Sandalwood Research Newsletter

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Introduction to the Sandalwood Research Newsletter

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As a result of an increasing global demand on *Santalum* species wood and oil products there is increasing exploitation pressure on existing *Santalum* species populations. However, through-out the Asian and Pacific Island countries there is a concerted effort to increase *Santalum* species conservation and plantation establishment (Daruhi 1993, Cherrier 1993, Jiko 1993, Applegate and McKinnell 1993 and Harisetijono and Suramihardja 1993).

Santalum species have high cultural and economical value to a large number of Asian and Pacific Island nations, such as *Santalum album* in Nusa Tengarra Timur, Indonesia. Within Indonesia efforts are being currently undertaken to increase the population size of this valued resource through plantation establishment. Other island nations such as New Caledonia and Fiji also acknowledge the importance of plantations of their native *Santalum* species.

The Sandalwood Research Newsletter (SRN) aims to distribute *Santalum* species literature from nations and their respective research and management organisations. Within many 'isolated' nations their *Santalum* species research and management programs will be enhanced through increasing access to international *Santalum* species literature.

The SRN is an initiative conceived by the Australian Centre for International Agricultural Research (ACIAR).

Increasing *Santalum* species awareness through the distribution of international *Santalum* species literature will:

i) promote *Santalum* species conservation.

ii) stimulate *Santalum* species plantation establishment.

iii) increase *Santalum* species research and management liaison between interested organisations.

iv) increase *Santalum* species literature exposure.

The SRN will become a bimonthly newsletter with distribution ranging through-out all nations currently involved in *Santalum* species research and management.

It is hoped that articles will be received for a range of *Santalum* species research and management issues. If you wish to contribute an article to the SRN or wish to be included on the SRN mailing list, please write to the Editor giving your name, organisation and postal address. All articles on relevant *Santalum* species topics are welcomed.

Address of Department of Conservation and Land Management Sandalwood Research Officer and Editor:

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Self and cross pollination in *Santalum Spicatum* and *S. album*

Acharee Rugkhla and Dr Jenny McComb

Acharee Rugkhla, a Thai national, is currently undertaking her Phd studies at the division of Biological and Environmental Sciences, Murdoch University, Perth, Western Australia.
Supervisor: Associate Professor Dr. Jenny McComb.
Phd Thesis: Interspecific hybridization between Santalum spicatum and S. album.
Dr. Jenny McComb is an Associate Professor at the division of Biological and Environmental Sciences, Murdoch University, Perth, Western Australia.

Interspecific hybrids of *Santalum spicatum* and *S. album* may combine the cold and drought tolerance of *S. spicatum* with the high oil content and fast growth of *S. album.* We are attempting to produce a sexual hybrid between the species by hand pollination and investigated pollen tube growth and fertilisation.

Trees at Curtin University, Perth W.A., were selected using the criteria of higher growth rate, disease resistance and early flowering. Unopened inflorescenses were enclosed in 2 layers of perforated thin polyethylene bags containing a wire spiral to avoid contact between flowers and the bags. Enclosed unpollinated flowers and enclosed emasculated flowers were used as controls. Five to 15 samples in each treatment were harvested 3 days after pollination. The pistil was dissected from the flower and squash preparations were stained for fluorescence microscopy using aniline blue fluorochrome (Martin 1959). The initial fruit set was recorded at 14 days after pollination for *S. album* and 21 days for *S. spicatum.* Mature fruit set was recorded 1 month before harvesting.

In intraspecific crosses of *S. album*, initial fruit set was 20% (Table 1), which supported the results of Jyothi *et. al.* (1991). In *S. spicatum*, initial fruit set was 5%, final set 1.3%, which was higher than natural fruit set 0.14 - 0.45% (Barrett 1987). Although the chance of fertilisation was increased by hand pollination, the fruit set obtained remained low which is a common characteristic of most woody perennial species producing hermaphrodite flowers (Sutherland 1986). In *S. spicatum*, only a small percentage of pollen tubes were found to have penetrated the embryo sac. Fertilisation was also reported to be poor in *S. acuminatum*, but in this species possible reasons included the fact that some ovaries had no developed embryo sacs and the plants had an inadequate supply of nutrients and water (Sedgley 1982). Some authors have suggested that fruit abscission of *S. spicatum* was associated with overcast conditions, inadequate nutrition (Fox and Reeve 1991) and inherent characteristics (Barrett 1987).

