Sandalwood Research Newsletter

November 2011 | Issue 26 | ISSN 1321-0022X

EDITOR'S NOTE

It is certainly an interesting time to be involved in sandalwood research and development. A growing number of people are now engaged in establishing resources and initiatives to ensure natural sandalwood products will be available in the coming generations. The papers contained within this issue of the Sandalwood Research Newsletter demonstrate this diversity of activity. With growing awareness of sandalwood plantations in China our first paper reveals the details of their current success in these endeavours. The growth and heartwood development achieved in these young plantings are very encouraging for the establishment of an alternative source for sandalwood products in the future. Our second paper outlines the recent establishment of an industry-first International Sandalwood Foundation. This non-profit organisation has a number of programs aimed at supporting sandalwood research, development and conservation. Our participation in The Foundation can help the industry better communicate within and between sectors and with consumers and the public

Tony Page

Preliminary analysis of growth and oil composition from a 6-year-old sandal (*Santalum album* L.) plantation in Gaoyao, Guangdong, south China.

Liu Xiaojin[1] Xu Daping[1*] Yang Zengjiang[1] Zhang Ningnan[1] Yang Lijun[2]
[1]Research Institute of Tropical Forestry, Chinese Academy of Forestry, Guangzhou, Guangdong 510520, China.
[2]Dragon Pearl Island Sandalwood Industrial Technology Corporation Ltd, Gaoyao, Guangdong 526113, China

Abstract

The commercial value of sandalwood lies within its heartwood oil. Heartwood development in planted sandalwood is therefore important to ensure the commercial success of such ventures. With only recent experience in planting sandalwood in China, it is important to quantify heartwood development in the young estate. In this study we examine the growth, heartwood presence and oil composition in a 6-year-old sandal plantation located in Gaoyao, Guangdong province, south China using a sample survey method. The results demonstrated an encouraging growth rate, including an average height of 5.26m, diameter at breast height (DBH) of 8.70cm and diameter at ground level (DGL) of 11.60cm. Heartwood development was recorded in 20% of trees sampled and oil concentration in these trees ranged from 0.64% to 1.78%. The level of total santalols was 32.1%-39.2%, which does not meet with the quality of international standard sandalwood oil, these levels are however encouraging for such young trees.

Figure 1. Six-year-old sandal plantation at D.P.I. Gaoyao, Guangdong Province

Introduction

Santalum album L. (sandal) is an evergreen root hemi-parasite tree species of great economic value. It is renowned for its scented heartwood which contains essential oil that has wide applications in fragrances, costumes, carvings, medicines as well as other products (Srinivasan *et al.* 1992). *S. album* was first introduced to mainland China in 1962 from Indonesia, and then was cultivated sporadically in south China during 1980's (Li 2003; Ma and Bunn 2007). Due to

a shortage of funds and lacking a set of effective cultivation technology, establishment of large-scale sandal plantations failed during that time. The development of cultivation techniques and policy support from State Forestry Administration (SFA) of China, combined to resolve these issues and sandal cultivation commenced in 2004 over a large area in south China (Liu *et al.* 2009; Liu *et al.* 2010a; Liu *et al.* 2010b).

While these developments are encouraging, more information is required on heartwood formation, oil content and oil composition, because the value of sandalwood at harvest will depend largely on the volume of heartwood, oil concentration and santalol percentage within the tree (Doran *et al.* 2005; Jones *et al.* 2006). In India, sandalwood generally formed heartwood at the age of 10-13 years (Rai 1990), but results from a study carried out in western Australia on a 14-year old sandal plantation showed that 25% of the individuals did not form heartwood (Brand *et al.* 2006), and approximately 14% of trees had not formed heartwood by the age of 20 years from a field survey in Bangalore, India (Arunkumar *et al.* 2011).

The aim of this study is to provide information on growth, heartwood formation, oil content and oil composition in a 6-year old sandal plantation in Guangdong province of south China.

