grafted on 7 August 2004. Trees were pruned to a major upright stem with all lateral branches within 30 cm of the graft point removed. Dis-bladed scions of about pencil thickness were selected from quandong trees growing on site. These scions were grafted to the rootstock by carefully matching stem diameters and top wedge grafting a few nodes of scion material. The graft area was tightly wound with laboratory sealing film (Parafilm, Whatman Cat. No. 2150663) and the scion covered completely with a thin layer of Parafilm. Previous experience indicated that insect and wind damage of the graft can be significant and although not essential, the graft can be covered with a bubble of wax paper (30 cm by 30 cm.), with pin holes for ventilation, and fastened with plasticized wire "garden ties". All developing shoots below the graft point were removed. The grafts were monitored for three months.

Results and Discussion

The trials showed that it is possible to field graft quandong onto either sandalwood or quandong with reasonable efficiency (Photographs 2 and 3). Indicators of graft success were timed as: leaf petiole loss at one month of age and bud burst at approximately two months of age. There was an obvious correlation between rootstock vigour and wound healing. Trees with significant new shoot growth prior to grafting outperformed slower growing trees.

It may be possible, under less favourable conditions, to further improve wound healing rates by cincturing of shoots while still on the source tree. (see Sedgley 1984).

The problems with nursery propagation and transplantation that have plagued the quandong industry, due mostly to the lack of vigour of potted plants, could be well supplemented with direct seeding and field grafting of quandongs. Improvements in orchard management for better vigour of trees over longer periods (Lethbridge 2003) may allow other grafting techniques to be used. (see McE Alexander 1999). Further experiments will refine the timing of grafting with vigour of the rootstock, scion, species and site used.

Acknowledgements

Elizabeth Gordon Mills for useful discussion and use of her quandong orchard trees at Langhorne Creek S.A.

References

Bentley D. (1997) Field grafting of Quandong. Acuminatum Summer 2-3. (Newsletter of the Australian Quandong Industry Association)

Brand J. and Jones P. (1999). Growing sandalwood (*Santalum spicatum*) on farmland in Western Australia. Sandalwood Information Sheet No1. Conservation and Land Management (Perth WA)

Jones P. (2004) Essential Oils: Sandalwood oil. In The New Crop Industries Handbook. Edited by Salvin S., Bourke M. Byrne A. Rural Industries Research and Development Corporation.

Lethbridge B. (2001) Grafting compatability of Quandong (*Santalum acuminatum*) Sandalwood Research newsletter, Issue 12, January, page 2.

Lethbridge B. (2004a) Do Our Own Research (DOOR) quandong production. RIRDC publication No. W04 / 111

Lethbridge B.(2004b) Native Foods : Quandong. In The New Crop Industries Handbook. Edited by Salvin S., Bourke M. Byrne A. Rural Industries Research and Development Corporation.

Lethbridge B. and Randell B.(2003) Genetic and agronomic improvement of Quandong. RIRDC Publication No. 03 / 110

McE. Alexander D. and Lewis W.J. (1999) Grafting and Budding Fruit and nut trees . A practical guide. CSIRO Australia.

Moody H. (1999) How to graft *Grevilleas*, Australian Horticulture June 44-46.

Possingham J.(1986) Selection for a better Quandong. Australian Horticulture February 55-59

Sedgley M.(1984) Australia's First Commercial fruit. Australian Horticulture Oct. 52-53, 56-59

Woodall G., Robinson C.(2003) Direct seeding of Acacias of different form and function as hosts for Sandalwood (*Santalum spicatum*) Conservation Science West Australia 4(3): 130-134.

Photo 2. Three-month-old graft (7/8 /04 to 7/11/04) of selected scion on quandong rootstock. (wax paper removed). Four of five successful grafts on distinct trees.

Photo 3. Three-month-old graft (7/8/04 to 7/11/04) of selected quandong scion on sandalwood (*S. spicatum*) rootstock (wax paper removed). Two of three successful grafts on the same tree.

