SANTALUM ALBUM L. PLANTATIONS:

A COMPLEX INTERACTION BETWEEN PARASITE AND HOST

This thesis is presented for the degree of Doctor of Philosophy of Murdoch University

1998

Submitted by

Andrew M. Radomiljac

Bachelor of Science (Forestry), Australian National University
I declare that this thesis is my own account of my research and contains as its main content work, which has not previously been submitted for a degree of any tertiary education institution.

Andrew M. Radomiljac


**ABSTRACT**

This thesis examines a broad spectrum of physiological and silvicultural features of the highly valued woody angiosperm hemi-parasite *Santalum album* L. (Indian sandalwood) in relation to its culture in plantations in northern Western Australia. Topics covered include allometry of host and *Santalum* when grown as single plant pairings in both field and pot culture, nutritional interactions between *Santalum* and beneficial and non-beneficial hosts, deleterious influences of parasitism on plantation productivity and heartwood induction in young trees.

In Western Australia sandalwood is grown in the nursery for 8 months before establishment in the field and during this time a pot host is introduced. Survival of *Santalum* after field establishment and its subsequent growth were significantly affected by the time of introduction of the pot host, *Alternanthera nana*. Increasing the period of the *Santalum : Alternanthera* association in the nursery to 109 days prior to field establishment markedly increased early growth of *Santalum* plantations. Introduction at 134 days prior to field establishment was detrimental to the parasite as the *Alternanthera* was too vigorous for the small *Santalum* seedlings. *Santalum* plants had a lower root : shoot ratio lower when cultured with *Alternanthera* in the nursery prior to field establishment compared with seedlings grown without *Alternanthera*. *Alternanthera* survival in the field was high when it had been grown with *Santalum* for 12 weeks or more in the nursery prior to field establishment. After 11 weeks in the field a strong negative linear relationship was shown between *Santalum* root : shoot ratio and *Alternanthera* dry weight, and a positive linear relationship between *Santalum* DW and *Alternanthera* DW.

In Western Australia *Santalum* is established in the field with an intermediate host which nourishes the parasite for 3-5 years before *Santalum* becomes dependent on its long-term host and the intermediate host dies. The relationship between *Santalum* and several species tested as intermediate hosts was examined by pairing *Santalum* seedlings with intermediate host seedlings in 25 litre pots over a 10 month period. Growth of *Santalum* in pot culture with three N\textsubscript{2}-fixing woody intermediate hosts (*Sesbania formosa, Acacia trachycarpa* and *A. ampliceps*), the woody non N\textsubscript{2}-fixing *Eucalyptus camaldulensis* or without a host varied considerably between host treatments. *Santalum* growth was greater and root : shoot ratio lower for seedlings grown with N\textsubscript{2}-fixing hosts compared with seedlings grown with *E. camaldulensis* or with no host. The root : shoot ratio of unattached *Santalum* increased exponentially over time,
whereas for all other treatments it remained relatively constant. An assessment of the value of the hosts, termed host use efficiency, was computed as *Santalum* shoot DW / host shoot DW. The host use efficiency of *A. trachycarpa* was greater than that of the other hosts.

The xylem sap of hosts and *Santalum*, and ethanolic extracts of endophytic tissue of haustoria of *Santalum* were analysed for amino acids, organic acids and sugars to determine which solutes were available in the host and which were extracted by the *Santalum* haustoria from different hosts. There were similarities between *Santalum* and legume hosts in concentration and composition of xylem sap amino acids, and in the amino acid spectra of the corresponding *Santalum* endophytic tissue, whereas there were low N levels in xylem sap of *E. camaldulensis* and dissimilarities between its amino acid composition and that of *Santalum*. This indicated substantial direct intake of xylem N by *Santalum* from legume hosts but little N from the xylem sap of *E. camaldulensis*. There were high concentrations of asparagine, glutamate, aspartate and γ-amino glutamate in the xylem sap of the legume hosts, while in the non-legume the most common amino acids were glutamate, aspartate, glutamine and arginine. Proline, the predominant amino acid in the xylem sap of *Santalum acuminatum* growing in natural vegetation (Tennakoon et al. 1997) was not detected or present in very low concentrations in *Santalum album* under these conditions. in the non-legume. Xylem sap of hosts contained variable amounts of sugars (sucrose, glucose and fructose) and organic acids (fumaric, citric and malic acid), whereas that of the parasitic *Santalum* was dominated by fructose and malic acid. Dissimilarities in the proportional amounts of xylem-borne sugars and organic acids were particularly evident for the *E. camaldulensis* : *Santalum* partnership.

Diurnal profiles of photosynthesis and transpiration of *Santalum* were closely similar to those for corresponding hosts, whereas the midday leaf water potential of *Santalum* was consistently more negative than that of corresponding hosts. Net photosynthesis and water use efficiency was lower, but transpiration rates were similar to that of corresponding hosts. Nitrogen concentrations of foliage of *Santalum* were higher than their hosts, and higher when on legume hosts than on *E. camaldulensis*, or without a host. Nitrogen concentrations of *Santalum* foliage was strongly correlated with net photosynthesis and water use efficiency of *Santalum*. $\delta^{13}$C values of shoot dry matter of *Santalum* were poorly correlated with instantaneous water use efficiency of *Santalum*. Tissue water relations of *Santalum* were similar to that of water-stress tolerant species.

*S. formosa* proved the best host followed by *Acacia ampliceps* and *A. trachycarpa* based on dry matter gains of *Santalum*. Estimates of heterotrophic gain of C of *Santalum* when grown in association with the legume hosts over a nine week period indicate 57.9% of C was derived
from *A. ampliceps*, 45.5% from *A. trachycarpa* and 34.6% from *S. formosa*. Abundance of haustorial attachments on roots of hosts was poorly correlated to *Santalum* shoot DW. Root nodules of legume hosts were parasitised by a small proportion of *Santalum* haustoria.

Sodium and phosphorus concentrations of foliage of *Santalum* were generally higher than that of corresponding hosts. Net gains of calcium, potassium, phosphorus and sodium in *Santalum* was greatest when grown in association with hosts richest in the corresponding element. Net losses or only small gains of calcium, potassium, phosphorus and sodium were recorded when *Santalum* was grown with *E. camaldulensis* or without a host suggesting that *Santalum* has limited ability for uptake of those minerals through its own root system.

To understand the effect of hosts on the productivity of a *Santalum* plantation a young plantation of *Santalum* with three host species *Cathormion umbellatum*, *Sesbania formosa* and *Acacia anuera* was selected to study the relationship between host quality and distance of hosts from *Santalum* on *Santalum* health. The selected plantation showed marked decline in health and vigour of both *Santalum* and hosts between years 3 and 5. Parameters of the host plants were assessed to select the best predictor of *Santalum* crown health. The height and diameter growth increment of *Santalum* between years 3 and 5 was strongly correlated to *Santalum* crown health. *Santalum* crown health and growth increased as host quality increased, and the distance of host from *Santalum* decreased. An index, which combined host quality and the distance of the host from that of *Santalum*, was a better predictor of *Santalum* crown health than host distance or quality alone.

The age at which heartwood is initiated in *Santalum album* under plantation conditions in Western Australia is unknown, but in natural stands in India it occurs between 10-13 years of age (Rai 1990). A field experiment was conducted to determine the efficacy of stem injections of paraquat and/or ethrel in initiating heartwood formation in five year old *Santalum* trees in a plantation. Trees injected with paraquat alone had a significantly greater extension of induced heartwood, both radially and vertically, than those trees injected with ethrel alone or distilled water. Eight months after treatment with paraquat or ethrel or a combination of these chemicals induced heartwood was formed, which had high lipid, and low starch and polysaccharide concentrations compared to the sapwood. Induced heartwood from both chemical treatments and their combinations contained total volatile oil and santalol oil (alpha and beta santalol) concentrations that were equal to or greater than that of naturally formed heartwood and greater than that of sapwood. Moisture content, and concentrations of K and Mg, and in some treatments Ca of induced heartwood were significantly lower than that of
sapwood.

The thesis concludes with a synthesis of the findings and suggestions for future research, with special reference to mid-rotation aspects of *Santalum* plantation silviculture.
# Table of Contents

Abstract ........................................................................................................................................ iii

Table of Contents .......................................................................................................................... vii

Acknowledgments ......................................................................................................................... xii

List of Publications ....................................................................................................................... xiii

Chapter 1 General Introduction .................................................................................................. 1

1.1 Background ............................................................................................................................. 1

1.2 The issue .................................................................................................................................. 2

1.3 Nomenclature of *S. album* ................................................................................................. 2

1.4 Botanical features ................................................................................................................... 3
   1.4.1 Habit ................................................................................................................................. 3
   1.4.2 Foliage ............................................................................................................................... 3
   1.4.3 Inflorescence, flowers and fruits ....................................................................................... 3
   1.4.4 Phenology ......................................................................................................................... 4

1.5 Geographic distribution .......................................................................................................... 4

1.6 Environmental amplitude ....................................................................................................... 5
   1.6.1 Climate .............................................................................................................................. 5
   1.6.2 Soil and physiography ...................................................................................................... 5

1.7 Physiology of *Santalum* and other root hemi-parasites ..................................................... 7

1.8 Cultural, commercial and religious importance of sandalwood ........................................ 9

1.9 Uses ......................................................................................................................................... 10
   1.9.1 Wood ............................................................................................................................... 10
   1.9.2 Oil, bark, leaves and fruit ............................................................................................... 10
   1.9.3 Land use, environmental and service aspects .................................................................. 11

1.10 Management and status of *Santalum* in natural stands ..................................................... 11

1.11 Silviculture and management ............................................................................................... 12
   1.11.1 Natural regeneration ...................................................................................................... 12
   1.11.2 Plantation silviculture .................................................................................................... 14
   1.11.3 Host selection for plantation culture ............................................................................. 17

1.12 Growth rate of *Santalum* in plantations and natural stands ............................................. 17

1.13 Heartwood formation in *Santalum* .................................................................................... 19

1.14 Heartwood heritability .......................................................................................................... 21

1.15 Formation of santalol oils ...................................................................................................... 22

1.16 Thesis objectives .................................................................................................................... 22
Chapter 5 Gas exchange and water relations of the root hemi-parasite Santalum album L. in association with legume and non-legume hosts. ........................................... 84

5.1. Abstract................................................................................................................. 84

5.3. Materials and methods ....................................................................................... 85
  5.3.1. Pot culture ....................................................................................................... 85
  5.3.2. Leaf gas exchange and leaf water potentials of Santalum and hosts ............... 86
  5.3.3. Nitrogen and chlorophyll contents of foliage .................................................. 87
  5.3.4. Carbon isotope discrimination ($\delta^{13}C$) of shoot dry matter ......................... 87
  5.3.5. Tissue water relations of Santalum ................................................................. 87

5.4. Results ................................................................................................................. 88
  5.4.1. Leaf nitrogen and chlorophyll contents .............................................................. 88
  5.4.2. Relationship between %N, Pn, Ei and WUE of parasite foliage ....................... 90
  5.4.3. $\delta^{13}C$ values for shoot dry matter and instantaneous WUE ......................... 90
  5.4.4. Matched diurnal profiles of $Pn$, $En$, WUE and $\psi$ for different host : parasite pairings .............................................................. 94
  5.4.5. Tissue water relations of Santalum on different hosts or without a host .......... 101

5.5. Discussion .............................................................................................................. 101

Chapter 6 Heterotrophic carbon gain and mineral nutrition of the root hemi-parasite Santalum album L. in pot culture with different hosts. ................................................. 107

6.1. Abstract................................................................................................................. 107

6.2. Introduction .......................................................................................................... 108

6.3. Materials and methods ....................................................................................... 109

6.4. Results ................................................................................................................. 111
  6.4.1. Haustorial numbers and sitings of attachments ................................................. 111
  6.4.2. C and N content of Santalum and parasitised and unparasitised hosts .......... 112
  6.4.3. Estimates of proportional heterotrophic gain (H) of C by Santalum on N2-fixing hosts .............................................................. 116
  6.4.4. Mineral nutrient distribution of parasite and host .............................................. 116
  6.4.5. Increments in dry weight, N, P, K, Ca and Na of Santalum on different hosts .... 127

6.5 Discussion .............................................................................................................. 127
Chapter 9 General discussion and synthesis .................................................. 182

References ........................................................................................................... 190

Appendices ......................................................................................................... 218
ACKNOWLEDGEMENTS

First and foremost I wish to express my sincere gratitude to my wife, Alison, for encouragement to undertake this thesis, providing moral support and sacrificing so much during the course of this work, during which we were blessed with the arrival of Declan.