Methods

Site description

Field survey was conducted in a sandalwood plantation of Dragon Pearl Island Sandalwood Industrial Technology Corporation Limited (D.P.I.), which located in Gaoyao, Guangdong province, south China (22°54′ N, 112°36′ E). The plantation which we investigated was established in April, 2004 in a laterite soil type with a sandalwood spacing of 3×3 m (Figure 1). The main hosts were *Caesalpinia sappan* (Caesalpiniaceae), *Clausena lansium* (Rutaceae), *Ligustrum lucidum* (Oleaceae), *Murraya exotica* (Rutaceae), one of which were planted between each 2 sandalwoods also with a spacing of 3×3m respectively.

Sampling method

Tree height, clear bole height, diameter at ground level (DGL), diameter at breast height (DBH) and crown width across north to south of the trees in the above sandal plantation were measured in October 2010. Generally the sandal crowns were symmetrical, so measuring in this fixed direction was considered to accurately represent the whole crown. A subsample of 20 trees were selected randomly for determining heartwood presence and oil quality.

Figure 2. Wood core sample extraction from a 6-year old sandal at 30cm above ground level using an increment borer.

The presence of heartwood was determined through a core sampling method according to Jones and Plummer (2007). Wood cores were taken using an increment borer (Figure 2) at 30cm and 100cm above ground level. The percentage of heartwood was determined roughly as the square of the ratio of heartwood width/total wood width at 30cm height above ground level. The samples were used to determine oil content as well as oil composition.

Oil distillation and analysis

Sapwood was removed from the cores, and the remaining heartwood was ground into fine particles. Oil was extracted from the powder using solvent (diethyl ether) extraction method. Oil content was determined by dry weight and oil composition was determined by gas chromatography-mass spectra (GC-MS) according to the methodology of Jones *et al.* (2007) and Howes *et al.* (2004).

The GC-MS analysis was performed on a Finnigan TRACE GC-2000-MSTM (USA) instrument equipped with a DB-5 column (Agilent, USA, 30m length, 0.25mm inside diameter and 0.25μm film thickness). Injection port temperature was 220℃ and oven initial temperature was 45℃. Oven temperature was ramped from 45℃ at 3℃/min to 220℃ and held for 5 min (Total run time 63 min), carrier gas was helium with a flow rate of 1.0ml/min and no split was used. All oils were diluted to 1.0% (v/v) with diethyl ether prior to analysis. Total injection volume was 1μl. The Mass Spectra were fitted with an EI source operated at 70 eV with a source temperature of 200℃, mass spectra were recorded in the range m/z 35-335amu at 1 scan/0.75s. Each compound (peak) was identified either from retrieving the NIST standard mass spectrometry library or by comparing retention indices and/or mass spectra with published data (Howes *et al.* 2004; Jones *et al.* 2006; Sciarrone *et al.* 2011; Shellie *et al.* 2004; Verghese *et al.* 1990). Relative content of each compound was estimated according to the relative area through an area normalization method.

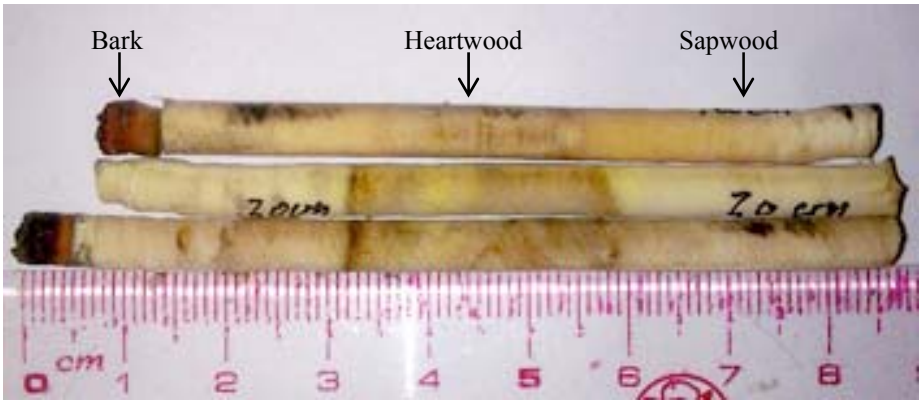

Figure 3. Cores taken from young sandal at different heights within a tree (lower core at 15cm, middle at 30cm and upper at 100cm)

