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Sandalwood (*Santalum spicatum* (R.Br.) A. DC.) excavation study

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Jonathan E Brand[a], Syd R Shea[a] and Ted Spadek[b]

[a] *Department of Conservation and Land Management, Locked Bag 104, Bentley Delivery Centre, Western Australia, 6983*
[b] *Chemistry Centre, 125 Hay Street, East Perth, Western Australia, 6004*

Abstract

A mature *Santalum spicatum*, growing near Kalgoorlie, Western Australia, was excavated to determine its total biomass and the oil concentration in certain sections of the tree. A high proportion (38%) of the total tree weight was found in the root system, which consisted mainly of shallow lateral roots. The concentration of extractable oil in the heartwood varied between different sections of the tree and was highest in the butt, then large stems and large roots. Extractable oil from the butt, large stems and large root sections had the greatest proportion of β-santalol.

Introduction

Santalum spicatum (R.Br.) A. DC. (Western Australian sandalwood) is exported to Southeast Asia, mainly for use in incense manufacture (Richmond, 1977). *S. spicatum*, a root hemi-parasite, occurs naturally throughout a large proportion of Western Australia (Loneragan, 1990). The tree is harvested mainly from pastoral leases and vacant crown land in semi-arid regions. Western Australian sandalwood is normally pulled from the ground to retrieve the heartwood from the tree's root (Shea *et al.*, 1997). This method of harvesting may result in large roots breaking close to the main stem and thus remaining in the soil. The amount of root material not harvested may be high due to the extensive lateral root system of the tree (Herbert, 1925).

Excavation of mature plants of *S. spicatum* will help determine the proportional weight of commercial wood contained in the root system. If a high proportion of wood is contained in the roots then new harvesting techniques may be warranted.

The fragrance of sandalwood oil is derived from sesquiterpenes, especially α-santalol and β-santalol (Adams *et al.*, 1975). Piggott *et al.* (1997) examined the composition of *S. spicatum* oil in different sections of a tree and identified five sequiterpene alcohols: epi-α-bisabolol; α-santalol; farnesol; β-santalol; and nuciferol.

The aims of this study were to: (i) excavate and examine the root system of a mature *S. spicatum* tree; (ii) determine the biomass contained within different sections of the tree; and (iii) determine the oil percentage and composition from different sections.

Methods

Excavation

The root system of a mature *S. spicatum* tree was excavated at Lakeside reserve (30° 50' S, 121° 36' E) near Kalgoorlie, in May 1997. The tree was growing near a creek, on a plain with *Acacia* and *Eucalypt* woodlands. The tree was 380 cm tall and had a stem diameter of 155 mm, at 150 mm above the ground. Pressurised water from was used to wash away the soil and expose the entire root system (Figure 1). The length and depth of the roots were measured and haustorial connections to host plants recorded.

The tree was divided into nine different groups: butt; large stems (> 40 mm diameter); large roots (> 40 mm diam.); medium stems (20-40 mm diam.); medium roots (20-40 mm diam.); fine stems (5-20 mm diam.); fine roots (5-20 mm diam.); dead branches; and leaves and twigs. The butt consisted of the main stem to a height of 15 cm above the ground, and roots to a length of 20 cm from the centre of the base. Stem and root diameter measurements included bark. Twigs with a diameter less than 5 mm were grouped with leaves. Each group was dried in an oven at 80°C for 48 hours, and then the dry weight was measured using a Mettler balance.

Oil extraction

Fresh wood samples from each group, except twigs and leaves, were

Exposed root system of a S. spicatum tree near Kalgoorlie, Western Australia

analysed for oil content at the Chemistry Centre, Western Australia. Seven cross-sectional wood pieces of 20-50 g were cut from each of the eight groups and ground through a crossbeater mill. Total oil from three of the ground samples was extracted using steam-distillation in a Dean and Stark apparatus for 24 hours. The mixed oil and water phases were extracted with dichloromethane, which was removed at 40°C by rotary evaporation under reduced pressure. Residual solvent was removed from the extracted oil using a high vacuum for 60 minutes. The weight of the total extractable distilled oil from each sample was expressed as a percentage of the wood dry weight.