I am indebted to my academic supervisor Professor Jen McComb for her unwavering motivation and encouragement, which is particularly significant when one considers the work for this thesis was performed at Kununurra (3000 km from Murdoch University).

Thanks go to my co-supervisors Drs Syd Shea and John McGrath for their discussions and critical comments on early manuscripts.

Special thanks go to Professor John Pate for the enthusiasm and expertise contributed to chapters 4, 5, 6 and 7 of this thesis.

The assistance of Craig Palmer with establishment, maintenance and assessment of nursery and field experiments is gratefully acknowledged. Peter Dart and Stephen Eldridge are thanked for supplying the *Bradyrhizobium* inoculum used in nursery experiments. Ed Rasins and Dr Kushan Tennakoon are thanked for performing xylem solute analysis. I thank Lin Wong for performing mineral nutrient analysis and Paul Moretta for performing oil analysis. I thank Tanya Vernes, Libby Burgess, Matt Giraudo and John Fairhead for their assistance with work in Kununurra and Perth.

Staff at Agriculture Western Australia’s Frank Wise Institute for Tropical Agricultural Research are gratefully acknowledged for generously providing a range of experimental equipment.

Finally, I am grateful for the opportunity to undertake these studies and the financial support provided by the Western Australian Department of Conservation and Land Management.
LIST OF PUBLICATIONS

The following publications have arisen from the research conducted for my PhD candidature and are presented in this thesis as individual chapters. As the papers are multi-authored, the contribution of each author is stated.

CHAPTER 1


Vernes contributed to the literature search.

CHAPTER 2


McComb and Shea were the supervisors for this thesis.

CHAPTER 3


McComb and McGrath were the supervisors for this thesis.
CHAPTER 4


McComb was the supervisor for this thesis. Pate contributed to compilation of the manuscript and Tennakoon assayed the organic solutes.

CHAPTER 5


McComb was the supervisor for this thesis. Pate contributed to compilation of the manuscript.

CHAPTER 6


McComb was the supervisor for this thesis. Pate contributed to compilation of the manuscript.
APPENDIX 1


APPENDIX 2


McComb and Shea were the supervisors for this thesis. McKinnell contributed to compilation of the manuscript.

APPENDIX 3


Bosimbi was involved with the field work.

CONFERENCE PROCEEDINGS PUBLISHED IN FULL


McComb was the supervisor for this thesis

McComb was the supervisor for this thesis.


Shea was the supervisor for this thesis. Brand and Jones contributed to compilation of the manuscript.
CHAPTER 1

GENERAL INTRODUCTION

1.1 BACKGROUND

*Santalum album* L. (Indian sandalwood) has formed the basis of the global sandalwood industry for 20 - 30 centuries (Srinivasan *et al.* 1992). Due to its high heartwood santalol oil content, diverse utilisation and inherent religious significance, Indian sandalwood is the most important species from the widely distributed and economically important *Santalum* genus (Radomiljac *et al.* 1998a; Appendix 1).

World sources of *S. album* and other *Santalum* species are declining rapidly (Harisetijono and Suriamihardja 1993; Srinivasan *et al.* 1992; Radomiljac *et al.* 1998a) and most sources will be exhausted over the next decade (Havel and McKinnell 1993). Australia has maintained an entirely export orientated sandalwood industry for the past 150 years, based on the native *S. spicatum* (R. Br.) DC (Stratham 1990), which is sparse but widespread in the arid interior of Western Australia (Hewson and George 1984).

The tropical *S. album* has shown potential as an irrigated plantation species within the Ord River Irrigation Area (ORIA), near Kununurra, northern Western Australia (McKinnell 1990; Havel and McKinnell 1993; Radomiljac *et al.* 1998a) and a *S. album* plantation resource may ultimately supplement the *S. spicatum* (R. Br.) A.D.C. harvest from natural stands (Kealley 1991).

As with all *Santalum* species *S. album* is a xylem tapping, obligate root hemi-parasite (Srinivasan *et al.* 1992). This was first recognised by Scott (1871). As the term hemi-parasite suggests, the species has the potential to meet a considerable proportion of its net requirements for carbon through its own photosynthesis (Pate *et al.* 1990a; Press *et al.* 1993; Cechin and Press 1993; Tennakoon and Pate 1996a; Tennakoon *et al.* 1997a; Press 1995a). *Santalum* taps directly into the host plants’ transpiration stream via haustoria (Rao 1942; Kuijt 1977; Webb 1984; Riopel and Timko 1995; Fineran 1991). It is widely accepted that successful haustorial attachment on suitable host species can greatly increase *Santalum* growth, both in early pot
culture and in the field (Nayar et al. 1988; Subbarao et al. 1990; Surata 1992a; Taide et al. 1994; Fox et al. 1996; Radomiljac 1998 (Appendix 2); Radomiljac et al. 1998a). Along with other root hemi-parasites, Santalum exhibits a strictly obligate parasitic habit and fails to grow in the absence of a host beyond a pre-parasitic period (Nagaveni and Srimathi 1985; Loneragan 1990; Barrett and Fox 1997). Due to this parasitic habit, plantation silviculture of Santalum is vastly more complex than traditional monocultural plantation systems.

1.2 The issue

Despite the commercial and cultural importance of S. album an appropriately detailed understanding of its silvicultural requirements has still to be elucidated (Havel and McKinnell 1993). Because of the paucity of information and the fundamental importance of understanding Santalum plantation silviculture, the present project investigated biological and physiological aspects of the parasite: host interaction with the objective of promoting Santalum growth under plantation conditions.

The remainder of this chapter will be devoted to summarising the general literature on S. album in relation to its importance, management, heartwood formation and physiology and introducing the problems that have been investigated in this thesis.

1.3 Nomenclature of S. album

The genus Santalum belongs to Santalaceae, a family in the Order Santales. Santalum comprises 16 known species (Hamilton and Conrad 1990; Barrett and Fox 1995) from India, Indonesia, Australia, Hawaii and the south Pacific Islands, as far East as the Juan Fernandez Islands near South America (Applegate and McKinnell 1993). Linneaus used the name Santalum album in 'Species Plantarum' (1753), but the name had been used by earlier writers. Santalum originates from the Greek word santalon, which in turn was derived from Chandana, a Sanskrit term meaning the tree as well as the wood and oil derived from it. The specific name album is derived from the Latin albus meaning white and refers to the wood colour, which is lighter than some other Santalum species.
1.4 Botanical Features

1.4.1 Habit

*S. album* is a tree of straight form to 15-18m in height, typically with a high bushy crown when grown in dense shade, evergreen and glabrous with slender drooping branchlets. The bark is reddish-brown to dark brown, smooth in young trees, rough and fissured in older trees (Luna 1996).

Even though *S. album* seedlings may grow photoautotrophically for up to 12 months its growth is greater in association with host plants (Ananthapadmanabha *et al.* 1984; Ananthapadmanabha *et al.* 1988a; Kharisma and Suriamihardja 1988; Rai 1990; Harisetijono and Suriamihardja 1993; Fox *et al.* 1995c; Radomiljac 1998; Radomiljac *et al.* 1998a).

*S. album* has good coppicing ability at an early age, however this capacity decreases with increasing age. Trees are sensitive to fire (Radomiljac 1995; Singh 1995).

1.4.2 Foliage

Leaves are ovate-elliptic, varying between 37.5 - 62.5 mm in length, being acute at the base and acute at the apex. Leaves are pale below, which are membranous and veined. On the one branch leaves vary in form from ovate to ovate-elliptic or ovate-lanceolate (De Candolle 1857). Several morphological forms based on leaf size and shape occur in India (Bagchi and Veerenda 1985; Barrett and Fox 1995).

1.4.3 Inflorescence, Flowers and Fruits

Panicles are terminal, lateral and are shorter than leaves, which are many flowered, whereas the pedicels are subequal in length to the tube of perianth. Bracts almost are non-existent or very small. Pedicels are shorter than leaves, which are reddish inside with simple white hairs at base of the anthers. Nectaries are thick and obovate-rotund in shape, whereas the filaments are slender and subequal in length to nectaries. Anthers are smaller than filaments (De Candolle 1857).

Flowers are faintly sweet scented with four triangular to ovate sepals about 2mm long, turning
from green through pink to dark red. The flower consists of four stamens, a half inferior ovary and a large central disk with prominent ovate lobes alternating with those of the perianth (Barrett and Fox 1995). Although the flower structure is designed for self-pollination, *S. album* is a predominantly outbreeding species (Bagchi and Veerendra 1987; Bhaskar 1992; Jyothi *et al.* 1991; Rugkhla *et al.* 1997). Pollinating agents are bees, butterflies and beetles (Veerendra and Padmanabha 1996).

The fruit is a drupe, which is globose in shape, about the size of a small cherry, which is about 7 - 8 mm in diameter. When fully ripened the drupe becomes black (De Candolle 1857). Fruiting begins when trees area around 2 - 3 years of age. The mesocarp is a creamy yellow colour and the endocarp is more or less spherical in shape, up to about 6 - 8 mm diameter. The kernel is firm, white and edible (Barrett and Fox 1995).

1.4.4 PhenoLOGY

Flowering and fruiting seasons vary depending on locality. In India flowering begins in May at the end of the dry season, with fruit maturity commencing in September (end of the wet season) (Srimathi and Nagaveni 1995). A second flowering commences in November with fruit maturity commencing in February. Most trees usually flower and fruit twice a year, however some have been observed to flower once a year and others throughout the year.

Flowers, buds and mature fruit can be found on a single tree at the same time (Barrett 1988; Luna 1996). Natural regeneration is via bird dispersal of seeds, and root suckers.

1.5 Geographic distribution

The origin of the Indian *S. album* is disputed, and it has been suggested that the species was introduced to India from Nusa Tenggara Timur, Indonesia (Brand 1994). However, references to sandalwood utilisation from natural stands in India can be traced to 2300 years ago (Srinivasan *et al.* 1992) so it is likely to be natural. The natural distribution of *S. album* is in the tropical belt of the Indian peninsula, the highland regions of eastern Indonesia, primarily on the islands of Nusa Tenggara Timur (Venkatesan and Srimathi 1981; Rai 1990; Harisetijono and Suriamihardja 1993) and the coastal areas of northern Australia, near Darwin, occurring on sandy soils behind mangrove communities and near small coastal waterholes (Barrett and Fox 1995) (Figure 1.1).
From the tropics of India it has been introduced to Central and Northern India (Srinivasan et al. 1992) and from Eastern Indonesia it has been introduced to Java and Bali (Barrett and Fox 1995). Experimental introductions have been performed in China (Li and Yu 1984), Fiji (Bulai 1995), New Caledonia (Chauvin 1988), Hawaii (Merlin and VanRavenswaay 1990), Tonga (Kaufusi 1995), Papua New Guinea (Paul 1990), Nepal (Neil 1990), Sri Lanka (Tennakoon pers. comm. 1997), East Indonesia (Harisetijono and Suriyamirdja 1993) and North Queensland, Australia (Keenan pers. comm. 1996). Commercial irrigated plantations are currently being established near Kununurra, northern Western Australia (Radomiljac et al. 1998a). S. album has also been introduced to Kenya, Nigeria, Zimbabwe, Tanzania and Uganda with varied success (Streets 1962). There is no recent published literature on these introductions.

1.6 ENVIRONMENTAL AMPLITUDE

1.6.1 CLIMATE

In India the natural distribution of S. album occurs mainly throughout the Deccan Plateau. The species is capable of growing where summer rainfall is between 500-5000mm, the dry season duration is 6-7 months, and the elevation is 0-1800m (Venkatesan and Srimathi 1995). However S. album can occur outside this climatic zone and tolerates extreme temperatures from 4°C to 46°C (Singh 1995).

The islands of Nusa Tenggara Timur are subject to a 2-3 month wet season with an average annual rainfall of 900mm in the lowlands to 2000mm in the highlands. In the dry season the average daily temperature reaches a maximum of 31.6°C (Harisetijono and Suriyamirdja 1993).