Variation in heartwood oil composition of young sandalwood trees in the South Pacific (*Santalum yasi*, *S. album* and F1 hybrids in Fiji, and *S. yasi* in Tonga and Niue)

John Doran[1], Lex Thomson[1], Joe Brophy[2], Bob Goldsack[2], Ponijesi Bulai[3], Tevita Faka'osi[4] and Terry Mokoia[5]

[1]CSIRO Forestry and Forest Products, PO Box E4008, Kingston ACT 2604. AUSTRALIA

[2]School of Chemical Sciences, University of New South Wales, UNSW Sydney 2052. AUSTRALIA

[3]Silvicultural Research Division, Department of Forestry , PO Box 2218, Suva. FIJI

[4]Forestry and Conservation Division, Ministry of Agriculture and Forestry, PO Box 14, Nuku'alofa. KINGDOM OF TONGA

[5]Division of Forestry, Department of Agriculture, Forestry and Fisheries, PO Box 74, Alofi. NIUE

Abstract

This study was undertaken during 2003 as part of AusAID's SPRIG (South Pacific Regional Initiative in Forest Genetic Resources) project. It had the primary aim of extending the knowledge base on the production of heartwood and heartwood oils in young Pacific Island sandalwoods, *Santalum yasi*, the introduced *S. album*, and the spontaneous F1 hybrid, *S. album* × *yasi*. A solvent (pentane) extraction technique was used to determine heartwood oil chemistry, following verification against steam distillation. The heartwood was obtained from trees by non-destructive coring.

Ages of the trees sampled ranged between 5 years and more than 25 years. Many of them had not yet started to lay down heartwood at their base. For those that had, heartwood was restricted to the lower most cores i.e. 0.1m or 0.2m above ground or very occasionally extending to 0.3m in older trees. Tree-to-tree variation in oil quality in *S. yasi*, as determined by allowable α-santalol and β-santalol levels in the International Standard (2002) for *S. album*, was substantial indicating a potential of improvement through selection and breeding if genetic parameters are favourable. Trees in Fiji of the spontaneous F1 hybrid, *S. album* × *yasi*, were very vigorous and the heartwood oil of two (out of three) of the 7-year-old trees with heartwood was of excellent quality.

The results suggest that rotation lengths of 25 to 30 years for the Pacific sandalwoods may be more realistic than the 15 to 20 year rotation lengths suggested by some workers.

Introduction

Sandalwood species are renowned for their fragrant heartwood and oil to the extent that they have been over-exploited in many parts of their natural distribution. This is true for *Santalum album* L. from India, Indonesia and East Timor which is the bench mark of commercial sandalwood species, but it is also true for the sandalwoods of the South Pacific. Indigenous Pacific sandalwoods like *Santalum yasi* Seeman from Fiji and Tonga, *S. austrocaledonicum* Vieillard from Vanuatu and New Caledonia and *S. insulare* Bertero from French Polynesia are now the subject of conservation and sustainable management studies and planting trials in an urgent attempt to ensure these genetic resources are available for future generations.

The commercial value of sandalwood depends on its heartwood oil content and composition, and the quantity of heartwood per tree. Information on these characteristics for the Pacific Island sandalwoods is scant. The comparison in composition between the South Pacific sandalwood oils and oils of *S. album*, the benchmark species with an International Standard, will be a determining factor in assessing the quality of the South Pacific oils in the market place. Two compounds and their percentage of total oils are highlighted in the International Standard ISO/DIFS 3518 (2002) for *S. album* , viz. α-santalol (41-55%) and β-santalol (16-24%). Assessing the percentage of these two compounds in the heartwood oils of *S. yasi*, *S. album* and their F1 hybrids was the main focus of this project.

This survey reports on variation in the composition of heartwood oils of relatively young trees of *Santalum yasi* Seeman, *S. album* L. and their spontaneous F1 hybrids in Fiji and *S. yasi* in Tonga and Niue. It was undertaken during 2003 as part of AusAIDs SPRIG (South Pacific Regional Initiative in Forest Genetic Resources) project which is assisting Pacific partners to identify trees with good heartwood formation for inclusion in conservation and improvement programs.

Materials and Methods

Sandalwood samples: Details of the individual trees of *S. yasi*, *S. album* and the spontaneous F1 hybrid, *S. album* × *yasi* in Fiji and *S. yasi* in Tonga and Niue sampled in this project are given in Tables 1 and 2. All trees were relatively young (most under 20 years) and had been either planted or established from natural regeneration from mature trees that had been removed from the site in commercial harvests. Sampling took place during January and May-July 2003.