Total extractable oil in the remaining four ground pieces in each group were extracted with ethyl acetate in a soxhlett apparatus, for 12 hours. After extraction, the extract was made up to 200 mL with ethyl acetate and a 100 mL aliquot was taken to determine the weight of extractable oil. Solvent was removed at 40°C by rotary evaporation under reduced pressure and residual solvent was removed from the extracted oil using a high vacuum for 60 minutes. Total weight of extractable oil was expressed as a percentage of the wood dry weight. The mean oil percentage from each tree section was multiplied by wood dry weight to estimate total oil content in the tree.

A 10 mL aliquot was also taken from each solvent extracted sample to determine the percentage of β-santalol and farnesol in sandalwood oil. Internal standard (octanol) was added to the solution and then analysed by Gas-Liquid Chromatography with a Flame Ionisation detector. The concentrations of β-santalol and farnesol were determined using peak area mode by comparison to authentic standards.

Results

Total dry weight of the tree was 74.4 kg, which included 45.4 kg of de-barked wood (Table 1). The proportion of wood was highest in the stems (46%), followed by the roots (38%), butt (9%) and dead wood (7%).

The root system consisted of one main lateral root, with a diameter of 7 cm at 100 cm from the stem base, and numerous smaller lateral roots. These extended up to 12 m from the stem base, but no deeper than 40 cm. The root system also contained a small tap-root to a depth of 90 cm. Fine feeder roots extended from the lateral roots and had haustoria attached to three different species: *Acacia burkittii* F.Muell. ex Benth., *Senna artemisioides* (DC.) Randell subsp. *filifolia* and *A. hemiteles* Benth. A total of 123 haustoria of 5-20 mm in length were recorded attached to the three host species. Two haustoria were also attached to the sandalwood's own root system.

The amount of oil obtained from solvent extraction was greater than steam distillation for each tree section (Table 2). Distillation recovered only

Tree Section	Wood		Bark		Total	
	(kg)	(%)	(kg)	(%)	(kg)	(%)
1. Butt	4.1	9.0	1.0	6.3	5.1	6.9
2. Large stems	12.1	26.7	3.5	22.1	15.6	21.0
3. Large roots	8.5	18.7	2.8	17.7	11.3	15.2
4. Medium stems	3.9	8.6	1.4	8.9	5.3	7.1
5. Medium roots	6.1	13.4	3.3	20.9	9.4	12.6
6. Fine stems	4.7	10.4	1.8	11.4	6.5	8.7
7. Fine roots	2.7	5.9	2.0	12.7	4.7	6.3
8. Dead wood	3.3	7.3	-	-	3.3	4.4
9. Leaves and twigs	-	-	-	-	13.2	17.7
Total	**45.4**	**100**	**15.8**	**100**	**74.4**	**100**

Table 1: Total dry weight of wood (heartwood and sapwood) and bark for each tree segment and proortion of wood and bark for each segment of the total tree.

Tree Segment	% Total oil extracted		% Compounds	
	Solvent	Distilled	β–santalol	Farnesol
1. Butt	7.3±0.5	1.6±0.1	9.8±0.2	1.2±0.1
2. Large stems	4.7±0.2	0.9±0.1	11.6±0.7	2.3±0.1
3. Large roots	3.8±0.2	0.7±0.1	10.5±0.5	1.5±0.2
4. Medium stems	1.8±0.1	0.2±0.1	6.7±0.5	2.0±0.3
5. Medium roots	2.8±0.3	0.4±0.1	5.4±0.2	ND
6. Fine stems	2.0±0.1	0.2±0.2	ND	ND
7. Fine roots	1.9±0.2	0.1±0.1	1.5±0.3	ND
8. Dead wood	0.8±0.2	0.1±0.1	ND	ND

ND - Not detected

Table 2:
Mean oil percentage of each *S. spicatum* tree segment as determined by solvent extraction and steam distillation and proportion of β–santalol and farnesol in solvent extracted oil within each *S. spicatum* tree segment.