1.6.2 SOIL AND PHYSIOGRAPHY

S. album is capable of growing on a range of soil types from gravelly, loam, sand and clay soils (Jain et al. 1968). The most common soil type in India on which S. album occurs is the red ferruginous loam, with underlying gneiss (Luna 1996), which has a poor nutrient status (Rangaswamy et al. 1986a). It is able to tolerate soils with a pH up to 9.0 but is unable to
Figure 1.1: World-wide distribution of *Santalum album* in natural stands in relation to the location of the Ord River Irrigation Area (modified from Rai 1990; Srinivasan *et al.* 1992; Harisetijono and Suriamihardja 1993; Applegate and McKinnell 1993).
tolerate waterlogged sites.

In Indonesia *S. album* occurs in the rugged topography of the Outer Banda Arc islands of Nusa Tenggara Timur, which are composed of uplifted sea floor deposits. The parent material gives rise to heavy textured soils, which tend to be stony, often sodic, alkaline (pH 8-9) and saline. The dominant soil types are alfisols, inceptisols, vertisols and entisols. Presently, *S. album* occurs only on poor soil types due to over exploitation and the conversion of more fertile soils to agricultural production (Harisetijono and Suriamihardja 1993).

1.7 Physiology of *Santalum* and Other Root Hemi-Parasites

Despite the commercial significance of *Santalum* and recent attempts to culture several species in plantations there is surprisingly limited information on the physiological relationship between *Santalum* and its hosts. From the limited information that is available from Webb (1984); Struthers *et al.* (1986); Subbarao *et al.* (1990); Barrett and Fox (1994); Barrett and Fox (1997) and Tennakoon *et al.* (1997a, b) and from information on other root hemi-parasites, some careful generalisations may be drawn.

It is widely accepted that growth of root hemi-parasites is enhanced following attachment to host plants (Seel and Press 1993; 1994; Fer *et al.* 1994; Press and Seel 1996; Tennakoon and Pate 1996a; Radomiljac 1998). Moreover growth is generally far greater when parasites are grown in association with N₂-fixing hosts than when grown with non-N₂-fixing hosts. This is a result of the higher concentration of N in the transpiration stream of legume hosts compared with non-legumes and the associated benefit of greater N uptake by *Santalum* (Schulze and Ehleringer 1984; Rai 1990; Seel and Press 1993; Seel *et al.* 1993; Cechin and Press 1993; Taide *et al.* 1994; Tennakoon and Pate 1996a). Selection of superior host species to give maximum benefit to *Santalum* may be critical for economically effective plantation culture (Rai 1990; Srinivasan *et al.* 1992; Havel and McKinnell 1993; Fox *et al.* 1996; Radomiljac 1998).

A large proportion of the information on root hemi-parasite : host relations deals with *Striga* and *Orobanchaceae*, largely due to their importance as weeds of agricultural crops (Press *et al.* 1987; Graves *et al.* 1989; Graves *et al.* 1990; Seel *et al.* 1992; Cechin and Press 1993; Graves *et al.* 1992; Ehleringer and Marshall 1995; review by Press 1995a). This body of literature deals extensively with the changes in the allometry and growth of parasitised hosts. Conversely, little literature exists on the influence of host species on the growth of the parasite. This aspect and other related effects of host species on *Santalum* seedlings in pot culture or
Host responses to parasitism and the deleterious influences of parasitism on host plants have been reviewed extensively by Graves (1995). The damage is due to uptake of water and organic solutes from the host transpiration stream, reduction in host photosynthesis and changes in dry matter allocation to favour root growth, all of which result in a marked reduction in the overall biomass and health of the host (Press and Stewart 1987; Press et al. 1990; review by Graves 1995; Tennakoon et al. 1997c). Deleterious influences of root hemi-parasitism on host plants has important implications for the effective culture of *Santalum* in plantations. This topic is covered in chapter 7 where a young *Santalum* plantation exhibited declining vigour of host and parasite.

The recent studies by Tennakoon et al. (1997a, b) on *Santalum acuminatum* (R.Br.) A.DC. eco-physiology and haustorial uptake of organic solutes from its associated hosts provide fundamental information on parasite : host relations from which further work on *Santalum* can be based. Transpiration and photosynthetic rates of *S. acuminatum* were found to be consistently less than those of the host *Acacia rostellifera* Maslin. while water use efficiencies and δ¹³C values of foliage were closely similar to the host (Tennakoon et al. 1997a). δ¹⁵N values for shoot dry matter of N₂-fixing hosts suggest these were strongly dependent on atmospheric N and the δ¹⁵N values for *Santalum* showed that the parasite derived N principally from these species. Solute composition of *S. acuminatum* and host xylem sap indicated that there was only limited direct flow of amino compounds between xylem streams of hosts and parasite (Tennakoon et al. 1997b). Proline, the amino acid which predominated in the haustorium and xylem sap of *S. acuminatum* was found in only negligible levels in the xylem stream of hosts. The passage of organic solutes from the host to *S. album* is examined in chapters 4 and 5.

This information on *Santalum* can be compared with data from other root hemi-parasites, such as *Olax phyllanthi* (Labill) R. Br. for which there are recent studies on the biology (Pate et al. 1990a), water relations (Pate et al. 1990b), haustoria morphology and anatomy (Pate et al. 1990c), amino acid transfer from host to parasite (Pate et al. 1994), heterotrophic carbon gain of parasite from hosts (Tennakoon and Pate 1996a), parasite influences on growth and partitioning of C and fixed N in host (Tennakoon et al. 1997c) and xylem fluxes of fixed N of through haustoria on nodules and roots of *Acacia* (Tennakoon and Pate 1997). Host : parasite N relations have been reported for *Odontites* (Grovier et al. 1967), *Rhinanthus* (Seel et al. 1993; Seel and Press 1993), *Bartsia* and *Parentucellia* (Press et al. 1993) and *Striga* (Cechin and
Despite numerous studies on the carbon metabolism and mineral nutrition of parasitic plants and their hosts in native habitats (Hocking 1980; Lamont and Southall 1982; Glatzel 1983; Schulze and Ehleringer 1984; Struthers et al. 1986; Pate et al. 1991a; Seel and Press 1993; Pate 1995a and references therein; Tennakoon and Pate 1996a; Veenendaal et al. 1996) limited information exists on the benefit to the parasite in terms of mineral uptake and heterotrophic carbon gain when attached to beneficial and non-beneficial hosts. This aspect is studied using pot cultured *Santalum* partnered singly with beneficial and non-beneficial woody hosts in chapter 6.

1.8 CULTURAL, COMMERCIAL AND RELIGIOUS IMPORTANCE OF SANDALWOOD

Sandalwood is the highly valued aromatic heartwood from most species of the *Santalum* genus, which has significant cultural, medicinal and commercial importance in Asian, Middle Eastern and Pacific Island countries (Havel and McKinnell 1993). Srinivasan et al. (1992) indicate sandalwood was an article of perfumed cosmetics and toiletry in Buddhist Jataka of the 7th century B.C. However, western culture's interest in sandalwood is more recent dating back only about 250 years (Stratham 1990), where utilisation was predominantly for cosmetic and medicinal purposes. Oil distilled from the heartwood is an important fixing agent used in the perfumery and cosmetics industry and oil is also widely used for aromatherapy (McKinnell 1992). Sandalwood powder is used for incense or joss stick manufacture (Havel and McKinnell 1993). Large sandalwood billets are increasingly rare and are sought after for furniture and carvings (Srinivasan et al. 1992).

*S. album* has the highest heartwood santalol oil content and growth rates of all *Santalum* species. The oil derived from the heartwood of this species contains approximately 90% α- and β- santalol. These compounds accumulate in the heartwood as the tree matures, but do not accumulate extensively in the sapwood (Hillis 1987). Consequently, whole stem utilisation is uncommon, and trees require a critical volume of heartwood to have high commercial value (Srinivasan et al. 1992).
1.9 Uses

1.9.1 Wood

The heartwood of *S. album* was used for many centuries for carvings, prayer poles and other religious artifacts, valuable handicrafts, fuel for funeral pyres, coffins and joss sticks (Jhingan 1966; Muralidhara 1977; Jayappa *et al.* 1981). The heartwood is close-grained, very fine and even textured, hard (specific gravity 0.92, weight 897-1137 kg/cubic metre), durable and renowned as a carving material (Luna 1996). The timber seasons well when dried slowly. The wood can be worked to a smooth finish and takes a satin-like polish.

1.9.2 Oil, Bark, Leaves and Fruit

Sandalwood oil is extensively used in the perfumery and cosmetics industries. It has been used as a coolant, astringent, antipyretic and aphrodisiac (Dastur 1962; Jain 1968). The oil is also used to treat migraines (Luna 1996), erysipelas, gonorrhea and cystitis (Kulkarni 1995). Oil is widely used in the agarbathi, cosmetic, fragrance and soap industries and, in terms of volume consumed, its use as a base for fragrance far outweighs its use in medicine (Srinivasan *et al.* 1992).

Oil from the heartwood is produced commercially by steam distillation, with an average yield between 4-6% (Rai 1990; Harisetijono and Suriamihardja 1993). However, individual tree estimates can vary between 0.2-7.25% (Haffner 1993). Variation in oil content is attributable to tree age, site and position of heartwood within the tree. Haffner (1993) presents a method for estimating the content of oil for individual trees by Soxhlet extraction. Srinivasan *et al.* (1992) details the chemical characteristics of *S. album* oil and extraction techniques.

The bark of *S. album* contains approximately 12-14% tannin (Shankaranarayana *et al.* 1980) and although it has potential in the tanning industry, is not widely used (Srinivasan *et al.* 1992). Some villagers use fresh bark as a substitute for betel-nuts (Gupta 1993). Leaves are used for fodder and green manure. The tree is useful as hedge plantings or for shade or windbreaks (Venkatesan 1995b).

Fruits and kernels are edible and may have potential as a horticultural crop until mature trees are harvested (Barrett 1989).
1.9.3 Land Use, Environmental and Service Aspects

As a plantation species within the Ord River Irrigation Area, northern Western Australia (Figure 1.2), *Santalum* may also offer a cost effective method to control the early onset of rising water tables within irrigated farming systems. In India *Santalum* is envisaged as a practical species for agroforestry on marginally productive agricultural lands to improve productivity and provide wind breaks, green manure, fodder, fuelwood, timber and fruits (Venkatesan 1995b).

1.10 Management and Status of *Santalum* in Natural Stands

A period of sandalwood scarcity is looming in both India and Indonesia, the main producers of *S. album*. The decline in *S. album* population is due to heavy pressure for clearing forested land for food production, the destruction of host trees for wood products, diseases such as spike disease, and illegal harvesting (Srinivasan *et al.* 1992; Havel and McKinnell 1993). Plantations are the only way to redress increasing problems of resource availability (Hamilton and Conrad 1990).

Annual global sandalwood heartwood production estimates are approximately 5100 tonnes (Radomiljac *et al.* 1998a). Assuming a heartwood yield of 20 tonnes per hectare with a rotation length of 25 years, a plantation resource of around 6400 hectares is suggested to meet current global sandalwood consumption. That is, an annual planting programme of 255 hectares (Radomiljac *et al.* 1998a).

India and Indonesia supply about 70% and 30% of the world's sandalwood oil, respectively (Harisetijono and Suriamihardja 1993). It is important that sandalwood supplies from India are maintained at a certain level to satisfy the needs of the sandalwood oil, joss stick and carving industries. If the sandalwood market collapses due to declining supply there are potentially serious repercussions for other small sandalwood producing countries (Appendix 3). A decline in supply may lead to (1) an overwhelming pressure to over-harvest remaining *Santalum* species populations, (2) a lost opportunity to develop viable and sustainable industries based on other *Santalum* species and (3) the intrusion of sandalwood oil synthetics into traditional markets. In this context a coordinated sandalwood marketing approach is needed to ensure remaining natural stands are sustainably managed until a plantation resource becomes available (Shea *et al.* 1998). Most countries with indigenous *Santalum* species have established *in situ* conservation and plantation establishment programmes (Daruhi 1993; Cherrier 1993; Jiko
Several factors prevent the adequate regeneration and protection of natural stands including unsustainable exploitation (Rai 1990), uncontrolled fire and grazing (Havel and McKinnell 1993), illegal harvesting (Murthy 1985), regulations that are a disincentive to *Santalum* conservation (Husain 1983) and spike disease (Rai 1990). Spike disease, caused by a phytoplasma type organism, causes trees to produce small stiff needle like leaves and as a result disrupts the trees' metabolism (Iyengar 1969; Iyengar 1972a). It ultimately causes tree death (Srinivasan *et al.* 1992). Detailed accounts of the theories proposed in the incidence, spread and control of spike disease in India are given by (Luna 1996; Srinivasan *et al.* 1992; Iyengar 1972b). Spike disease has not been detected outside of India at present.