The majority of trees were non-destructively sampled using a CSIRO Trecor Wood Corer with 500mm long drill bit that leaves a 22mm diameter hole (Figure 1). Bark to bark cores were taken from each tree at the heights given in the table. Most trees were sampled at the base (0.1m or 0.2m above ground) and some selected for coring at higher positions (e.g. 0.3m and 1.3m) to study the extent of heartwood formation up these trees. After removal of the cores the holes were sealed to the atmosphere using broomstick timber dowels. Exceptions were the two samples (N1 & 2) of *S. yasi* from Niue which were destructively sampled with wood biscuits (discs) provided for chemical analysis.

To allow verification of the solvent extraction method, *S. yasi* tree 1 and *S. album* tree 12 from Fiji were cored and then destructively sampled at 0.1m to give sufficient heartwood for steam distillation to verify the solvent extraction method.

After collection the samples were labelled, air dried, placed in calico or plastic bags and sent to Australia for oil extraction and chemical analysis.

Isolation of oils: Approximately 0.1g of sandalwood was removed from the core sample with a 5mm doweling bit. Both heartwood and sapwood were drilled. It was soon found that no oil was present in the sapwood and from then on only the darker heartwood was drilled. The (accurately weighed) sample of drill shavings was added to a 2ml vial fitted with a Teflon coated septum and a screw top. To this vial was added (by weight) 1ml of a solution containing 0.0685 g of n-hexadecane in 100ml (63.5456g) of pentane. The resulting mixtures were allowed to stand for 1-3 weeks and were shaken gently every day. Trial analyses at the end of one week and at the end of three weeks showed there was no difference in the composition of the oil, nor was there any increase in the amount of oil extracted as measured by the ratios of the α- and β-santalol peaks relative to that of n-hexadecane. The pentane solutions were analysed as outlined below and peak identifications were confirmed by GC/MS, also as set out below.

To verify the solvent extraction method, two ground heartwood samples from wood biscuits taken adjacent to core A of *S. yasi* tree 1 and adjacent to core A of *S. album* tree 12 were steam distilled with cohobation for 24 hours as set out in Brophy *et al.* (1991). In this case the oils obtained were removed from the aqueous distillate by dissolution in pentane. The pentane was dried over anhydrous sodium sulphate and the pentane allowed to evaporate at room temperature over night. The residual oil was weighed and analysed in the same manner as the solutions.

Identification of components: Analytical gas chromatography (GC) was carried out on a Shimadzu GC17 gas chromatograph. A WCOT DB-Wax [60 m x 0.5 mm, film thickness 1 µm] was used, programmed from 50°-225° C at 3°C/min with helium at 3.5 mL/min as carrier gas. GC integrations were performed on a SMAD electronic integrator without the use of correction factors. GC/MS was performed on a VG Quattro mass spectrometer operating at 70 eV ionization energy; the column used was DB-Wax [60 m x 0.32 mm, film thickness 0.25 µm] programmed from 35°-220° C at 3°C/min, with helium at 35 cm/sec. Compounds were identified by their identical GC retention times to known compounds and by comparison of their mass spectra with either known compounds or published spectra (Adams, 2001; Heller and Milne 1978, 1980, 1983; Joulain and König 1998; Stenhagen *et al.* 1974; Swigar and Silverstein 1981).

Figure 1. Heartwood coring in Fiji.

Table 1. α-Santalol and β-santalol percentages of heartwood oils of individual trees of *S. yasi* and *S. album*. SOL is the determination by solvent (pentane) extraction of a micro-sample of heartwood in wood cores, while SD is steam distillation of heartwood from wood biscuits (discs) taken adjacent to the position of wood coring (i.e. at 0.1m above ground).

Species	Extraction Method*	Tree no.	% of total oil	
			α-santalol	β-santalol
S. yasi	SD	1	33.7	14.7
	SOL	1B	30.5	15.5
S. album	SD	12	58.6	23.0
	SOL	12A	55.5	22.1

* SD is steam distilled and SOL is solvent extract

Results

Verification of the solvent extraction method based on sub-sampling cores: Comparison of the estimation of the percentage of α- and β-santalol of total oil in sub-sampled cores and matching steam distilled samples for two trees is given in Table 1. The α- and β-santalol percentages of total oils among pairs of samples of both *S. yasi* and *S. album* were in very good agreement.