14% of oil when compared to solvent extraction, but was indicative of the solvent results (r = 0.99). Oil percentage varied between tree sections and was highest in the butt, large stems, large roots and medium roots. The estimated total volume of extractable oil in the tree was 1.6 L and most of this was contained in the stem (0.73 L), root (0.54 L) and the butt (0.30 L).

The percentage of β-santalol was highest in the extracted oil from the butt, large stem and large root, ranging from 9.8% to 11.6% (Table 2). A low percentage of farnesol was detected in the oil from the butt, large stem, large root and medium stem, ranging from 1.2% to 2.3%.

Discussion

The *S. spicatum* root system consisted almost entirely of lateral roots, except for one small tap-root which had penetrated to a depth of only 90 cm. The lateral roots extended over 12 m from the base of the tree stem, no deeper than 40 cm. The fine feeder roots had numerous haustoria, which were attached to a variety of host plants, including: *A. burkittii*, *S. artemisioides* subsp. *filifolia* and *A. hemiteles*.

The root system contained a high proportion (38%) of the total wood weight. Small roots (5-40 mm), which are not normally harvested, represented 51% of the root system weight. A reasonable amount of extractable oil was present in this root material (1.9-2.8%), when compared to large root material (3.8%). The estimated total volume of extractable oil in the tree was 1.6 L and 34% of this was contained in the root system. However, the percentage of β-santalol contained in the oil from the small root material (1.5-5.4%) was much lower than in the butt, large root and large stem (9.8-11.6%). These findings are consistent with Piggott *et al.* (1997) found that the proportion of sequiterpene alcohols in *S. spicatum* oil varied between different sections within the tree. Since the fragrance of sandalwood is partly derived from the amount of β-santalol (Adams *et al.*, 1975), the oil contained in the smaller root material may be less fragrant than in other sections of the tree. This study, albeit limited to a single tree, showed that a large amount of wood and oil is contained in the root system.

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Somatic embryogenesis in *Santalum album* L.

V A Bapat and P S Rao

Nuclear Agriculture and Biotechnology Division, Bhabha Atomic Research Centre, Trombay, Mumbai 400-085, India

In India, a range of pathogens, of which a mycoplasma-like organism (MLO), causes Spike disease is the most serious, plagues *Santalum album* in natural stands and plantations. In some areas local populations of *Santalum* have disappeared as a result of Spike disease. Vegetative propagation of *Santalum* by conventional methods (cuttings or grafting) has not been successful. In this respect, the development of tissue culture techniques for mass multiplication of superior genotypes, such as Spike resistant *Santalum*, has important practical benefits. The occurrence of somatic embryogenesis in *Santalum* is one of the earliest reports of somatic embryogenesis in forest trees. Somatic embryogenesis in *Santalum* is governed by a multivariable process influenced by several physical and chemical factors, coupled with other parameters, such as genotype, age of the source tissue and location of the source material. This article deals with various *in vitro* strategies developed for the mass multiplication of this *Santalum*.

Hypocotyl segments from aseptic *Santalum* seedlings and stem segments from 30 year-old trees when cultured on an appropriate nutrient medium produced actively growing callus tissue. The callus tissue on transfer to an appropriate medium produced highly regenerative embryogenic tissue that contained numerous somatic embryos. The somatic embryos through successive ontogenic stages of development exhibited globular, heart and torpedo shape stages similar to zygotic embryos of seeds. Perpetually regenerating callus tissue cultures could be maintained by sub-culturing the tissue on fresh medium of the same composition. A sequential change in the media induced differentiation of somatic embryos in the callus tissue and the somatic embryos developed into viable plantlets. In many cultures the somatic embryos differentiated again and produced a callus mass which showed secondary embryogenesis. An extract of cyanobacterium (*Plectonema boryanum*) when added to the nutrient medium as a supplement stimulated somatic embryogenesis and plantlet development.