It appears that the development of a plantation resource is the only means of maintaining or increasing *S. album* production (Radomiljac 1995; Shea *et al.* 1998). In this respect, research is needed on *Santalum*: host allometry and physiological relations, influences of host species on *Santalum* growth under plantation conditions and heartwood formation in plantation grown *Santalum*.

### 1.11 Silviculture and Management

#### 1.11.1 Natural Regeneration

Under natural conditions *S. album* preferentially parasitises leguminous species (Rai 1990). Natural regeneration can be abundant, through both seed germination and root-suckers, if there are suitable host plants present, there is a low incidence of vigorous weed species, lateral shading from hosts, protection from grazing and fire, provision for expansion of the crown and limited water stress (Gupta 1993; Luna 1996). With adequate vegetative cover and moist, well-drained soils, seed germination is profuse. Under optimal conditions (suitable hosts and high soil moisture) growth is rapid with up to 30cm height growth by the end of the first wet season (Luna 1996).
1.11.2 PLANTATION SILVICULTURE

The silviculture of *Santalum* plantations poses unique and complex issues for successful field establishment and sustained plantation productivity. *Santalum* must always exist in a multi-species environment, which has profound implications for its silviculture.

A wide range of plant species are parasitised by *S. album* (Srinivasan et al. 1992). However, under plantation conditions *S. album* growth is greater when parasitising leguminous species compared to those plants grown in association with non-leguminous species (Rai 1990; Shinde et al. 1993; Taide et al. 1994). *Santalum* requires a host which is neither too vigorous and out competes the tree, nor too weak so that the host is exhausted (Havel and McKinnell 1993).

In practice, three or more types of host are required when establishing seedling plantations, a short term host in the nursery stage, which is termed the pot host (Radomiljac 1998), and two or more longer-term hosts in the field, termed the intermediate and long term hosts (Radomiljac et al. 1998a).  

A bio-diverse farm forestry system appears possible due to *S. album*'s parasitic silvicultural requirement for a series of host species. That is, a range of species is required to perform as hosts at various stages in the plantation rotation (Radomiljac et al. 1998a). Long-term host species that produce a valuable timber may offer the potential for two or more timber products from one plantation system. Successful long term host species introductions within the ORIA to date include the CITES-listed *Swietenia macrophylla* King, *S. mahogani* Jacq. and *Dalbergia melanoxylon* Guill. and Perr. (Radomiljac et al. 1998a).

Seed germination of *Santalum* has been extensively studied and it has been shown that *Santalum* seed has a dormancy period that can be overcome by nicking the testa or soaking in gibberellic acid (Ovcharov 1977; Nagaveni and Srimathi 1981; 1985; Nagaveni and Ananthapadmanabha 1986; Ananthapadmanabha et al. 1986; 1988b; Kagy 1987; Nagaveni et al. 1989; Brand et al. 1993; Fox et al. 1995a; Nasi 1995a). Techniques for seed collection, processing, storage, nursery techniques and establishment of *S. album* in India are given by Fox et al. (1995a) and Luna (1996).

In India, direct sowing seeds under potential hosts plants is commonly practised (Rai and Kulkarni 1986) although there has been some success with plantation establishment using *S. album* seedlings raised in the nursery (Rai 1990; Srinivasan et al. 1992). However *Santalum*
plantation establishment has not achieved operational status in India.

Establishment requirements of *S. album* plantations in Timor are reported in detail by Fox *et al.* (1995b).

In New Caledonia research on *Santalum* plantation establishment using *S. austrocaledonicum* Vieillard has been performed (Quemin 1988; Cherrier 1993). A routinely used establishment technique has been developed and is described by Ehrhart and Fox (1995). At present research is being directed to aspects of mid-rotation *Santalum* silviculture (Nasi 1995b).

In recent years field establishment research has commenced on *S. album* in northern Western Australia (Figure 1.3) (Applegate and McKinnell 1993). Nursery propagation for *S. album* plantations commences in October and it usually takes about nine months to raise robust *S. album* seedlings (Radomiljac *et al.* 1998a). Two to three months following germination, cuttings of a herbaceous pot host are planted into each *S. album* seedling container (Radomiljac 1998; Radomiljac *et al.* 1998a). The relationships between the pot host and the parasite are studied further in chapter 2.

The parasite : host combination continues throughout the nursery phase and into the early stages of field establishment (up to 18 months following plantation establishment). Intermediate and long-term host seedlings are raised simultaneously in the nursery and are strategically placed in the field at establishment. Field establishment occurs in June-July (during the dry season), and the site is frequently flood irrigated. In India field establishment also occurs in June-July, following the commencement of the monsoon season (Srinivasan *et al.* 1992).

The long term host, which must persist as final host for the entire rotation length (25-30 years), is planted up to four metres from the *S. album* and pot host combination. This is to avoid obstruction of the growth of the *S. album* as both *S. album* and long term hosts have large crowns. An intermediate host is planted between the *S. album* and long term host seedling. The intermediate host is parasitised for about 4-5 years, until it dies (Radomiljac *et al.* 1998a). Ideal intermediate hosts appear to be fast-growing, short lived leguminous trees.
1.11.3 HOST SELECTION FOR PLANTATION CULTURE

As with other root hemi-parasites, host plants influence Santalum growth and vigour (Rangaswamy et al. 1986b). Several studies have investigated the gross influences of host species on Santalum growth (Table 1.1).

1.12 GROWTH RATE OF SANTALUM IN PLANTATIONS AND NATURAL STANDS

In India and Indonesia S. album grows slowly in natural stands (Fox et al. 1995d), increasing in stem diameter between 0.3-1.5 cm year\(^{-1}\) (Rai and Kulkarni 1986) or between 1-1.5 cm year\(^{-1}\) (Srinivasan et al. 1992). Rajagopal Shetty (1977) suggests that a growth rate of S. album diameter at breast height is highly variable, where diameter growth in certain parts of India is as low as 0.33 cm year\(^{-1}\) but under favourable conditions diameter growth is as high as 5-6 cm year\(^{-1}\) in natural stands. However, studies by Rai and Sarma (1986) conclude that S. album on poor sites take about 130 years to reach a diameter at breast height of 20 cm. The growth rate of plantation grown S. album has been recorded as being higher than that of trees in natural stands, with a mean annual diameter increment between 0.29 and 0.20 cm in natural stands in Tamil Nadu and Andhra Pradesh, respectively (Sarma and Rai 1986). Even though plantation growth is usually faster than that of naturally grown Santalum large variability exists between trees (Fox et al. 1995d).

Whole stem utilisation in Santalum is uncommon and the commercial value of Santalum depends on the heartwood content of trees. In this respect, it is difficult to assess total growth rates of Santalum without reference to heartwood formation. Venkatesan (1995a) and Srinivasan et al. (1992) present average heartwood yields for different diameter classes of S. album. Rai and Sarma (1986) developed a model to predict heartwood yield based on diameter classes. The model was developed from only six samples, thus its accuracy might be questioned. Further study on heartwood yield modelling is clearly called for. For a predictive model to be developed for plantation grown Santalum, sampling for heartwood yield from trees of known age needs to be performed. Unfortunately, Santalum plots with a range of stocking rates, established on various sites of similar tree age and host species are not available for modeling purposes. Heartwood yield modelling is then dependent on reconciling the various sources of published information on individual tree yield. This has many associated pitfalls, not the least being the large variation in heartwood yield from individual trees.
Table 1.1: Summary of research on *Santalum* host species selection. Asterisks indicate N₂-fixing species.

<table>
<thead>
<tr>
<th><em>Santalum</em> species</th>
<th>Country</th>
<th>Pot host</th>
<th>Intermediate host</th>
<th>Long term host</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S. album</em></td>
<td>India</td>
<td><em>Cajanus cajan</em></td>
<td></td>
<td><em>Albizia lebbeck</em></td>
<td>Sarma and Wilson (1936)</td>
</tr>
<tr>
<td></td>
<td>India</td>
<td><em>Cajanus cajan</em></td>
<td><em>Calotropis gigantea</em></td>
<td><em>Cassia siamea</em></td>
<td>Harikrishnan (1972)</td>
</tr>
<tr>
<td></td>
<td>India</td>
<td><em>Cajanus cajan</em></td>
<td><em>Calotropis gigantea</em></td>
<td><em>Acacia auriculiformis</em></td>
<td>Rangaswami and Griffith (1939)</td>
</tr>
<tr>
<td></td>
<td>India</td>
<td></td>
<td></td>
<td><em>Cassia siamea</em></td>
<td>Ananthapadmanabha et al. (1988)</td>
</tr>
<tr>
<td></td>
<td>India</td>
<td></td>
<td></td>
<td><em>Acacia auriculiformis</em></td>
<td></td>
</tr>
<tr>
<td></td>
<td>India</td>
<td></td>
<td></td>
<td><em>Casuarina equisetifolia</em></td>
<td></td>
</tr>
<tr>
<td></td>
<td>India</td>
<td></td>
<td></td>
<td><em>Pongamia pinnata</em></td>
<td></td>
</tr>
<tr>
<td></td>
<td>India</td>
<td></td>
<td></td>
<td><em>Terminalia alata</em></td>
<td></td>
</tr>
<tr>
<td></td>
<td>India</td>
<td></td>
<td></td>
<td><em>Dalbergia sisso</em></td>
<td></td>
</tr>
<tr>
<td></td>
<td>India</td>
<td></td>
<td></td>
<td><em>Cassia siamea</em></td>
<td>Shinde et al. (1993)</td>
</tr>
<tr>
<td></td>
<td>India</td>
<td></td>
<td></td>
<td><em>Azadirachta indica</em></td>
<td></td>
</tr>
<tr>
<td></td>
<td>India</td>
<td></td>
<td></td>
<td><em>Casuarina equisetifolia</em></td>
<td>Taide et al. (1994)</td>
</tr>
<tr>
<td></td>
<td>India</td>
<td></td>
<td></td>
<td><em>Pongamia glabra</em></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Australia</td>
<td><em>Desmanthus virgatus</em></td>
<td></td>
<td><em>Cassia siamea</em></td>
<td>Rai (1990)</td>
</tr>
<tr>
<td><em>S. austrocaledonicum</em></td>
<td>Indonesia</td>
<td><em>Cajanus cajan</em></td>
<td><em>Acacia villosa</em></td>
<td></td>
<td>Fox et al. (1996)</td>
</tr>
<tr>
<td></td>
<td>Australia</td>
<td><em>Alternanthera nana</em></td>
<td></td>
<td><em>Cassia siamea</em></td>
<td>Harisetijono &amp; Suriarnihardja (1993)</td>
</tr>
<tr>
<td>New Caledonia</td>
<td><em>Alternanthera sessilis</em></td>
<td></td>
<td></td>
<td><em>Dalbergia latifolia</em></td>
<td>Radomiljac (1998)</td>
</tr>
<tr>
<td>New Caledonia</td>
<td></td>
<td></td>
<td></td>
<td><em>Casuarina equisetifolia</em></td>
<td>Kagy (1987)</td>
</tr>
<tr>
<td></td>
<td>Vanuatu</td>
<td><em>Alternanthera sp.</em></td>
<td></td>
<td><em>Cassia siamea</em></td>
<td>Nasi (1995b)</td>
</tr>
<tr>
<td><em>S. yasi</em></td>
<td>Fji</td>
<td><em>Calliandra calothyrsus</em></td>
<td></td>
<td><em>Acacia spirorbis</em></td>
<td>Vira and Smith (1995)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td><em>Casuarina collina</em></td>
<td>Bulai (1995)</td>
</tr>
<tr>
<td></td>
<td>Australia</td>
<td></td>
<td></td>
<td><em>Gymnostoma deplancheana</em></td>
<td></td>
</tr>
<tr>
<td><em>S. spicatum</em></td>
<td>Australia</td>
<td></td>
<td></td>
<td><em>Acacia acuminatum</em></td>
<td>Struthers et al. (1986)</td>
</tr>
</tbody>
</table>
For instance, near Sholapurum only 40% of eight-year old trees with a diameter growth rate between 0.9-1.8 cm year$^{-1}$ contained heartwood. By 14 years of age the growth rate dropped to between 0.7-1.5 cm year$^{-1}$ and only 50% of trees contained heartwood (Srimathi and Kulkarni 1979). Similarly, near Mudimalai, nine year old Santalum growing on poor sites had a diameter growth rate between 0.5-0.9 cm year$^{-1}$, of which about 42% contained heartwood.