Although not reported here, oil concentration was also determined by solvent extraction methods and steam distillation on these two pairs of samples. Oil concentration values were substantially different between pairs of samples, indicating the need for more work in using solvent extraction methods to reliably determine this trait.

Variation in oil composition within and between species: Ten of the thirteen *S. album* trees sampled in Fiji had no detectable heartwood oil at their base despite one of them of unknown age having a core length of 28 cm. Of the three trees that gave heartwood oils at their base, the quality of the oils met or exceeded the standard (Table 2a). In the ten-year-old tree there was no detectable heartwood oil at the 0.3m sampling height.

The eight F1 hybrid trees, *S. album* × *yasi*, sampled in Fiji were very vigorous and, where recorded, three trees had a mean basal core length of 15.6 cm in only c.5–7 years from planting. Only three trees gave heartwood oil at the base (Table 2b). The quality of this oil was equivalent to best-quality *S. album* oil in the case of two trees, but below par for one of the 7-year-old trees at Rotuma.

The *S. yasi* samples cover a range of trees of different ages from 10 years to more than 25 years, and were sourced from different countries (Tables 2c–Fiji, 2d–Tonga, 2e–Niue) and sites. As expected oil quality was highly variable with, in general, the basal heartwood oils of the older trees (i.e. 15 years or greater) matching the *S. album* standard but the oils from younger trees falling below the standard for α-santalol. The tendency for *S. yasi* to give oils with relatively high levels of β-santalol (at near 30%), as first noted by Alpha (1997) in samples from Tonga, is evident in this data. Similar to the experience with the other species heartwood oils do not extend very far up the stems of these relatively young trees. Four *S. yasi* trees in Fiji, two aged 10 years with a core length average of 9.5 cm and two aged 15 and 21 years with a core length average of 17.8 cm, gave no detectable basal heartwood oil.

Discussion

Very considerable tree-to-tree variation in age of commencement of heartwood formation in stems of *S. album* and *S. yasi* was demonstrated by this study which is consistent with other work. Haffner (1993), for example, reported a range of 14 to 46 years for *S. album* in Timor. While the economics of growing sandalwoods commercially for oil production in the Pacific will be improved by short rotation lengths, the results of this survey throw into question the predictions of some that rotation lengths of 15 years to 20 years might be viable. Certainly, in any one stand there will be some trees of *S. yasi* and *S. album* that have started to lay down heartwood at this age but many that have not. It would be prudent, therefore, to delay harvest until the majority of trees had formed substantial proportions of heartwood up the stem. This was expected to take 25 to 30 years for plantation grown *S. album* in north-western Australia (Radomiljac *et al.* 1998), and could well be a more realistic rotation length for the Pacific sandalwoods.

For the main species sampled, *S. yasi*, heartwood oils of six of the seventeen trees cored had lower α-santalol and/or β-santalol levels than allowed by the standard for *S. album* (International Standard ISO/DIFS 3518 2002). Admittedly, at least two of these trees were only 10-years-old and their young age might be playing a part, but there is an indication in the data of substantial variation between trees in oil quality. If α- and β-santalol levels (% of total oils) prove to be heritable, as well they might given oil quality is moderately to highly heritable in foliar oils in other genera (e.g. Doran 2002), then there will be considerable scope for their improvement by selection and breeding.

The spontaneous F1 hybrid, *S. album* × *yasi*, trees are typically very vigorous, as indicated by the core lengths against age given in Table 2b compared to the core lengths against age of representatives of the parent species (Table 2a and 2c). Only three of the eight c. 5 to 7-year-old hybrids sampled gave heartwood oil and, in the case of two trees, it proved to be of very good quality. This bodes well for sandalwood growers in Fiji who are in the process of propagating and establishing stands of this hybrid.

References

Adams, R.P. (2001). *Identification of Essential Oil Components by Gas Chromatography/ Quadrupole Mass Spectrometry.* Allured Publishing Corp., Carol Stream, IL.