Santalum callus tissue on transfer to agitated liquid medium produced good cell suspensions. During the growth phase of cell suspension, thousands of cell per ml were developed, which on alteration in the hormonal composition organised into embryos. Mature organised embryos developed into plantlets on transfer of the embryos from liquid to solid medium. *Santalum* cells growing in flasks were used as the inoculum for cultivating cells in bioreactors. A seven-litre bioreactor was used for the conversion of non-embryogenic cells to embryogenic cells and then a bell jar reactor was used for the development of pro-globular embryos to mature embryos and plantlets.

Protoplasts could be isolated from a variety of sources such as stem callus, cell suspensions and leaf mesophylls. A high percentage of protoplasts divided and developed into cell colonies. Embryos regenerated from these colonies, which subsequently developed into plantlets. The regeneration of plants from protoplasts in *Santalum* is the first report of regeneration of plants from protoplasts in any tree species.

The process of maturation and desiccation of zygotic embryos in seeds is controlled by a variety of external and internal factors. For the optimum conversion of somatic embryos to plants it is necessary to study and apply these factors in somatic embryogenesis. Various studies in *Santalum* revealed that desiccation of embryos promote more embryos to develop into plants. This demonstrates the ability of the desiccated *Santalum* embryos to survive and regenerate into plants. Dried callus kept in aseptic conditions for 4-weeks rejuvenated and formed embryos when transferred to the fresh medium.

Somatic embryogenesis offers rapid delivery of tissue cultured plants, the storage of important germ plasm and easily transported plant material. However, for successful implementation of large-scale somatic embryogenesis a number of potential difficulties need to be addressed. Firstly, prior to encapsulation, synchronization of embryogenesis and development of well-developed somatic embryos must be achieved. Secondly, there needs to be the successful conversion of somatic embryos to plantlets in large numbers and thirdly, there needs to be clear commercial gains in the use of somatic embryogenesis.

Somatic embryogenesis is an important research tool for *Santalum* breeding, as it might be used in investigations on the physiological and genetic influences of santalol oil formation in *Santalum*.

Santalum macgregorii F. v. Muller in Papua New Guinea

Andrew Radomiljac[1] and Derek Bosimbi[2]

[1]*Department of Conservation and Land Management, CALMScience, Locked Bag 104, Bentley Delivery Centre 6983, Western Australia*

[2]*Papua New Guinea Forest Authority, National Tree Seed Centre, PO Box 134, Bulolo, Morobe Province, PNG*

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Introduction

The fragrant heartwood from *Santalum* is of high commercial significance for many rural based economies. However, as a result of acute over-exploitation most *Santalum* species are now faced with declining populations. *Santalum macgregorii*, endemic to southern Papua New Guinea, is one of the least known *Santalum* species (Paul 1990), and the extent of exploitation until recently was unknown.

S. macgregorii's former distribution extended continuously along the PNG southern savanna region.

Santalum macgregorii for plantations

Morphologically, *S. macgregorii* appears quite close to *S. album* (native to southern India and eastern Indonesia). *S. album* produces the most widely utilised heartwood of all *Santalum* species, Indian sandalwood. If *S. macgregorii* and *S. album* are closely related then *S. macgregorii* may become a useful plantation species, both within and outside PNG.

As with all *Santalum* species *S. macgregorii* is an obligate root hemi-parasite. This parasitic habit complicates nursery propagation and plantation culture. However, the silvicultural protocols developed for *S. album* in India, Indonesia and Australia and for *S. austrocaledonicum* in New Caledonia might be transferred to *S. macgregorii*. Thus making it feasible to consider *S. macgregorii* as a native plantation species in PNG.

Santalum macgregorii heartwood oil analysis

The percentage content and composition of *S. macgregorii* heartwood oil is unknown. Oil analysis is being undertaken on a number of heartwood samples at the Department of Chemistry, University of Western Australia, Perth. This analysis will be compared with a number of exotic *Santalum* species and will assist in understanding differences between *S. album* and *S. macgregorii*.