For *S. austrocaledonicum* growth rates have been estimated on naturally grown mature trees (Nasi 1995c). Comparisons between naturally and plantation grown trees show plantation growth rates (2 cm year$^{-1}$) are usually higher than that of naturally grown trees (1.1-1.5 cm year$^{-1}$) (Nasi 1995c). A poor relationship exists between tree age and sapwood width (Nasi 1995c). Sapwood width is generally greater on favourable sites than that of poor sites (Quemin 1988; Cherrier 1991). In this respect, trees growing in plantations on good sites should exhibit greater sapwood widths than when growing in natural stands. Nasi (1995c) details methodologies for heartwood yield prediction.

Genetic variation between *S. album* populations in India and West Timor has been evaluated by Brand (1994), who suggested that the two populations may be separate varieties or races. In addition, growth and heartwood volume in natural stands of *S. album* is not uniform (Barrett 1988).

### 1.13 Heartwood Formation in Santalum

The commercial value of *Santalum* depends entirely on its heartwood santalol oil content and the quantity of heartwood per tree. In India and elsewhere there is considerable literature, albeit ephemeral and in some instances anecdotal, on the formation of heartwood in *Santalum* (Nayar 1974; Srimathi *et al.* 1980; review by Srinivasan *et al.* 1992; Jiko 1993; Cherrier 1993; Srimathi *et al.* 1995; Gjerum *et al.* 1995). Mostly, this work investigates the influence of host species, site factors, soil type, tree age and growth rate on heartwood formation. Despite these investigative attempts the conclusions drawn are contradictory. The general consensus from this body of literature is that heartwood formation is genetically controlled as there is the accumulated rejection and contradiction of the various environmental factors studied as being important influences on heartwood formation. Despite the strong overtures of genetic factors significantly influencing heartwood formation in *Santalum* virtually no information exists on this subject. Studies on heartwood: sapwood relations in *S. spicatum* and *S. album* were investigated with both genetic and environmental influences as variable factors (Haffner 1993).
Haffner (1993) concluded that it is unlikely that the estimates of the age at which heartwood was initiated were accurate since the number of growth rings are known to either under or over-estimate tree age by 53%. To answer many of the questions that relate to 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.

Luna (1996) discusses the rate of growth and heartwood production from different localities in India. The tree age at which heartwood formation commences varies considerably (Haffner 1993). Heartwood formation may commence between 14-46 years of age in Timor (Haffner 1993). In contrast Rai (1990) states that in India heartwood formation commences at 10-13 years of age. The findings of Rai (1990) and Haffner (1993) may indicate that environmental influences may play an important role in heartwood formation. Radmiljac et al. (1998a) reports from limited sampling within plantations in northern Western Australia most trees have initiated heartwood formation by the age of 10 years. The rotation length of these S. album plantations in Australia is still unclear, but is estimated to be 25-30 years. These estimates are based on average harvesting cycle of natural stands of Santalum in Timor.

When grown in natural stands under favourable conditions heartwood formation normally commences at age 10 years when tree diameter is approximately 24 cm at ground level and total height 3 m (Barrett 1988). Heartwood formation is rapid from about 20 years onwards when stem girth is between approximately 40 to 60 cm (Lakshmi Sita 1991). An 8-year old tree, growing near Mysore, Karnataka, with a diameter of 14.3 cm and height of 8.5 m yielded approximately 15 kg of heartwood (Barrett 1988).

In Indonesia the rate of heartwood formation of Santalum increases from age 20 years, with a sandalwood oil content of up to 10% in heartwood of roots, whereas it ranges between 4.5-6.5% in the heartwood of the stem (Harisetijono and Sutarjo 1991). About 108 S. album candidate plus trees have been selected from natural stands based on measurements of sapwood radius and stem diameter (Effendi 1992). Trees at the upper end of percentage heartwood, between 60-70% of basal area as heartwood, are further considered on the basis of form and bole length (Fox et al. 1995d). Heartwood : sapwood relations of naturally grown Santalum were also examined by Fox et al. (1995d). This study found that the percentage of the bole that was heartwood varied greatly, between 20-70%. In this study heartwood volume was found to be strongly related to heartwood weight, tree diameter, total tree volume, bole length, proportion of heartwood cross-sectional area at base, sapwood radius and tree height. In New Caledonia studies on heartwood formation in naturally grown S. austrocaledonicum suggest heartwood volume can be predicted by simple measures of tree girth and sapwood width (Nasi
1995c). Other studies on *S. austrocaledonicum* show that sapwood width (and indirectly heartwood) is related to growing conditions (Cherrier 1993), suggesting that the more favourable the growing conditions, the better the growth and the wider the sapwood band. A number of studies in softwood species have identified a positive correlation between early growth rates and the extent of future heartwood production (Hillis and Ditchburne 1974; Hillis 1987; Wilkes 1991; Climent et al. 1993). Hillis and Ditchburne (1974) showed that *Pinus radiata* with the most rapid growth in the first five years had the greatest heartwood diameter at all future ages. It is important to determine if this relationship exists for *Santalum*. This relationship is described by the equation:

$$H_d = -2.83 + 0.1055d + 0.03114aD_5$$

Where $H_d$ : heartwood diameter, $d$ : DBHOB at future age $a$, $a$ : future age and $D_5$ : DBHUB at age five.

Both Quemin (1988) and Cherrier (1993) developed predictive models for heartwood formation in *S. austrocaledonicum*, but the accuracy of these models are questioned by Nasi (1995c).

Heartwood volume in individual *Santalum* differs between trees of the same age and size (Fox et al. 1995d), in some instances heartwood formation in individual mature trees can be negligible (Sen-Sarma 1977). Clear economic benefits exist in inducing early heartwood formation in young *Santalum*. Heartwood induction in *Santalum* will be discussed in Chapter 8.

### 1.14 Heartwood Heritability

It is widely reported that age of heartwood initiation, extent of formation and santalol oil content in *Santalum* is strongly influenced by inherent traits, more so than that of site factors (Srimathi and Kulkarni 1979; Shankaranarayana *et al.* 1985; Shankaranarayana and Parthasarathi 1987; Fox *et al.* 1995d; Venkatesan 1995a). Shankaranarayana *et al.* (1985) report that santalol oil varies with wood colour, with higher concentrations in lighter coloured wood, and with tree age. Srimathi and Kulkarni (1979) suggest *Santalum* can form heartwood under varied climatic and edaphic conditions. In this respect it is paramount that research is directed toward the influence of environmental factors on wood quality (i.e. santalol oil content of heartwood).

A study on the composition of santalol oil from trees samples in several locations in Karnataka
and Tamil Nadu showed that oil yield varied considerably and in some cases β-santalol were unusually higher than that found for α-santalol (Jayappa et al. 1981). If heartwood variation between populations is strongly influenced by a genetic component then examining the growth characteristics of Santalum progeny may assist in determining trees suitable for commercial cultivation. Conjecture exists on the influence of host species and site on Santalum heartwood formation (Srinivasaya and Rangaswami 1931; Rangaswami 1941; Venkata Rao 1941). This area remains unresolved and further study in these respects under field conditions with controlled genetic components is clearly called for. The establishment and analysis of well designed Santalum progeny experiments is dependent on an appropriate silvicultural system to ensure high survival and uniform effects of the hosts within the study area.

1.15 Formation of Santalol Oils

Santalol compounds, like many other heartwood constituents, form from starch, sugars and other primary metabolites at the heartwood : sapwood boundary (Hillis 1987), where there is likely to be an intermediate precursor between the sapwood constituents and that of the santalol oils. The region in which heartwood constituents form is very narrow and the concentration of heartwood products depends on the amount of sapwood constituents that are available to be transported to the heartwood : sapwood interface.

Heartwood is the central part of the secondary xylem in woody plants containing non-functional tracheary elements. Santalols are large complex compounds (Pigott et al. 1997) that are relatively immobile. In this respect santalol oils are formed and deposited within the heartwood in situ near the heartwood : sapwood boundary. Heartwood in Santalum is sharply differentiated from sapwood by scent and its resistance to fungal decay, termite attack and other degradation processes (Venkatesan 1995a; Harisetijono and Sutarjo 1991).

1.16 Thesis Objectives

Despite the commercial importance of most species of Santalum the above precis of literature on Santalum clearly indicates large deficiencies exist on Santalum physiology and silviculture which hamper the development of reliable systems for the culture of Santalum in plantations. The above information also shows the experimental potential Santalum may provide for investigating the influence of host plants on root hemi-parasite growth.
The central theme of this thesis is to address significant issues to provide the means for the effective culture of *Santalum* on flood irrigated sites on Cununurra clay within the Ord River Irrigation, northern Western Australia. This theme will be explored with six working hypotheses:

- that there is an optimum period of the association between *Santalum* and the pot host during the nursery phase prior to field planting that improves *Santalum* field survival and growth.

- that *Santalum* growth is enhanced when attached to N$_2$-fixing woody hosts.

- that xylem sap composition of host and *Santalum*, especially in respect of nitrogenous solutes, may provide key information on host quality and that C and N concentration of xylem sap of *Santalum* are enhanced when attached to N$_2$-fixing woody hosts.

- that the net assimilation and transpiration rates and water use efficiencies of *Santalum* are enhanced when attached to N$_2$-fixing woody hosts.

- that it is possible to develop a model to quantify the impact of hosts on the growth of *Santalum* in plantations.

- that stem injections of plant growth regulators may induce heartwood formation in young *Santalum* trees.
FIELD ESTABLISHMENT OF SANTALUM ALBUM L. – THE EFFECT OF THE TIME OF INTRODUCTION OF A POT HOST (ALTERNANTHERA NANA R. BR.)

2.1. ABSTRACT

Field establishment of the root hemi-parasite Santalum album L. under large-scale plantation conditions, until recently, has been largely unsuccessful. In this experiment the growth of S. album seedlings grown with the herbaceous pot host Alternanthera nana R. Br. for 134, 109, 84, 60 and 35 days in a nursery container prior to field establishment was examined after 11, 16 and 23 weeks in the field. S. album survival and growth was greater, and root : shoot ratio was lower for the 23 weeks for S. album seedlings grown with A. nana compared with seedlings grown without a host. Seedlings grown with A. nana for 134 days in the nursery prior to field establishment had greater stem diameter, height and root, shoot and total plant DW over the 23 weeks in the field than all other treatments. Seedlings grown with A. nana for 109 days in the nursery prior to field establishment had greater field survival than all other treatments. A. nana survival in the field remained high when grown with S. album for 134 and 109 days in the nursery prior to field establishment whereas survival within remaining treatments declined significantly and A. nana growth was significantly less. S. album grown with A. nana for 134 days in the nursery prior to field establishment had a lower root : shoot ratio than all other treatments at all assessments. A strong negative linear relationship exists between S. album root : shoot ratio and A. nana DW, whereas a positive linear relationship exists between S. album DW and A. nana DW. Foliar phosphorus and sodium concentrations for S. album were lower and foliar potassium concentration higher when seedlings were grown with A. nana for 134 days in the nursery prior to field establishment compared with the remaining treatments at the 16 week assessment. The period of the S. album : A. nana association in the nursery significantly influenced S. album survival and growth following field planting.
2.2. Introduction

It is widely accepted that the presence of a pot host increases the success of establishment of *Santalum* L. species in plantations (Srinivasan et al. 1992; Surata 1992a; Shinde et al. 1993; Taide et al. 1994; Nasi 1995b; Barrett and Fox 1995; Fox et al. 1996; Radomiljac 1998). *Santalum* species are xylem tapping obligate root hemi-parasites with most species producing a highly-valued aromatic heartwood, known as sandalwood. Due to its high heartwood santalol content, diverse utilisation and inherent religious significance *S. album* (Indian sandalwood) is the most important species from the widely distributed and economically important genus (Radomiljac et al. 1998a). There is interest in cultivating *S. album* as a plantation species in the Ord River Irrigation Area (ORIA), in northern Western Australia (McKinnell 1993) and elsewhere in the tropics (see Hamilton and Conrad 1990; Gjerum et al. 1995). Plantation silviculture for parasitic species is more complex than traditional monocultural plantations due to the need to provide a range of host plants (Radomiljac et al. 1998a). The initial host, known as the pot host, is introduced into the pot during the nursery phase with the main function of preventing *S. album* water and nutrient deficits at the time of field establishment. The presence of a host changes the nutrient status of the parasite tissues (Struthers et al. 1986), but it is not known whether there is a carryover effect of the time of the presence of the pot host on nutrient levels in plants transferred in the field.