The low fertility following self pollination (Table 1), assessed by both initial fruit set and pollen tube growth, confirmed the self incompatibility previously reported in *S. album* (Bhaskar 1980, 1992; Jyothi *et al.* 1991). *S. acuminatum* has also been reported to be cross pollinated (Keighery and Dixon 1986). Sindhuveerendra and Sujatha (1989) indicated that some trees of *S. album* partly self pollinated but up to 75% of the selfed fruit abscised before maturity. Sites of pollen tube inhibition at the upper style, lower style and around the embryo sac suggested a complex mechanism for genetic control of self incompatibility which was also found in *Banksia* (Fuss and Sedgley 1991).

Interspecific hybrids were extremely difficult to obtain (Table 1) as most pollen tubes were inhibited in the style (Table 2). *Santalum* flowers are initially green and change through pink to dark red. Pollen tubes only penetrated the embryo sac in red flowers which were the only flower

Table 1 Production of inter and intra-specific hybrids following hand pollination of *Santalum album* and *S. spicatum*

Cross	No. of Flowers Pollinated	Initial Fruit Set		Mature Fruit Set	
		No.	%	No.	%
S. album x *album*	533	113	20.1	39	7.3
S. spicatum x spicatum	1165	60	5.2	15	1.3
S. album selfed					
among flowers	220	4	1.8	2	0.9
same flower	180	0	0	0	0
S. spicatum selfed					
among flowers	250	1	0.4	1	*0.4
same flower	210	0	0	0	0
S. album x *spicatum*	680	3	0.4	0	0
S. spicatum x album	940	4	0.4	0	0
Bagged, unpollinated flower	1200	0	0	0	0
As above but also emasculated	150	0	0	0	0

*The fruit size was small and abnormal in shape.

Table 2 Pollen tube numbers in the style, ovary or embryo sac of pistils fixed 3 days after pollination

Cross	*S. Album*			*S. spicatum*		
	Style	Ovary	Embryo sac	Style	Ovary	Embryo Sac
Intraspecific	22.4±6.1	5.2±1.5	0.3±0.1	16.6±5.2	0.8±0.5	0.2±0.2
Self	6.8±0.6	1.2±0.8	0.2±0.2	2.7±4.0	0.7±0.3	0
Interspecific	3.6±1.2	0.2±0.2	0.1±0.1	3.0±2.0	0.3±0.3	0

types from which hybrid fruits developed in *S. album* trees. All the hybrid fruit abscised within 1 to 3 months. It is likely that there is more than one mechanism involved in interspecific incompatibility. In addition to the inhibition of pollen tube growth in the stigma and style, a post fertilisation mechanism may be involved.

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THE WESTERN AUSTRALIAN SANDALWOOD RESEARCH INSTITUTE

The Sandalwood Research Institute was formed in 1980 by the Australian Sandalwood Company. The intention of the Company was to put some of the returns from the Western Australian sandalwood export industry back into research which would benefit the industry. The role of the Institute was to channel funds from the industry back into research on sandalwood in tertiary institutions.

At the time the SRI was formed, there was some concern about the long term future of the sandalwood harvesting industry. There was justifiable concern about the lack of regeneration of the Western Australian sandalwood (*Santalum spicatum*), which is found over a large area of the semi-arid part of the State. For that reason, early research projects concentrated on various aspects of the regeneration of *S. spicatum.*

Following reports in the literature of success with tissue culture of *S. album* in India, research on the potential for use of this technique with *S. spicatum* was funded at Murdoch University in Perth. It became apparent that *S. spicatum* was a most recalcitrant species in this regard and this line of research was dropped in favour of tissue culture of *S. album.*

Currently, the Sandalwood Research Institute is supporting research on heartwood formation in *S. spicatum* and *S. album* at Melbourne University and further research on tissue culture in *S. album.* It is also contributing some funding to the sandalwood research program at Kununurra reported elsewhere in this Newsletter.

F H McKinnell

Determining Heartwood Formation within *Santalum album* and *S. spicatum*

Deanne Haffner

Deanne Haffner has completed her M(ForSc) thesis at the University of Melbourne, Victoria.
M(ForSc) Thesis: The Quantity and Quality of Heartwood in two species of sandalwood.
Supervisor: Dr W E Hillis
Deanne Haffner's M(ForSc) thesis is divided into two sections for the SRN. Part 2 (Determining heartwood oil content within Santalum album and S. spicatum) will be published in the second issue of the SRN.

Commercially, the value of any sandalwood tree depends upon the quantity and quality of heartwood it contains. To date, there has been much dissension about the age at which heartwood formation is initiated in sandalwood and there is little information about the rate of which heartwood is subsequently developed. Many theories exist as to which factors may affect these processes but very few have been concluded from experimental evidence.