Indices	Mean	Standard deviation	Maximum	Minimum
Height (m)	5.26	0.53	6.50	4.40
DBH (cm)	8.70	0.75	10.0	7.50
DGL (cm)	11.60	1.00	13.30	9.40
Clear bole height (m)	2.10	0.26	2.50	1.80
Crown width (m)	2.77	0.50	3.40	1.95

Table 1. Growth indices of 6-year old sandal plantation in D.P.I, Guangdong, China

Results

Tree Development

The morphology of the 6-year old sandal plantation planted in D.P.I, Gaoyao in Guangdong province of south China was presented in Table 1.

Heartwood formation and oil content

In the sampled 20 trees, 4 trees were found to have visible heartwood at the height of 30cm above ground, of which, one tree was detected to have heartwood at 100cm height above ground. Heartwood can be easily distinguished from sapwood because of its darker colour (Figure 3). Heartwood proportion on the sample cross-sections ranged from 8.52% to 15.67% in area (estimated by sample cores), oil content of these samples ranged from 0.64 to 1.78%.

Oil composition

Total ion chromatogram of sandal essential oil was shown in Figure 4, and 27 compounds were isolated, compound names and the corresponding relative content were listed in Table 2. From Table 2, we found that the main ingredients in sandalwood oil such as *α*-santalol, *β*-santalol, *α*-santalene, *β*-santalene, epi-*β*-santalol , *α*-*trans*-bergamotenol and teresantalol were detected in these young sandal trees (Jones and Plummer 2007; Sciarrone *et al.* 2011), but the relative content of each compound was low. Total santalol content ranged from 32.1% to 39.2%.

Discussion

Compared to earlier more sporadically planted sandalwood in Guangdong province (Huang *et al.* 1989; Li 2003), these more recent, larger-scale plantings exhibit a better growth performance (including height and diameter growth, data not shown). A total of 20% of sampled trees have formed heartwood naturally, this ratio is very encouraging in the 6-year old plantation, which may indicate that sandal can not only planted in large scale in China, but also show an inspiring growth performance and a high ratio of forming heartwood at an early age (6-year-old have started to form heartwood naturally). This phenomenon may have something to do with improved cultivation techniques such as site management, host configuration and management, fertilization, irrigation, weed control etc.