An earlier study by Radomiljac (1998) showed *Alternanthera nana* R. Br. to be a suitable pot host for *S. album*, which promoted high survival and growth following field planting. This chapter tests the hypothesis that there is an optimum period of time of the *S. album : A. nana* association in a nursery container prior to field establishment and that longer periods may improve early survival, growth and nutrition of *S. album* when transplanted to the field under flood irrigation conditions.

2.3. Materials and Methods

The effect of the length of *S. album : A. nana* pot host parasitism prior to field establishment on the growth of *S. album* seedlings after field establishment was initially studied in a shadehouse and then on a flood irrigated Cununurra clay site near Kununurra (lat. 15° 46’ S. long. 128° 44’ E.), Western Australia.

*S. album* seedlings were propagated following Radomiljac (1998). On 7 February 1996, 360 uniform seedlings, about 7 weeks old, in 1.4 litre pots were selected. *A. nana* cuttings were
placed into sixty pots containing *S. album* seedlings on 12 February 1996, 8 March, 2 April, 26 April and 21 May. This provided 134, 109, 84, 60 or 35 days growth of *S. album* and *A. nana* together in pots in the nursery before transfer to the field. *A. nana* shoots were harvested from vigorous parent plants and stems 5 – 8 mm diameter were prepared as 110 – 150 mm long cuttings. Leaves were removed up to the apical tip. Cuttings were then rinsed in 4% sodium hypochlorite solution, washed and placed vertically into pre-formed 40 mm deep holes 40 - 50 mm from the *S. album* seedling. There were 60 control pots with no *A. nana*. Treatment rows were randomly allocated to a position within each replicate and the position of the replicate and the treatment row within each replicate were randomly changed every 14 days.

Immediately prior to field planting *S. album* height and diameter at 2cm and *A. nana* ground cover area were assessed. Ground cover area was estimated by calculating the area of a circle from the average of the maximum diameter of the *A. nana* clump and the diameter at right angles to this. *S. album* seedlings within one randomly selected complete replicate (10 seedlings per treatment) were harvested. One randomly selected *S. album* seedling in each treatment was carefully removed from its pot and a 2 cm x 2 cm grid was placed onto the surface of the undisturbed pot medium at three positions and the total number of connected haustoria in all three areas were recorded. This technique was used rather than attempting to count the total number of connected haustoria as to do so would involve washing the soil from the root systems and physically separating the two species root systems. This may have caused young haustoria connections to break. The template positions were subjectively selected on the basis of the three highest concentrations of connected haustoria. The single replicate of plants was then harvested and *S. album* leaf, stem, root and *A. nana* shoot dry weight (DW) was measured after the plant material had been oven dried at 80°C for 48 hours.

*S. album* and *A. nana* plants remained in the nursery until field establishment. On 25 June 1996 all parasite : host association treatments were planted in the field in a fully randomised complete block design. Each treatment plot consisted of a single row of ten seedlings planted at 3m spacings along the row, replicated five times. A 1.8m buffer separated treatment rows. The site, establishment procedures and irrigation regime follows Radomiljac (1998).

At 11, 16 and 23 weeks after planting, *S. album* survival, height and diameter at 10 cm above ground level and *A. nana* survival and ground cover area were assessed. At each assessment three *S. album* seedlings were selected (one seedling equal to, one +1 standard deviation (SD) and one –1 SD of the mean *S. album* diameter) from three replicates. *S. album* plants were harvested by cutting the stem at ground level and the shoot partitioned into stems and leaves. *A. nana* shoots were cut at ground level. *S. album* roots were carefully excavated by hand,
separated from *A. nana* roots and then washed. *S. album* stem, leaf, root and *A. nana* shoot DW was measured after the plant material had been oven dried at 80°C for 48 hours.

At each harvest, following DW assessment, *S. album* leaves from each treatment were bulked within each replicate, a sub-sample taken and then passed through a 1.0 mm mill. Nitrogen content was determined by the Kjeldahl method (McKenzie and Wallace 1954). Material was then digested with nitric, sulphuric and perchloric acid (Piper 1942) and the concentration of Ca, Mg, Cu, Mn and Zn analysed using an atomic absorption spectrophotometer. Potassium and Na were analysed using a Corning flame photometer. Phosphorus was determined colorimetrically using a spectrophotometer (Kitson and Mellon 1944).

Survival and growth data were analysed using ANOVA of plot means and Tukey’s pairwise t-test. Survival data was transformed using the log_{10} transformation. All analyses were performed using SYSTAT® statistical software (Systat 1992). Linear regressions were fitted using the linear regression procedure of SYSTAT®.

### 2.4. Results

The growth of *S. album* seedlings in the nursery and the number of connected haustoria per plant prior to field establishment were not significantly influenced by the time of *A. nana* introduction into *S. album* containers. As expected, *A. nana* growth in pots was greater for treatments in which it was introduced earliest (Table 2.1).

*S. album* field survival was close to 100% for all treatments for the first 16 weeks, after which the time of introduction of the *A. nana* pot host influenced *S. album* survival. Survival of *S. album* seedlings without a pot host was significantly lower than seedlings grown with *A. nana* for 134 and 109 days prior to field establishment. *S. album* seedlings with the least advanced *A. nana* pot host treatments declined while those with the longest established pot hosts showed high survival. *S. album* survival in the field was highest at all of the three harvests for plants grown with *A. nana* for 109 days prior to field establishment (Figure 2.1i).
Table 2.1: The effect of *Alternanthera nana* sowing time on *Santalum album* height, stem diameter at 2cm, leaf, stem and root dry weight (DW), *A. nana* ground cover area and shoot DW and number of connected haustoria per plant immediately prior to field establishment.

<table>
<thead>
<tr>
<th>Age of <em>S. album</em> at the time of <em>A. nana</em> introduction (days)</th>
<th>Length of <em>S. album</em> : <em>A. nana</em> association in a pot prior to field planting (days)</th>
<th><em>Santalum album</em></th>
<th><em>Alternanthera nana</em></th>
<th>No. of connected haustoria per plant&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Height (mm)</td>
<td>Diameter (mm)</td>
<td>Leaf DW (g)</td>
<td>Stem DW (g)</td>
</tr>
<tr>
<td>54</td>
<td>134</td>
<td>374.1 ± 142.2 a</td>
<td>3.9 ± 1.30 a</td>
<td>20.6</td>
</tr>
<tr>
<td>79</td>
<td>109</td>
<td>398.2 ± 80.4 a</td>
<td>4.4 ± 0.73 a</td>
<td>21.8</td>
</tr>
<tr>
<td>104</td>
<td>84</td>
<td>390.0 ± 96.2 a</td>
<td>4.2 ± 0.78 a</td>
<td>24.3</td>
</tr>
<tr>
<td>128</td>
<td>60</td>
<td>329.5 ± 56.7 a</td>
<td>4.0 ± 0.79 a</td>
<td>20.9</td>
</tr>
<tr>
<td>153</td>
<td>35</td>
<td>379.5 ± 89.6 a</td>
<td>4.1 ± 0.60 a</td>
<td>24.3</td>
</tr>
<tr>
<td>Control&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0</td>
<td>394.5 ± 116.1 a</td>
<td>4.2 ± 0.92 a</td>
<td>25.0</td>
</tr>
</tbody>
</table>

*S. album* height and diameter and *A. nana* surface area data are from 6 replicates and DW and haustoria data are from 1 replicate (see text). Means followed by the same letter are not significantly different from each other (p > 0.05 using Tukey's pairwise t-test).<sup>a</sup> Connected haustoria numbers are the total on the surface of the undisturbed pot medium within three 4cm<sup>2</sup> areas (see text).<sup>b</sup>, Control is *S. album* without an *A. nana* pot host.
Figure 2.1: Santalum album (i) mean survival, (ii) mean stem diameter at 10cm and (iii) mean shoot height for 23 weeks after field establishment. Seedlings were grown with Alternanthera nana as a pot host for (■) 134, (△) 109, (▲) 84, (□) 60, (◇) 35 days prior to field establishment or had no pot host (▽). Data are from 5 replicates. See Table 2.2 for statistical data.
Seedling stem diameter and height increased over time, irrespective of the pot host treatment (Figures 2.1ii and 2.1iii). In relative terms, for all harvests the diameter of seedlings grown with *A. nana* for 134 and 109 days prior to field establishment was almost double that of seedlings grown with *A. nana* for 35 days prior to field establishment or that of seedlings without a pot host. A comparison of *S. album* growth 16 weeks after field planting when grown with *A. nana* for 134 and 84 days prior to field establishment and without a pot host is shown in Figure 2.2.

Seedling root and shoot DW increased over time, irrespective of treatment. The absolute difference in the root and shoot DW of seedlings grown with *A. nana* for 134 days prior to field establishment rather than for shorter periods increased between the 16 and 23 week harvests. During this period the root and shoot DW of seedlings grown with *A. nana* for 109 days and less prior to field establishment remained relatively constant (Figures 2.3i and 2.3ii).

For seedlings grown with *A. nana* for 109 and 134 days prior to field establishment the leaf component was between 35 - 50% of the plant DW over time (Figure 2.4). For seedlings with no pot host the leaf component was between 15 - 25% of plant DW over time. For root DW the inverse relationship exists. The proportion of dry matter in stems remained relatively constant for all treatments, between 30 - 40% of the plant DW. Due to this relationship the root : shoot ratio for seedlings grown with *A. nana* for 134 and 109 days prior to field establishment remained lower than all other treatments (Figure 2.3iii). Seedling root : shoot ratio remain relatively stable over time, except for seedlings grown with no *A. nana* pot host, for which the root : shoot ratio increased steadily up to the 16 week harvest and then declined down to the 23 week harvest.

Survival of *A. nana* after transfer to the field remained constant for plants grown with *S. album* for 134 and 109 days prior to field establishment, however survival for the remaining treatments declined over time (Figure 2.5i). Pot host shoot DW and ground cover area increased over time except for the shoot DW of the *A. nana* propagated 60 days before field establishment and the ground cover area of the *A. nana* propagated 109 days before field establishment (Figures 2.5ii and 2.5iii).

The length of time of *S. album : A. nana* association in a nursery container prior to field establishment affected growth of all parasite and host parts and survival after 23 weeks in the field (Table 2.2).
Figure 2.2: Growth of *Santalum album* seedlings 16 weeks after establishment in the field on a flood irrigated Cununurra clay site, Ord River Irrigation Area, north Western Australia. Plants had *Alternanthera nana* (seen at base of the *S. album* seedlings in i. and ii.) as a pot host for (i.) 134 days before transfer to the field, (ii.) 84 days and (iii.) no pot host. Rule is 1 metre.
Figure 2.3: Changes in *Santalum album* (i) mean root dry weight (DW), (ii) mean shoot DW and (iii) mean root: shoot ratio for 23 weeks after field establishment. Seedlings were grown with *Alternanthera nana* as a pot host for (■) 134, (Δ) 109, (●) 84, (□) 60, (◆) 35 days prior to field establishment or had no pot host (◇). Data are from 5 replicates. See Table 2.2 for statistical data.
Figure 2.4: The proportion of the total *S. album* plant dry weight from (i) leaf, (ii) stem and (iii) root for 23 weeks after field establishment. Seedlings were grown with *Alternanthera nana* as a pot host for (A) 134 and (B) 109 days prior to field establishment or had no pot host (C). Data are from 3 replicates.
Figure 2.5: Response of Alternanthera nana pot host (i) mean survival, (ii) mean shoot dry weight (DW) and (iii) mean surface area for 23 weeks after field establishment. A. nana were grown as a pot host in association with Santalum album for (■) 134, (△) 109, (●) 84, (□) 60, (◆) 35 days prior to field establishment. Survival and ground cover area data are from 5 replicates and shoot DW data are from 3 replicates. See Table 2.2 for statistical data.
Table 2.2: The effects of the length of time *Santalum album* was parasitised to *Alternanthera nana* pot host in the nursery on plant growth for 23 weeks in the field. *, Treatment means are not significantly (ns) different from each other (p > 0.05). †, Numbers are the probability of accepting the null hypothesis of no difference in treatment means.