Seed collection and *ex situ* conservation

Two varieties of *S. macgregorii* may exist in southern PNG, a coastal and highland variety. The coastal variety has a broader leaf, a lighter coloured heartwood and less scented heartwood than that of the highland variety. However, detailed morphological examination on flowers and fruits is required to determine the actual extent of this variation.

Mature trees in natural stands produce moderate fruit crops, thus there should be reliable seed supplies for silvicultural research and species introduction, *ex situ* plantings and small-scale plantation establishment work.

Current status of *Santalum macgregorii*

S. macgregorii is considered scarce. Population decline is presumably a result of over-harvesting coupled with indiscriminate burning. Recent inventories have identified *S. macgregorii* as far west as the Paupala range, near Lese, in the Gulf Province. A complete inventory of remaining populations is urgently required to assist identifying areas that require germ plasm conservation measures.

As in most areas of PNG, the forested areas of Central and Gulf Provinces are relatively inaccessible. This inaccessibility impedes *S. macgregorii* harvesting and heartwood transport. By virtue of remoteness some remnant *S. macgregorii* populations have avoided exploitation.

There is limited traditional use of *S. macgregorii* in PNG, which is in contrast to the inherent utilisation of sandalwood for medicinal and religious purposes in Indonesia, India and several South Pacific countries.

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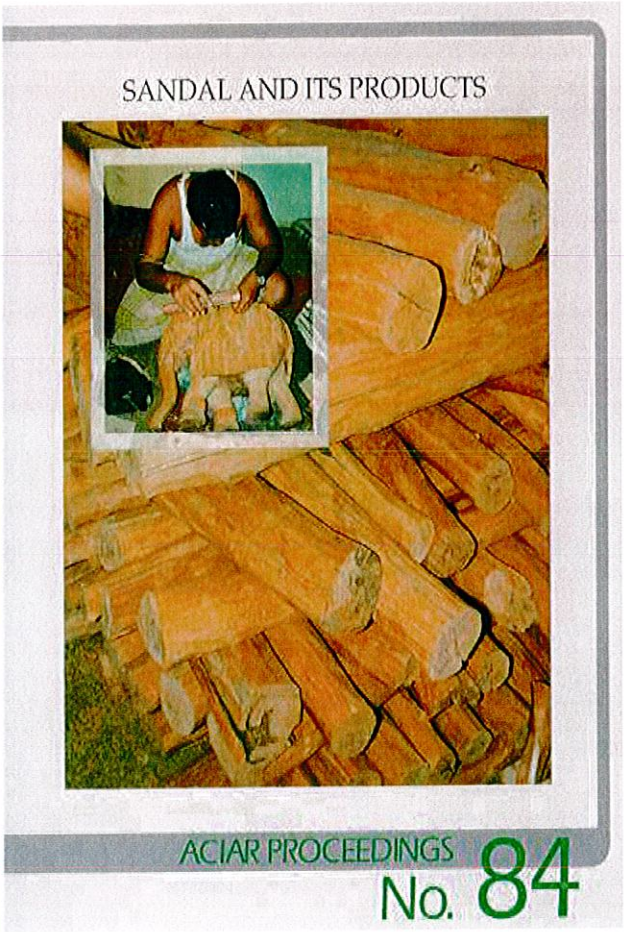

New Book

Sandal and its Products

ACIAR Proceedings No. 84

Sandal and its Products. AM Radomiljac, HS Ananthapadmanabha, RM Welbourne, KS Rao (eds.). Proceedings of an International Seminar held at Bangalore, India, 18-19 December 1997. Canberra, ACIAR Proceedings Number 84. 203 pp.

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Andrew Radomiljac
Department Conservation and Land Management
Locked Bag 104
Bentley Delivery Centre 6983
Western Australia

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Editor: Mr Andrew Radomiljac
Department of Conservation and Land Management
Locked Bag 104
Bentley Delivery Centre 6983
WESTERN AUSTRALIA

Phone: +61 8 9334 0327
Fax: +61 8 9334 0161
email: andrewr@calm.wa.gov.au