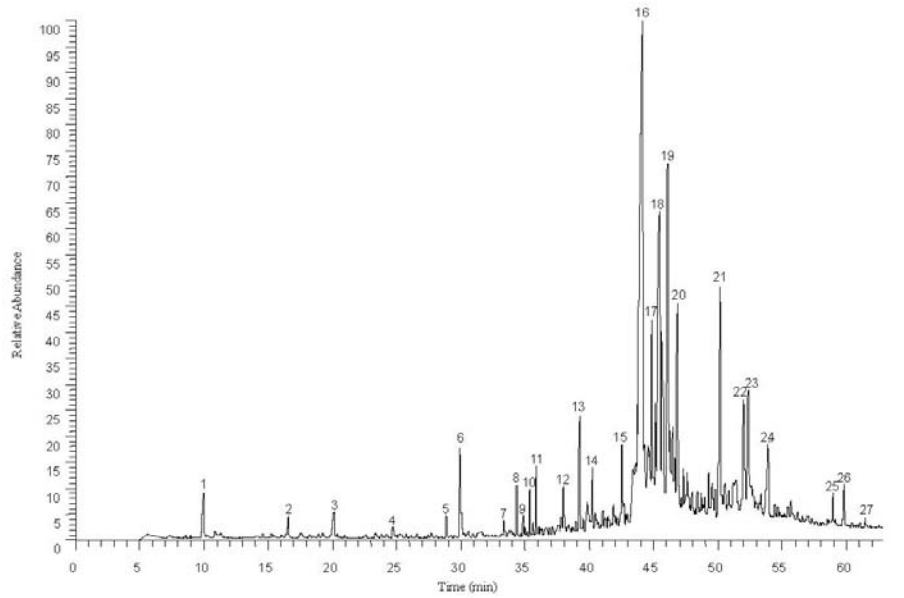

Figure 4. Total ion chromatogram of essential oil extracted from 6-year-old young sandal. Full descriptions for each numbered compound are on Table 2 (Page 4).

Although oils extracted from these young sandal trees contains the main ingredients of sandalwood oil, the content were low compared with 14-year and 15-year old trees planted in Australia (Brand *et al.* 2006; Brand *et al.* 2007) or mature trees planted in India (Jayappa *et al.* 1981). The *α*-santalol was 15.2%-22.9%. and *β*-santalol was 8.89%-12.46%, these have not meet the current ISO standards for *S. album* oil, which are 41-55% of *α*-santalol and 16-24% of *β*-santalol (ISO 2002). Apart from the main ingredients of sandalwood oil, many other hydrocarbons or carbohydrates such as eicosane, hexadecanoic acid, octadecanoic acid were isolated from the heartwood (Table 2), these compounds are possibly servicing as a precursor for sandalwood oil synthesis. As the growth and development progresses, these compounds may convert to santalols under the control of secondary metabolism or other metabolic path, so the content of santalols can accumulate gradually.

These results were encouraging, and provided an evidence that young sandal plantations grown in south China started to produce heartwood or essential oil as early as age 6 years. Although heartwood formation and oil accumulation are two independent processes (Arunkumar *et al.* 2011), we still should pay great attention on studying the mechanism of heartwood or oil formation. By doing this, we may develop an efficient technology to speed or accelerate the heartwood formation or oil accumulation.

Acknowledgements

This study was sponsored by a 948 introduction project (2008-04-02) from State Forestry Administration of China and a technology innovation special project from Forest bureau of Guangdong province (2009KJCX002-01). Staffs in Dragon Pearl Island are gratefully acknowledged for helping with the field survey and measurement.