<table>
<thead>
<tr>
<th>Dependent variable</th>
<th>Weeks after field establishment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>11</td>
</tr>
<tr>
<td><strong>Parasite</strong></td>
<td></td>
</tr>
<tr>
<td>Survival</td>
<td>ns*</td>
</tr>
<tr>
<td>Diameter</td>
<td>0.001b</td>
</tr>
<tr>
<td>Height</td>
<td>0.001</td>
</tr>
<tr>
<td>Shoot DW</td>
<td>0.003</td>
</tr>
<tr>
<td>Leaf DW</td>
<td>0.008</td>
</tr>
<tr>
<td>Stem DW</td>
<td>0.002</td>
</tr>
<tr>
<td>Root DW</td>
<td>0.023</td>
</tr>
<tr>
<td>R:S ratio</td>
<td>0.005</td>
</tr>
<tr>
<td><strong>Host</strong></td>
<td></td>
</tr>
<tr>
<td>Survival</td>
<td>0.007</td>
</tr>
<tr>
<td>Shoot DW</td>
<td>0.001</td>
</tr>
<tr>
<td>Ground cover area</td>
<td>0.001</td>
</tr>
</tbody>
</table>
After 11 weeks in the field *S. album* DW and root : shoot ratio were strongly correlated to *A. nana* DW (Figure 2.6). All *S. album* DW components increased when it was parasitised to large *A. nana* plants. Conversely, *S. album* root : shoot ratio decreased when *S. album* was parasitised to large *A. nana* plants. The length of time of the *S. album* : *A. nana* combination in the nursery affected the *S. album* foliar concentration of all nutrients except Ca, Mg and Mn during subsequent growth in the field (Table 2.3).

Foliar P and Na were at a maximum for seedlings grown without an *A. nana* pot host (Figures 2.7i and 2.7iii). There was a decrease in P and Na concentrations from seedlings grown with *A. nana* for 35 days and 134 days prior to field establishment with about a 0.6% and 25.6% decrease in P and Na concentrations, respectively, for each 1 day increase in the length of time seedlings are grown with *A. nana* prior to field establishment.

Foliar K concentration was higher for seedlings grown with *A. nana* for 134 days prior to field establishment (Figure 2.7ii). There was a linear increase in foliar K levels from seedlings grown with *A. nana* for 35 days and 134 days prior to field establishment with about a 1.71% increase in K levels for each 1 day increase in the length of time seedlings are grown with *A. nana* prior to field establishment. Seedling foliar Ca concentration was relatively constant, between 0.843 - 1.087%, irrespective of treatment. As a result seedlings grown for longer with *A. nana* pot hosts had a greater foliar K : Ca ratio and this difference was maintained over the 23 week period of growth in the field (data not shown). Foliar N concentration were highest for seedlings grown with *A. nana* for 134 days prior to field establishment (data not shown) but was not significantly different between treatments at the harvests at 16 and 23 weeks (Table 2.3). Foliar N concentrations remained constant over a 23 week period in the field, between 3.02 – 3.16% for *S. album* grown with *A. nana* for 134 days prior to field establishment and 2.20 – 2.71% for *S. album* grown without a pot host.
Figure 2.6: Relationship between (i) Santalum album plant, shoot and leaf dry weight (DW) and (ii) S. album root : shoot ratio to Alternanthera nana pot host dry weight (DW). Seedlings were grown with Alternanthera nana as a pot host for (■) 134, (▲) 109, (●) 84, (□) 60, (◆) 35 days prior to field establishment or had no pot host (▽). Data are from all six treatments from the harvest 11 weeks after growth in the field.
Table 2.3: The effects of the length of time *Santalum album* was parasitised to *Alternanthera nana* pot host in the nursery on *S. album* foliar nutrient concentration for 23 weeks in the field. *a*, Treatment means are not significantly (ns) different from each other (p > 0.05). *b*, Numbers are the probability of accepting the null hypothesis of no difference in treatment means.

<table>
<thead>
<tr>
<th>Dependent variable</th>
<th>Weeks after field establishment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>11</td>
</tr>
<tr>
<td>N</td>
<td>0.033&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>P</td>
<td>ns</td>
</tr>
<tr>
<td>K</td>
<td>0.001</td>
</tr>
<tr>
<td>Ca</td>
<td>ns</td>
</tr>
<tr>
<td>Mg</td>
<td>ns</td>
</tr>
<tr>
<td>Zn</td>
<td>0.003</td>
</tr>
<tr>
<td>Cu</td>
<td>0.020</td>
</tr>
<tr>
<td>Mn</td>
<td>ns</td>
</tr>
<tr>
<td>Na</td>
<td>0.001</td>
</tr>
<tr>
<td>K/Ca ratio</td>
<td>0.001</td>
</tr>
</tbody>
</table>
Figure 2.7: *Santalum album* foliar (i) phosphorus, (ii) potassium and (iii) sodium concentration 16 weeks after field establishment. *S. album* plants had different periods of association with *Alternanthera nana* as a pot host before transfer to the field. Treatment means followed by the same letter are not significantly different (p > 0.05) using Tukey's pairwise t-test. Bars show SE's. Data are from 3 replicates.
2.5. DISCUSSION

2.5.1. PLANT GROWTH

*S. album* seedlings parasitised to *A. nana* early during cultivation in pots in the nursery showed significantly higher growth when transferred to the field, than plants parasitised to *A. nana* for less time before field establishment. *S. album* seedlings not attached to *A. nana* prior to field establishment performed poorly. During 23 weeks in the field the *S. album* root : shoot ratio was highest for *S. album* which had no pot host and lowest for seedlings grown for 134 days in the nursery with *A. nana* before field establishment. The differences in *S. album* root : shoot ratio may be an adaptation of the parasite which maximises plant growth, giving priority to shoot growth over root growth once adequate haustorial contact is made and host mass and vigour are adequate. It appears that *S. album* uses *A. nana*’s root system as an extension of its true root system to support a large shoot biomass.

In this experiment, vigorous *S. album* seedlings had a root : shoot ratio between 0.29 - 0.34. This is a low root : shoot ratio compared to the root : shoot ratio of 1.28 – 1.46 for the root hemi-parasite *Olay phyllanthi* (Labill.) R. Br. (Pate et al. 1990a) and 1.4 – 2.9 for unattached *S. spicatum* (R. Br.) DC. 180 - 210 days following germination (Wijesuriya and Fox 1985). A high *S. album* seedling root : shoot ratio may increase survival following field establishment (Fox et al. 1995b). A low root : shoot ratio may lead to increased *S. album* mortality or at least a reduced growth rate if *A. nana* died. The death of the pot host would result in *S. album* losing its ‘artificial’ root extension, and in effect create a water and nutrient deficit for *S. album*. Root : shoot ratios increase in response to water (Begg and Turner 1976; Stoneman and Dell 1993) and nutrient deficits (Ericsson 1981; Cannell 1985; Nadelhoffer et al. 1985; Cromer and Jarvis 1990; Kirschbaum et al. 1992; Raison and Myers 1992; Cromer et al. 1993) and one would predict that if *S. album* experienced a water and nutrient deficit, biomass partitioning would change to favour allocation to roots, if the seedling did not perish.

Through root parasitism *S. album* establishes a large shoot early during the seedling stage at the expense of its own root system. This probably serves to increase its photosynthetic area to increase sunlight interception. Seeds of *S. album* germinate and establish naturally in Timor, eastern Indonesia, during the tropical monsoon season, which is the wettest and hottest period of the year, when competitive grasses and broadleaf species grow prolifically (Evans 1986). While these may be useful initial hosts they compete with *S. album* for light. Our observations are that, contrary to Barrett and Fox’s (1994) statement that *S. album* seedlings are shade
tolerant, within ORIA plantations *S. album* remains stunted under the heavy shade of hosts. That is, *Santalum* species are more heliophilic as seedlings than sciophilic (Nayar *et al.* 1988; Havel and McKinell 1993; Shinde *et al.* 1993; Veillon and Jaffre 1995). Barrett and Fox (1994) make the general observation that when *S. album* is grown in the open its canopy is often nearly bare whilst in dense shade, in association with other trees, it has large leaves which are a darker green and thicker. We suggest that this is not a shade related phenomenon. A sparse *S. album* canopy when grown without a host is a result of increased water and nutrient deficits, whereas when in association of hosts *S. album* develops a vigorous crown as a result of using the host roots as an extension of its own root system, therefore lowering its root : shoot ratio (A. M. Radomiljac and J. A. McComb unpublished data).

The optimal length of time for the *S. album* : *A. nana* association in a nursery pot prior to field establishment for subsequent high survival and growth of *S. album* in the field was between 109 - 134 days. Although several parameters suggest that growth is best given 134 days nursery association with *A. nana*, *S. album* field survival was higher at all harvests when it had been grown with *A. nana* for 109 rather than 134 days prior to field establishment. *S. album* seedlings were smothered by *A. nana* when it was introduced into the pot with *S. album* seedlings younger than 8 weeks old.

This period of pot host parasitism is considerably less than that practiced in other countries but the optimal time could vary with host species. In India, *Cajanus cajan* Huth. seed is sown directly into a pot at the same time as the germinated *S. album* seed, and the two species grow together in the nursery for 180 - 240 days until field establishment (Srinivasan *et al.* 1992). In Indonesia, either *Desmanthus virgatus* (L.) Willd., *Alternanthera* spp. Forsskal cv. (Fox *et al.* 1996) or *Acacia villosa* Willd. (Surata *et al.* 1995) are grown in combination with *S. album* for 210 days (Fox *et al.* 1996).

The role of the pot host at the time of field establishment is two-fold: to reduce *S. album* outplanting stress and to act as the initial host (Radomiljac 1998). The pot host is important until the intermediate host is parasitised. In this experiment the survival of *A. nana* in the field 6 months following field establishment fell to 30 – 60% for the three treatments giving least time in combination with *S. album* in the nursery, whilst *A. nana* survival of the two treatments giving greatest association time in the nursery remained around 98%. When grown as single parasite : host pairings *S. album* is a debilitating parasite causing earlier mortality in smaller and weaker host plants. Early *A. nana* mortality breaks the parasite : host relationship resulting in reduced *S. album* survival and/or growth (Radomiljac 1998), whereas larger *A. nana* plants
were beneficial to *S. album* survival and growth following field establishment.

This experiment indicated that an important aspect of the value of the pot host becomes manifested following field establishment. During nursery propagation the growth of unattached seedlings was similar to attached seedlings, suggesting that young *S. album* seedlings may remain photoautotrophic under nursery conditions, when water and nutrient supply is optimal. This therefore throws into question a number of nursery studies that draw conclusions about the suitability of pot host species (Surata 1992b; Fox and Doronila 1993) without testing the performance of parasite and host following field establishment.

### 2.5.2. Mineral concentration of the parasite

*S. album* seedlings parasitised to *A. nana* for longer periods prior to field establishment had a higher foliar K : Ca ratio. A high K : Ca ratio is a feature of parasitic angiosperms (Grovier *et al.* 1967; Tsivion 1978; Wolswinkel 1978; Lamont and Southall 1982; Struthers *et al.* 1986). K and Ca are phloem and xylem mobile elements, respectively. The difference between parasitic plants which depend mainly on host xylem-sap and those which depend on substances derived from the phloem becomes manifest in the K : Ca ratio (Tsivion 1987), a higher ratio suggesting a phloem feeding parasite (Ziegler 1976). In this experiment *S. album* attached to advanced *A. nana* pot hosts had a K : Ca ratio > 6 which suggests *S. album* is not solely reliant on xylem absorption. Struthers *et al.* (1986) recorded that *S. spicatum* attached to *A. acuminata* Benth. had a foliage K : Ca ratio of 1.9. By comparison the phloem feeding holoparasites *Orobanche ramoa* L. (Ernst 1986) and *O. australis* F. Muell. (Pate 1995a) had a shoot K : Ca ratio of 18.6 and 5.21, respectively.