It is know that there is large variation in the age at which *S. album* initiates heartwood formation (Srimathi and Kulkarni, 1979) and that some individuals never form any heartwood (Rai, 1990). Growth rate appears to be the best indicator of the proportion of heartwood, however, very little information is available about how the rate of heartwood formation is affected by tree age and growth rate.

In this study, 39 *S. spicatum* and 30 *S. album* trees were sampled from different sites in Western Australia and West Timor, Indonesia to examine the factors that affect the amount and quality of heartwood. From this work a method was established to estimate the heartwood content of a standing tree and to predict the error associated with such an estimate.

Methodology

Tree height and diameter at 150 mm above ground level were measured in all trees. In *S. album*, the crown size was also measured.

At 150 mm above ground level in each tree, a cross-section was cut, sanded and then painted with potassium iodide-iodine solution (equal volumes of 2% w/v potassium iodide/water and 2% w/v iodine/ethanol) (Kutscha and Sacha, 1962) to test for starch found only in the sapwood. The sapwood and heartwood area and the sapwood width were measured and the number of growth increments was counted.

Results and Discussion

Tree height and diameter

The mean tree height (10.2±1.9 m) and diameter over bark at 150 mm above ground level (309.8±74.1 mm) of *S album* were significantly greater than the mean height (3.2±0.7 m) and diameter (172.5±36.7 mm) of *S. spicatum*. The large variation in tree heights and diameters within each species reflects the differences in ages of the trees as well as the differences between sites or genotypes.

Amounts of heartwood and sapwood

At 150 mm above ground level, the mean total cross-sectional area of the *S. album* cross-sections was three times that of *S. spicatum* but the mean heartwood proportion was only 41.2% of the cross-sectional area in *S. album* compared with 81% in *S. spicatum* (Table 1). Although the heartwood area was only 1.5 times greater in *S. album* compared with *S. spicatum* (Table 1), the heartwood was formed in one third of the time (Table 2).

Two of the 30 *S. album* trees sampled appeared to contain no heartwood.

Factors related to sapwood and heartwood content

In both species, the amount of sapwood was positively correlated with the growth rate of the tree. In *S. spicatum* this also correlated with the heartwood content, however, no relationship could be found between the heartwood content of *S. album* and any of the parameters measured.

Predicting the heartwood content in stand trees

The heartwood area at 150 mm above ground level in *S. spicatum* may be best predicted by either the total cross-sectional area or the diameter over bark, which gave the regression equations.

HW area = 0.891 total area - 10.6
HW area = 1.43 diameter over bark - 132

The proportion of the cross-sectional area that was heartwood was considerably lower in *S. album* (Table 1) and hence the sapwood component had a significant effect on the regression analyses. For those *S. album* trees found to contain heartwood, the heartwood area could be calculated from the total cross-sectional area and the sapwood width (SWW) by the formula:

HW area = 92.9 - 4.47 SWW + 0.632 total area

It is essential that these regression equations are checked against a different sample of trees. More work is required to convert these values to volume estimates, particularly by measuring the height to which the

Table 1 **Comparisons of the mean area of the heartwood (HW), sapwood (SW) and the total cross-section, the sapwood proportion of the total area and the sapwood width (SWW) at 150 mm above ground level in cross-sections of *S. spicatum* and *S. album***

Variable	*S. spicatum*		*S. album*	
SW area (cm^2)	26.0	(11.4)	257.5	(126.2)
HW area (cm^2)	115.9	(66.8)	174.1	(102.1)
Total area (cm^2)	142.0	(74.4)	436.9	(157.0)
SW area (%)	19.0	(8.9)	58.8	(17.9)
SWW (mm)	6.5	(0.4)	45.7	(24.6)

The standard deviation is in parentheses.

Table 2 **Comparison of the mean number growth increments (GI) in the sapwood (SW), heartwood (HW) and total cross-section and the proportion of the total GI that are in the SW at 150 mm above ground level in cross-sections of *S. spicatum* and *S. album***

Variable	*S. spicatum*		*S. album*	
SW GI	11.7	(3.7)	23.6	(7.0)
HW GI	79.4	(24.9)	26.1	(12.7)
Total GI	91.8	(24.6)	48.6	(10.6)
SW GI (%)	13.5	(5.4)	48.2	(14.9)

The standard deviation is in parentheses.

heartwood extends and the heartwood taper.