References

Arunkumar AN, Srinivasa YB, Joshi G and Seetharam A (2011) Variability

Peak No.	Retention (minutes)	Compound Name	Molecular Formula	Relative Content (%)	Similarity (%)
1	10.31	2-Methoxy-1,3-dioxolane	$C_4H_8O_3$	0.51	81
2	16.63	Glycerol	$C_3H_8O_3$	0.31	84
3	20.17	Unidentified	NA	0.48	NA
4	24.72	Epi-*β*-santalol	$C_{15}H_{24}O$	0.18	83
5	28.91	Eicosane	$C_{20}H_{42}$	0.24	85
6	29.95	*trans*-2-isopropylbicylclo[4.3.0] non-3-ene-8-one	$C_{12}H_{18}O$	1.41	85
7	33.36	Dodecanal	$C_{12}H_{24}O$	0.22	94
8	34.35	*α*-Santalene	$C_{15}H_{24}$	0.51	91
9	34.88	Teresantalol	$C_{10}H_{16}O$	0.21	94
10	35.36	Epi-*β*-Santalene	$C_{12}H_{24}$	0.46	92
11	35.88	*β*-Santalene	$C_{15}H_{24}$	0.69	93
12	37.88	Unidentified	NA	0.49	NA
13	39.26	Bicyclogermacrene	$C_{15}H_{24}$	1.45	78
14	40.26	4-Ethenyl-4-methyl-1-(propan-2-yl)-3-(prop-1-en-2-yl) cyclohex-ene	$C_{15}H_{24}$	0.65	77
15	42.53	Bicyclo[2.2.1]heptane-7-bicyclo [2.2.1]hept-7-ylidene	$C_{14}H_{20}$	1.12	76
16	43.95	*α*-Santalol	$C_{15}H_{24}O$	18.62	92
17	44.54	*α*- *trans*-Bergamotenol	$C_{15}H_{24}O$	2.38	90
18	45.45	*β*-Santalol	$C_{15}H_{24}O$	9.82	89
19	46.09	*cis*-Lanceol	$C_{15}H_{24}O$	5.69	94
20	46.86	Unidentified	$C_{15}H_{24}O$	1.54	NA
21	50.18	Unidentified	$C_{15}H_{24}O$	3.69	NA
22	52.01	*cis*-(Z)-*α*-bisabolene eoxide	$C_{15}H_{24}O$	2.16	78
23	52.37	7-Oxabicyclo[4.1.0]heptane-2,2,6-trimethyl-1-(3-methyl-1,3-butadienyl)-5-methylene	$C_{15}H_{24}O$	1.57	80
24	53.92	Hexadecanoic acid	$C_{16}H_{32}O_2$	1.09	83
25	58.99	9-Octadecenoic acid	$C_{18}H_{34}O_2$	0.35	92
26	59.84	Octadecanoic acid	$C_{18}H_{36}O_2$	0.64	88
27	61.44	n-Docosane	$C_{22}H_{46}$	0.12	85

Table 2. Composition of sandal essential oil extracted from 6-year-old young sandal in south China

in and relation between tree growth, heartwood and oil content in sandalwood (*Santalum album* L.). *Current Science* 100, 827-830.

Brand J, Kimber P and Streatfield J (2006) Preliminary analysis of Indian sandalwood (*Santalum album* L.) oil from a 14-year-old plantation at Kununurra, Western Australia. *Sandalwood Research Newsletter* 21, 1 -3.

Brand JE, Fox JED, Pronk G and Cornwell C (2007) Comparison of oil concentration and oil quality from *Santalum spicatum* and *S. album* plantations, 8-25 years old, with those from mature S. *spicatum* natural stands. *Australian Forestry* 70, 235.

Doran J, Thomson L, Brophy J, Goldsack B, Bulai P, Faka'osi T and Mokoia T (2005) Variation in heartwood oil compositon of young sandalwood trees in the South Pacific (*Santalum yasi*, *S. album* and F1 hybrids in Fiji, and *S. yasi* in Tonga and Niue). *Sandalwood Research Newsletter* 20, 3-7.

Howes M-JR, Simmonds MJ and Kite G, C (2004) Evaluation of the quality of sandalwood essential oils by gas chromatography-mass spectrometry. *Journal of Chromatography A* 1028, 307-312.

Huang B, Feng MK, Chen SR and Liu LH (1989) Growth analysis of sandal plantation in Guangdong, China. *Chinese Herbal Medicines* 12, 7-11 (in Chinese).

ISO (2002) 'Oil of sandalwood (*Santalum album* L.) II Edition.' (Geneva, Switzerland: 3518:2002 (E), 2002-03-01).

Jayappa V, Nataraj BM, Shanbang PK, Patil KB and Srinivas A (1981) Regional variation in the yield and quality of sandalwood oil-a case study by GlC. *Pafai Journal* October-December, 27-31.

Jones CG, Ghisalberti EL, Plummer JA and Barbour EL (2006) Quantitative co-occurrence of sesquiterpenes; a tool for elucidating their biosynthesis in Indian sandalwood, *Santalum album*. *Phytochemistry* 67, 2463-2468.

Jones CG and Plummer JA (2007) Non-destructive sampling of Indian sandalwood (*Santalum album* L.) for oil content and composition. *Journal of Essential Oil Research* 19, 157-164.