Alpha, T.T.M.J. (1997). *Etudes des concretes et des essences du santals d'origine oceanienne- elucidation de Nouve.* A thesis presented for the award of Docteur en Sciences, de l'Universite Francaise de Pacifique.

Brophy, J.J, House, A.P.N., Boland D.J. and Lassak, E.V. (1991). *Digests of the essential oils of 111 species from northern and eastern Australia.* In *Eucalyptus Leaf Oils - Use, Chemistry, Distillation and Marketing.* Edits, D.J. Boland, J.J. Brophy and A.P.N. House, pp 29-155, Inkata Press, Melbourne.

Doran, J.C. (2002). *Genetic improvement of eucalypts: with special reference to oil-bearing species.* In *Eucalyptus: The Genus Eucalyptus*. Edit. J.J.W. Coppen, pp 75–101. Medicinal and Aromatic Plants - Industrial Profiles, Taylor & Francis, London and New York.

Haffner, D. (1993). *Determining heartwood formation within Santalum album and Santalum spicatum*. Sandalwood Research Newsletter 1: 4–5.

Heller, S.R. and Milne, G.W.A. (1978, 1980, 1983). *EPA/NIH Mass Spectral Data Base.* U.S. Government Printing Office, Washington D.C.

International Standard ISO/FDIS 3518 (2002). *Oil of sandalwood (Santalum album L.)*. ISO, Geneva (Final Draft).

Joulain, D. and König, W.A. (1998). *The Atlas of Spectral Data of Sesquiterpene Hydrocarbons.* E.B. Verlag, Hamburg.

Radomiljac, A.M., Shea, S.R., McKinnell, F.H. and McComb, J.A. (1998). *Potential for irrigated tropical forestry in northern Western Australia.* Australian Forestry 61: 70–75.

Stenhagen, E., Abrahamsson, S. and McLafferty, F.W. (1974). *Registry of Mass Spectral Data*, Wiley, New York.

Swigar, A.A. and Silverstein, R.M. (1981). *Monoterpenes*. Aldrich, Milwaukee.

Table 2. Details of the young sandalwood trees (*Santalum yasi*, *S. album* and F1 hybrids in Fiji, and *S. yasi* in Tonga and Niue) sampled by the project and variation in their heartwood oil composition.

Sample No.	Planted (P) Natural Regen. (NR)	Tree height (m)	Age (years)	Location	Core height (m)	Core length (cm)	% of Heartwood core length	Oil composition % of total oil: α-santalol	Oil composition % of total oil: β-santalol
(a) *S. album*—planted Fiji *									
12A	P	x	10	Vunimaqo	0.1	13.2	-	55.5	22.
12B					0.3	8.5	0	-	-
W7	P	x	16	Western Vitu Levu	0.1	9.4	10	47.5	26.1
W8	P	x	16	Western Vitu Levu	0.1	9.2	11	53.2	32.2
(b) *S. album* x *S. yasi*—putative spontaneous hybrid Fiji #									
4A	P	x	C.7	Nadera	0.1	17.0	2	54.4	27.3
4B					0.3	15.5	0	-	-
X1	P	x	7	Rotuma	0.1	-	-	32.4	13.7
X4	P	x	7	Rotuma	0.1	-	-	47.3	21.9
(c) *S. yasi*—Fiji @									
1A	P	x	c.18	Colo-1-Suva Nursery	0.1	10.5	37	30.5	15.5
1B					0.3	6.2	3	20.3	14.7
1C					1.3	7.2	0	-	-
2	P	x	C.15	Laucala Beach, Suva	0.1	11.0	23	48.6	27.4
5A	P	x	10	Vunimaqo	0.1	10.2	9	24.5	13.2
5B					0.3	11.5	9	15.6	10.4
5C					1.3	7.8	0	-	-
6A	6A	6A	10	Vunimaqo	0.1	11.0	4	38.6	20.4
6B	6B	6B			0.3	10.0	6	27.4	17.8
6C	6C	6C			1.3	9.0	0	-	-

Table 2 (continued)