Growth increments

The mean total number of growth increments was much higher in *S. spicatum* than in *S. album*, and both the number of growth increments and the proportion of the growth rings in the sapwood were much higher in the *S. album* than *S. spicatum* cross-sections (Table 2).

Tree age at heartwood initiation

Assuming that the number of growth increments in the sapwood at 150 mm above ground level is equivalent to the age of heartwood initiation, in *S. spicatum*, the age at which heartwood was initiated ranged from 4 to 22 years with a mean of 11.7 years (Table 2). In the *S. album* trees that contained heartwood, the number of growth increments in the sapwood varied from 14 to 46 with a mean of 23.6.

Rate of heartwood formation

No relationship was found between the number of growth increments in the sapwood and either the rate of growth or tree age. Therefore, it was assumed that once heartwood was initiated, it increased at the rate of one growth ring per year.

Conclusions

It is unlikely that the estimates of the age at which heartwood was initiated were accurate since the number of growth rings in sandalwood are known to either under or over-estimate tree age by 53% (Chowdhury and Ghosh, 1949). It was concluded that the data collected in this study were not appropriate to estimate either the age at which heartwood was initiated or the rate of heartwood formation.

To answer many of the questions that relate to age, especially the age at which heartwood is initiated and the rate of heartwood formation, trees of equal and known age (and genotype) should be compared on a range of sites over time.

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Introduction and Overview of *Santalum* Research in Kununurra, Western Australia

Andrew M Radomiljac

Andrew Radomiljac is a Research Officer with the Department of Conservation and Land Management involved with silvicultural and tree breeding research of Santalum album. Kununurra, Western Australia. This position is jointly funded by the Australian Centre for International Agricultural Research (ACIAR), the Sandalwood Research Institute (SRI) and the Sandalwood Conservation and Regeneration Program (SCARP).

Introduction

This paper outlines the background and present status of *Santalum album* and other *Santalum* species research within the Ord River Irrigation Area (ORIA), Kununurra, Western Australia (Figure 1.). *Santalum* species research occurs within a flood irrigation scheme, consequently allowing for the irrigation of *S. album* plantations.

Santalum species research is categorised into two components:

i. development of *S. album* nursery propagation and silvicultural techniques.

ii. germ plasm conservation of threatened and endangered *Santalum* species.

Background

Exploitation of Asian, Australian and Pacific island *Santalum* species has resulted in a severe decline of the *Santalum* species population. A strong demand exists for sandalwood, either for joss stick manufacturing or for the perfumery industry. Without the advent of an artificial sandalwood oil substitute (McKinnell 1990) and continual exploitation the differential between the supply and demand for sandalwood products will increase.

Australia contains 6 of the 29 *Santalum* species (Applegate and McKinnell 1993). One of the 6 species, *S album,* occurs along the northern extremities of the Australian coastline (Figure 1). This species is an exotic. Introduced by Indonesian fisherman or traders.

Of the native *Santalum* species only *S. spicatum,* occurring predominantly in Western Australia (Figure 1.), is considered to be of significant commercial value. Average heartwood oil content of *S. spicatum* is 2 percent (McKinnell 1990) and it is harvested for the production of joss sticks only.

Exploitation of *S. spicatum* has placed pressure on this species population. A proportion of remaining *S. spicatum* occurs in nature reserves and is removed from the harvestable population. Natural regeneration is low due to fire and grazing. Long rotation lengths, 50 - 100 years, dispels the possibility of establishing commercially viable *S. spicatum* plantations.

S. album plantations within the ORIA is perceived to release exploitation pressures from *S. spicatum.*

Climate

The ORIA climate (Table 1.) is tropical with monsoonal rains occurring during a four month period, December to March.

Soils

The most predominant soil type conducive to flood irrigation in the ORIA is a hard setting, self mulching clay soil, Cununurra clay.

Two other soil types exist, a sand and levee type soil. These two soil types have limited application to *S. album* plantations due to their inappropriateness for flood irrigation and the presence of *Mastotermes darwiniensis.*

Site preparation

Plantation establishment occurs on cleared agricultural land. Site preparation parallels intensive agriculture techniques. Intensive site preparation is required for two reasons:

i) flood irrigation requires the planting site to be levelled prior to planting bed and the irrigation furrow formation.

ii) planting beds, due to the Cununurra clay's hard setting nature, require a fine tilth. This is achieved by intensive cultivation.

Research developments

The first significant *S. album* planting at Kununurra occurred in 1986 where 180 seedlings were planted. Seed being of Indian origin. This planting is sexually mature and supplies seed for research purposes.