Li YL (2003) 'Introduction Research of Sandalwood (*Santalum album* L.).' (Science Press (in Chinese): Beijing).

Liu XJ, Xu DP, Xie ZS and Zhang NN (2009) Effects of different culture media on the growth of Indian Sandalwood (*Santalum album* L.) seedlings in Zhanjiang, Guangdong, southern China. *Forestry Studies in China* 11, 132-138.

Liu XJ, Xu DP, Zhang NN, Xie ZS and Chen HF (2010a) Effects of gibberellin on seed germination and seedling growth of sandalwood (*Santalum album* L.) *Seed* 29, 71-74 (in Chinese).

Liu XJ, Xu DP, Zhang NN, Xie ZS and Chen HF (2010b) Effects of pot host configuration on the growth of Indian sandalwood (*Santalum album*) seed-

lings in south China. *Forest Research* 23, 924-927 (in Chinese).
Ma GH and Bunn E (2007) Embryology and pollination trials support dichogamy in *Santalum album* L. *Sandalwood Research Newsletter* 22, 1-4.
Rai SN (1990) Status and cultivation of sandalwood in India. In 'Proceedings of the Symposium on Sandalwood in the Pacific'. Honolulu, Hawaii. (Eds L Hamilton and CE Conrad) pp. 66-71. (US Forestry Service General Technical Paper PSW-122).
Sciarrone D, Costa R, *et al.* (2011) Application of a multidemensional gas chromatography system with simultaneous mass spectrometric and flame ionization detection to the analysis of sandalwood oil. *Journal of Chromatography A* 1218, 137-142.
Shellie R, Marriott P and Morrison P (2004) Comprehensive two-dimensional gas chromatography with flame ionization and time-of-flight mass spectrometry detection: qualitative and quantitative analysis of West Australian sandalwood oil. . *Journal of Chromatographic Science* 42, 417-422.
Srinivasan VV, Sivaramakrishnan VR, Rangaswamy CR, Ananthapadmanbha HS and Shankaranarayana KH (1992) 'Sandal (*Santalum album* L.).' (Institute of Wood Science and Technology: Bangalore, India).
Verghese J, Sunny TP and Balakrishnan KV (1990) (+)- a-Santalol and (-)-P-Santalol(z) concentration, a new quality determinant of east Indian sandalwood oil. *Flavour and Fragrance Journal* 5, 223-226.

The International Sandalwood Foundation: A Non-Profit Organization Dedicated to Scientific Research and Sustainable Harvesting of Sandalwood

Danica T. Harbaugh-Reynaud
Executive Director, International Sandalwood Foundation.
P.O. Box 2924 El Cerrito, CA 94530, USA

Introduction

Like many of you, I've devoted much of my adult life to researching- and falling in love with- sandalwoods. There is an undeniable attraction to this culturally and ecologically important plant. Revered by millions, if not billions, of people throughout the world for its ethereal scent, sandalwood is one of the most valuable plants on earth. Its parasitic nature, requiring it to rely on its neighboring hosts for life and support, has inspired many to connect to sandalwoods in a spiritual way. Few plants on this earth have this heavenly status, and the demand for its golden oil (now as precious as gold) has had dramatic affects on its ability to survive and thrive in the wild. In fact, at least one species has gone extinct and several others are considered rare or endangered. In India, where it is an integral part of cultural and spiritual traditions, sandalwood is now banned from export in its raw form to help slow the impact on the declining populations, and now similar laws are being drafted in other regions.

For those of us that are interested in and devoted to sandalwood, there has never been a better time to focus on understanding this unique plant and how we can conserve it for future generations. Over the past decade, I've had the opportunity to meet and learn from many of you who are dedicated to growing, harvesting, conserving, and researching sandalwoods throughout the world. Throughout my experience, and reading through the almost 20 years of *Sandalwood Research Newsletter* issues, one thing that has become clear is: whether we are dedicated to scientific pursuits or in turning a profit, we all have many common goals- understanding how sandalwoods grow and thrive, determining why there is variability from tree to tree or island to island, and establishing the best practices for sustainable harvest.