Sample No.	Planted (P) Natural Regen. (NR)	Tree height (m)	Age (years)	Location	Core height (m)	Core length (cm)	% of Heart-wood core length	Oil composition % of total oil α-santalol	Oil composition % of total oil β-santalol
(c) *S. yasi*—Fiji[@] (continued)									
W1	P	?	C.25	Western Vitu Levu	0.5	17.4	43	47.6	24.6
W9A	P	8	16	Western Vitu Levu	0.1	16.3	45	44.9	21.4
W9B					1.0	8.7	Trace?	-	-
W10	P	8	16	Western Vitu Levu	0.1	18.8	7	50.4	21.6
W11				Western Vitu Levu	0.1	-	<2	49.1	28.8
W11A					1.3	7.2	0	-	-
B2	P	6	C.20	Bua, Western Vitu Levu	0.1	18.5	32	56.8	24.4
B6	P	6	15	Bua, Western Vitu Levu	0.2	17.4	<1	50.1	34.0
B7	P	8	15	Bua, Western Vitu Levu	0.2	12.6	19	43.5	25.9
B8	P	8	<20	Bua, Western Vitu Levu	0.2	25.1	92	35.1	14.8
(d) *S. yasi*—Tonga									
T1A	NR	8	C.20	Hafu, 'Eua	0.2	25.0	80	50.0	27.0
T5A	NR	6	C.20	Hafu, 'Eua	0.3	12.0	56	35.4	28.6
T6A	NR	10	C.20	Hafu, 'Eua	0.3	11.5	37	48.9	27.1
(e) *S. yasi*—mature trees Niue									
N1	P	x	>20	Malakava	base	x	x	43.1	26.6
N2	P	x	>20	Malakava	base	x	x	39.1	29.8

* Seven trees sampled at 0.1m (core length average of 10.2cm) and aged 10 years had no detectable heartwood. A further three trees assumed to be older from larger core lengths (average 19.1cm) also gave no oil.
[#]Two hybrids sampled at 0.1m – 0.2m (core length average of 14.8cm) and aged c.5-7 years at Nadera gave no detectable heartwood. Similarly, three, 7-year-old hybrids at Rotuma gave no heartwood oil.
[@]A further four trees sampled at 0.1m – 0.2m (core length average of 13.7cm) and aged c.10 – 21 years gave no detectable heartwood.

Indian Sandalwood: Genetic and oil diversity and biochemistry of the Australian germplasm collection

Chris Jones, School of Plant Biology, UWA, 35 Stirling Hwy Crawley, WA 6009

The University of Western Australia (UWA), supported by the Forest Products Commission of Western Australia (FPC) have commenced work on the genetic diversity and oil biochemistry of *Santalum album* L. This is an ARC-Linkage project (LP 0454919) between UWA and the FPC, with molecular genetics assistance from the WA Department of Conservation and Land Management (CALM) Science division. It is being undertaken by PhD student Chris Jones, under the supervision of Dr Julie Plummer (Plant Biology, UWA), Dr Emilio Ghisalberti (Chemistry, UWA), Dr Margaret Byrne (CALM Science) and Dr Liz Barbour (FPC).

The project consists of three main components. The first component is to determine the degree of genetic diversity and species relatedness within *Santalum album*, and two other closely related species; *S. austrocaledonicum* and *S. macgregorii*. It is hoped that other researchers in the field may provide additional data which will help piece together the fascinating phylogeny of *Santalum*. At this stage, restriction enzyme digests have been performed on sandalwood DNA, and hybridisation studies using previously determined *S. spicatum* probes (Byrne *et al.* 2003) have started.

Secondly, the essential oil composition of *S. album* growing in the Ord River Irrigation Area will be examined. It has been suggested that specific 'chemotypes' of *Santalum* exist (Butaud *et al.* 2003) and may well apply to *S. album*. This will be correlated to genotypes determined in the first part of the study. Some 100 wood cores have been taken from live trees, and it is hoped that the identification of 'superior' oil producing trees is in agreement with the genetic results.