Two *S. album* research trials were established in 1987 (McComb unpublished). Firstly, a site selection trial, concluding the appropriateness of the Cununurra clay soil for *S. album* plantations. Secondly, a

Table 1 Climatic data of the Ord River Irrigation Area

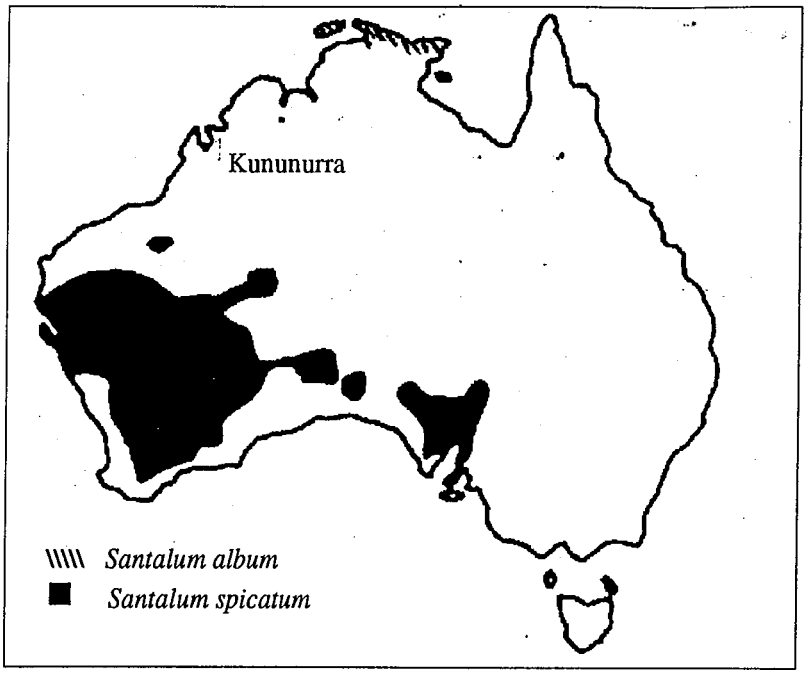
Rainfall (mm)	Mean Max Temp (C)	Mean Temp (C)	Mean Min Temp (C)	9am RH (%)	Evaporation (mm)
745	35.1	27.7	20.5	43	272

host selection trial, concluding *S. album's* host preference is positively correlated to the hosts' ability to fix nitrogen.

Favourable *S. album* host species include *Sesbania formosa, Acacia coriacea, Cassia siamea, Dalbergia sisso, Acacia trachycarpa* and *Acacia ampliceps.* Poor *S. album* host species are characteristic of their inability to fix nitrogen. Poor *S. album* hosts species include *Anacardium occidentale, Eucalypt spp, Gmelina arborea* and *Cordia sebstana*

A low level of quantitative research has been conducted on nursery propagation of *S. album.* Two areas of the nursery propagation that have been investigated include seedling potting mixture and preliminary pot host selection.

Figure 1 Distribution of *Santalum spicatum* and *S. album* in Australia



(Modified from Hewson and George 1984)

Present research status

Santalum species research in the ORIA is relatively new. Quantitative *S. album* research commenced in 1993 with the appointment of a research officer.

Areas identified of high priority for nursery propagation and silvicultural research include seed handling and storage, seedling nutrition, pot and field host selection, weed control, irrigation regimes and heartwood formation studies.

Germ plasm conservation research of *Santalum* species include provenance trials of *S. austrocaledonicum* and progeny trials of *S. album.* It is perceived that a larger representation of the *Santalum* species will be included into this research component. Germ plasm conservation is an important ACIAR research component as most *Santalum* species are threatened by extinction.

Forth coming Sandalwood Research Newsletter issues will summarise methodologies, results and conclusions of *S. album* and other *Santalum* species research conducted within the ORIA, Kununurra.

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The Sandalwood Research Newsletter invites all readers to write to the article authors to acquire additional information concerning *Santalum* species research and/or management work outlined in these articles. An aim of the SRN is to promote and increase the level of liaison between researchers and managers of *Santalum* species.

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ACIAR PROCEEDINGS OF SANDALWOOD SYMPOSIUM AVAILABLE

The Australian Centre for International Agricultural Research has published the proceedings of a symposium on sandalwood held at the XVII Pacific Science Congress in Hawaii, in June 1991. Edited by F H McKinnell, the proceedings contains papers from Australia, Indonesia, Vanuatu, Fiji, New Caledonia, French Polynesia and Hawaii. It is entitled Sandalwood in the Pacific Region, ACIAR Proceedings No. 49, and the publication is available from:

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