Foundation Establishment

To support the activities of sandalwood scientists and foster their communication with those in the sandalwood trade and the general public, I have created the International Sandalwood Foundation, a charitable nonprofit organization based in California, USA. The Foundation is unique in that it represents not only the interests of scientists and conservationists, but also responsible harvesters, growers and manufacturers. The hope is that by bringing together individuals and organizations from these many "angles" so to speak, we not only help guide our research initiatives, but also provide the wealth of knowledge and expertise that so many sandalwood researchers around the world can provide to those involved in the sandalwood trade.

Foundation Aim

Our ultimate goal is to promote sandalwood research and conservation, while at the same time promoting economic development through responsible and sustainable harvest. The Foundation's directors, advisors, and members represent not only some of the top sandalwood researchers in the world, but other sandalwood experts, growers, landowners, and representatives from major natural products companies.

Programs

Through its novel and ambitious three core programs, Sustainable Sandalwood™, SandalGeneBank™, and SandalSeedBank™ the International Sandalwood Foundation aims to ensure that sandalwood sold on the market today has originated from sustainable sources, increasing its marketability and value, and to guarantee that seeds and genetic resources are preserved for future generations. The SandalGeneBank™ is a novel and comprehensive database of sandalwood genetic data from sandalwoods around the world, which will allow us to assess the genetic diversity, population sizes, and taxonomy of sandalwoods worldwide. And lastly, in partnership with member individuals and organizations, the SandalSeedBank™ is a collection of seeds from sandalwood populations for the preservation of genetic resources of existing wild populations, as well as for developing nurseries for out-planting and re-introduction programs.

Outreach

Other activities of the Foundation include educational workshops and discussion forums, in person and online, to bridge-the-gap between individual and corporate members and the public, to help bring awareness of the research and conservation issues facing sandalwood today. The International Sandalwood Foundation, along with several co-sponsors (TBA) will be holding a Symposium on Sandalwood in the Pacific and Asia at the East West Center in Honolulu, HI in October 2012 (dates TBA); this symposium is open to all members of the Foundation and will include several days of scientific workshops, presentations, and field-trips focused on sandalwood. Additionally, we are currently developing a members-only webpage for announcements and advertisements, and publishing a quarterly newsletter with scientific and popular articles, literature reviews, and other timely matters related to sandalwood and the sandalwood trade. For more information, to share an idea, to participate in one of our programs, or to become a member, please visit the Foundation's website at www.sandalwoodfoundation.org or email me at Danica@sandalwoodfoundation.org. This organization is dedicated to representing you and your interests, so I look forward to hearing from you!

Corresponding Authors:

Xu Daping
Research Institute of Tropical Forestry, Chinese Academy of Forestry,
No.682 GuangShan Yi Rd, Tianhe District
Guangzhou, Guangdong 510520, P.R.China
Tel: + 86-20-8703 3626
Fax: + 86-20-8703 1622
E-mail: gzfsrd@163.com

Danica Harbaugh-Reynaud
International Sandalwood Foundation,
P.O. Box 2924 El Cerrito, CA 94530, USA
Tel: + 1-510-965-5310
Fax: + 1-510-590-3330
Email: danica@sandalwoodfoundation.org
Web: www.sandalwoodfoundation.org

If you would like to contribute an article to the SRN or wish to be included on the SRN email alerts, please contact the editor .

Editor: Tony Page
James Cook University, PO Box 6811,Cairns, 4870 Australia
Telephone: + 61 7 4042 1673, Facsimile: + 61 7 4042 1319
Email: tony.page@jcu.edu.au

Back issues of the SRN and individual articles can be downloaded from the SRN website: http://www.jcu.edu.au/mbil/srn/index.html

The Sandalwood Research Newsletter is published by James Cook University, Queensland Australia.