The third component of the project seeks to determine the biosynthetic pathway of essential oils in the heartwood of *S. album* and their mechanism of production. While the biosynthesis of sesquiterpenes in many plant species is well known (Cane 1990), it has only been assumed that a similar process is occurring in sandal-

wood (Adams *et al.* 1975; Parker *et al.* 1967). The lack of research into this aspect of sandalwood is probably due to the inherent difficulty of the location of biosynthesis; i.e. deep in the heartwood. In order to determine the pathway, a few approaches may be taken. One may take wood cores from live trees, and extract functional enzymes from the transitional zone between heartwood and sapwood (Hauch and Magel 1998). Isotopic labelling would confirm the origins of sesquiterpene metabolism. Alternatively, cell cultures of sandalwood can be used to determine metabolic pathways, or even extract and purify enzyme fractions from this tissue. This has been used in determining the biosynthetic origins of the terpenoid phytoalexin thujaplicin in the gymnosperm *Cupressus lucitanica* (Yamaguchi *et al.* 1997). Recently, much of the work on sesquiterpene biosynthesis has utilised primary structure similarity of key enzymes known as sesquiterpene synthases, or cyclases (Back and Chappell 1995; Liu *et al.* 2002; Schnee *et al.* 2002). It is anticipated that all three methods will be employed to determine both the biosynthetic pathway to the essential oils of sandalwood and to identify the key enzymes responsible. This will help in our understanding of why the sandalwoods produce essential oils, and whether it can be induced or selected for.

The research is to be published in peer-reviewed scientific journals and is expected to continue until 2007.

References

Adams DR, Bhatnagar SP, Cookson RC (1975) Sesquiterpenes of *Santalum album* and *Santalum spicatum*. *Phytochemistry* **14**, 1459-1460.

Back K, Chappell J (1995) Cloning and bacterial expression of a sesquiterpene cyclase from *Hyoscyamus muticus* and it's molecular comparison to related terpene cyclases. *Journal of Biological Chemistry* **270**, 7375-7381.

Butaud J-F, Raharivelomanana P, Bianchini J-P, Baron V (2003) A new chemotype of sandalwood (*Santalum insulare* Berto ex A. DC.) from Marquesas Islands. *Journal of Essential Oil Research* **15**, 323-326.

Byrne M, MacDonald B, Brand JE (2003) Regional genetic differentiation in Western Australian sandalwood (*Santalum spicatum*) as revealed by nuclear RFLP analysis. *Theoretical and Applied Genetics* **107**, 1208-1214.

Cane DE (1990) Enzymatic formation of sesquiterpenes. *Chemical Reviews* **90**, 1089-1103.

Hauch S, Magel E (1998) Extractable activities and protein content of sucrose-phosphate synthase, sucrose synthase and neutral invertase in trunk tissues of *Robina pseudoacacia* L. are related to cambial wood production and heartwood formation. *Planta* **207**, 266-274.

Liu Y, Ye H-C, Li G-F (2002) Cloning, *E. coli* expression and molecular analysis of a novel sesquiterpene synthase gene from *Artemisia annua*. *Acta Botanica Sinica* **44**, 1450-1455.

Parker W, Roberts JS, Ramage R (1967) Sesquiterpene biogenesis. *Quarterly Reviews* **21**, 331-363.

Schnee C, Kollner TG, Gershenzon J, Degenhardt J (2002) The maize gene *terpene synthase 1* encodes a sesquiterpene synthase catalyzing the formation of (*E*)-β-farnesene, (*E*)-nerolidol, and (*E,E*)-farnesol after herbivore damage. *Plant Physiology* **130**, 2049-2060.

Yamaguchi T, Itose R, Sakai K (1997) Biosynthesis of heartwood tropolones I. Incorporation of mevalonate and acetate into b-thujaplicin (Hinokitiol) of *Cupressus lusitanica* cell cultures. *J. Fac. Agr., Kyushu Univ.* **42**, 131-138.

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Authors contact details:

John Doran
CSIRO Forestry and Forest Products
PO Box E4008
Kingston ACT 2604
AUSTRALIA

Tel: + 61 8 6281 8319
Fax: + 61 8 6281 8266
Email: John.Doran@csiro.au

Ben Lethbridge
Lobopogon Research
62 Grants Gully Rd
Clarendon SA 5157
AUSTRALIA

Tel: + 61 8 8383 6181
Fax: + 61 8 8383 6181
Email: lethbm62@bigpond.com.au

Chris Jones
School of Plant Biology
University of Western Australia
35 Stirling Highway
Crawley WA 6009
AUSTRALIA

Email: jonescg@cyllene.uwa.edu.au