In this study K concentration of *S. album* foliage were significantly higher in attached compared with unattached plants, consistent with studies on other root hemi-parasites by Klaren and Jansen (1978), Struthers *et al.* (1986) and Pate *et al.* (1990a). In some root hemi-parasites (Webb 1984; Kuo *et al.* 1989) the parasite xylem - host xylem continuity is formed by the differentiation of endophytic cells into open-ended vessels which penetrate host vessels. There is no phloem continuity between parasite and host (Webb 1984). Since K is a phloem mobile element and in this experiment *S. album* parasitised to *A. nana* for longer periods had fivefold higher concentrations of K in foliage it is presumed there is a loading of K into *S. album* from host phloem. However the means of K enrichment remain unclear (Stewart and Press 1990).
In this experiment the *S. album* foliar Ca concentration was not significantly different between attached and unattached *S. album* with the concentration remaining constant over time. This is consistent with Klaren and Jansen (1978) who observed a gradual accumulation of Ca in both attached and unattached *Rhinanthus serotinus* (Schonh.) Oborny, whilst K increased considerably. As a result the K : Ca ratio was lower in the unattached *R. serotinus* in comparison to attached plants. In contrast Pate *et al.* (1990a) observed a marked increase in the Ca concentration of *O. phyllanthi* seedlings following attachment.

*S. album* parasitised to advanced *A. nana* pot hosts had a significantly lower concentration of foliar Na compared to unattached *S. album*. In contrast Struthers *et al.* (1986) observed that attached *S. spicatum* has a high foliar Na concentration, compared to unattached plants. Struthers *et al.* (1986) considers that a high Na concentration would serve to maintain a strong water potential gradient to assist with moisture absorption from its host. This study suggests that K may be a more important osmotically active compound. The coincident decline in *S. album* foliar Na concentration with increasing K concentration when attached to *A. nana* for longer periods suggests a substitution of one monovalent osmoticum for another.

Seedlings grown with *A. nana* for longer in the nursery prior to field establishment and those attached to more vigorous host plants in the field had greater growth than seedlings grown without a host. Greater *S. album* growth was associated with increased foliar K, lower foliar P and Na and a relatively constant foliar N concentration. This indicates that *A. nana* vigour or the length of time of the *S. album : A. nana* association did not significantly influence the nitrogen status of *S. album*.

**2.6. CONCLUSION**

This shadehouse and field experiment showed that manipulation of the utilisation of *A. nana* as a pot host leads to significant increases in *S. album* field survival and growth. The silvicultural treatment of increasing the period of *S. album : A. nana* association in the nursery prior to field establishment increased early *S. album* plantation growth markedly.
CHAPTER 3

INTERMEDIATE HOST INFLUENCES ON THE ROOT HEMI-PARASITE SANTALUM ALBUM L. BIOMASS PARTITIONING.

3.1. ABSTRACT

*Santalum album* L. seedlings parasitised on the N$_2$-fixing woody hosts *Sesbania formosa* (F. Muell.) N. Burb., *Acacia trachycarpa* E. Pritzel and *A. ampliceps* Maslin and the non N$_2$-fixing woody host *Eucalyptus camaldulensis* Dehn. were grown for 38 weeks in 25 litre nursery containers. *S. album* growth was greater and root : shoot ratio lower for *S. album* seedlings grown with N$_2$-fixing hosts compared with seedlings grown with *E. camaldulensis* or with no host. Seedlings grown with *S. formosa* had a greater stem diameter, height, leaf area, root and shoot dry weight (DW) than all other treatments. *S. album* grown with *S. formosa* and *A. ampliceps* had a lower root : shoot ratio than all other treatments at all assessments. The root : shoot ratio of unattached *S. album* increased exponentially over the 38 week period. Seedling growth declined for all treatments between the 33 and 38 week harvests, except for those seedlings attached to *A. trachycarpa*. A strong positive linear relationship was shown between *S. album* leaf area and shoot DW irrespective of host species. No relationship was found between *S. album* shoot DW and root : shoot ratio with host shoot DW. The combined *E. camaldulensis* and *S. album* root system supported a smaller *S. album* shoot biomass compared with the *S. album* shoot biomass supported by the combined root systems of the N$_2$-fixing hosts and *S. album*. Compared with all other host species the *A. trachycarpa* root system was more efficient in supporting its own shoot biomass and the total biomass of *S. album*. Host use efficiency (*S. album* shoot DW / host shoot DW) values of *S. formosa* and *A. trachycarpa* were greater than the host use efficiency values of *A. ampliceps* and *E. camaldulensis*. Values for unparasitised *S. formosa* leaf area, shoot and root DW and root : shoot ratio were greater than those for parasitised plants.
3.2. Introduction

Indian sandalwood is the highly valued aromatic heartwood from the sub-tropical root hemi-parasite Santalum album L. Sandalwood utilisation has occurred for 20 to 30 centuries in eastern cultures (Srinivasan et al. 1992) and over exploitation of most natural populations has generated an acute supply shortage (Havel and McKinnell 1993). Establishment of plantations of S. album in northern Western Australia is progressing (Radomiljac 1998; Radomiljac et al. 1998a, b). The parasitic requirement of S. album greatly increases the complexity of plantation silviculture (Radomiljac et al. 1998a).

The single most important silvicultural parameter influencing plantation survival and growth is the host plant. For plantations, host plants are divided into three categories: pot, intermediate and long term hosts (Fox 1996; Radomiljac et al. 1998a). The function of the pot host has been described (Radomiljac et al. 1998b). In northern Western Australia the herbaceous Alternanthera nana R. Br. has been shown be an ideal host as it promotes high S. album survival and growth following field establishment (Radomiljac 1998). The intermediate and long term hosts are propagated simultaneously in separate nursery containers. At field establishment both host types are strategically placed within the plantation. The intermediate host, usually a fast-growing short-lived perennial, acts as a ‘bridging agent’ between the pot host and long term host and should promote early S. album plantation growth. Intermediate hosts eventually die or becomes less important following S. album attachment to the long term host, which should persist as final host for the entire rotation length.

A number of studies on softwood species have shown a positive correlation between early growth rates and the extent of future heartwood production (Hillis and Ditchburne 1974; Hillis 1987; Wilkes 1991; Climent et al. 1993). Hillis and Ditchburne (1974) showed Pinus radiata Donn. trees with the most rapid growth in the first five years had the greatest heartwood diameter at all future ages.

It is important to determine if high early stem growth is positively correlated to high future S. album heartwood production as if so, the intermediate host is an extremely important silvicultural component being effective during the early phase of plantation growth.

Despite the importance of the S. album : host relationship on S. album plantation productivity, the relationship between growth and carbon allocation between the root hemi-parasite and its woody hosts has not been fully resolved (Nayar et al. 1988; Subbarao et al. 1990). Surprisingly little literature exists on the host influences on S. album biomass partitioning under controlled
conditions. Growth studies on root hemi-parasites have concentrated almost exclusively on the economically important agricultural weeds, such as Orobanchaceae (Orobanche or broomrape) and Scrophulariaceae (Striga or witchweed) (Press and Stewart 1987; Graves et al. 1989; Sauerborn 1991; Graves 1995) or species which have no economic importance, such as Olax phyllanthi (Labill) R. Br. (Tennakoon and Pate 1996a). Scant literature exists on experiments designed to promote growth of root hemi-parasites.

This chapter examines the effect of intermediate woody hosts on the growth of S. album seedlings and the null hypothesis tested is that S. album growth is not enhanced when attached to legume intermediate hosts.

3.3. MATERIALS AND METHODS

The effect of intermediate hosts on the growth of pot cultured S. album seedlings was studied in the Department of Conversation and Land Management’s nursery at Kununurra (lat 15° 46’ S. long 128° 44’ E), Western Australia. The experiment comprised 5 S. album : intermediate host species single plant pairings (intermediate host plants were : Sesbania formosa (F. Muell.) N. Burb, Acacia trachycarpa E. Pritzel, Acacia ampliceps Maslin, Eucalyptus camaldulensis Dehn. and a no host control) x 3 harvests (24, 33 and 38 weeks after the placing of S. album and the intermediate host together in pots) x 8 replicates in a randomised complete block design.

In July 1996, 200 uniform S. album seedlings (height; 40.5 ± 2.98 cm and basal diameter 3.9 ± 0.58 mm) about 6 months old growing in 1.4 litre pots with Alternanthera nana as a pot host, were selected. S. album seedlings were propagated following Radomiljac (1998). The A. nana was cut to soil level and the S. album seedling transplanted to 25 litre pots of coarse river sand : peat : perlite at 3 : 2 : 2. Pots were placed on mesh benches 10 cm above ground to avoid root contamination between treatments. A single intermediate host seedling inoculated with Bradyrhizobium where appropriate (Table 3.1) was transplanted to the pot and positioned about 150 mm from the single S. album seedling. Each intermediate host species was also transplanted into an unreplicated pot and remained as an unparasitised control plant. Intermediate host seedlings were propagated following Radomiljac (1998).
Table 3.1: Host plant origins and *Rhizobium* inocula.

<table>
<thead>
<tr>
<th>Host species</th>
<th>Family</th>
<th>CSIRO seedlot No.</th>
<th>Location</th>
<th><em>Rhizobium</em> inoculum strain No.</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Sesbania formosa</em></td>
<td>Papilionaceae</td>
<td>18833</td>
<td>Ivanhoe Crossing, WA</td>
<td>PMA 295/2 5/97</td>
</tr>
<tr>
<td></td>
<td>(Fabaceae)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Acacia trachycarpa</em></td>
<td>Mimosaceae</td>
<td>16774</td>
<td>Mt. Lockyer, WA</td>
<td>PMA 469 5/97</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Lat 22° 24' S; Long 118° 47' E</td>
<td></td>
</tr>
<tr>
<td><em>Acacia ampliceps</em></td>
<td>Mimosaceae</td>
<td>18648</td>
<td>N. shore Lake Nongra, NT</td>
<td>PMA 251/1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Lat 18° 10' S; Long 129° 46' E</td>
<td></td>
</tr>
<tr>
<td><em>Eucalyptus camaldulensis</em></td>
<td>Myrtaceae</td>
<td>15050</td>
<td>Gibb River, WA</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Lat 16° 30' S; Long 126° 10' E</td>
<td></td>
</tr>
</tbody>
</table>

*a*. Commonwealth Scientific and Industrial Research Organisation Forestry and Forest Products, Australian Tree Seed Centre, Canberra.

*b*. University of Queensland, Department of Agriculture, St. Lucia.
A slow release fertiliser, Scotts® Osmocote Plus (8-9M) (N 16.0%, P 3.5%, K 10.0%, S 2.4%, Mg 1.2%, B 0.02%, Cu 0.05%, Fe 0.4%, Mn 0.06%, Mo 0.02% and Zn 0.015%) was then applied at 10 g on the surface of each pot. Four weeks later A. nana was completely removed from the pots. Seedlings remained in the nursery for a further 10 months in full sun with overhead watering twice daily, for approximately 15 minutes, to near field capacity.

There were four replicates of five pots with each S. album : intermediate host treatment and S. album without a host within each replicate. All pots with unparasitised hosts were positioned in one replicate. All pots were randomised within each replicate. The position of the replicate and pots within each replicate were randomly changed only twice due to the large pot size and weight.

3.3.1. Assessment

Immediately prior to, and 13, 24, 33 and 38 weeks after planting the host and parasite together, S. album and host height and diameter at 2 cm were measured.

On weeks 24, 33 and 38 weeks of the association of parasite and host, three pots per treatment were selected on the basis of one seedling equal to, one + 1SD and one – 1SD of the mean S. album diameter. S. album and host plants were harvested by cutting the stem at soil level and the shoot partitioned into stem and leaf material. S. album and host leaf, stem and root dry weight (DW) was recorded after plant material had been oven dried at 80°C for 48 hours. Remaining pots were retained for further study.

Leaf areas of both S. album and hosts were determined by passing a leaf sub-sample through a planimeter (Paton Electronic Planimeter, Stepney, S. Aust.). The DW of the sub-sample was then obtained and the leaf area to DW ratio calculated. S. album specific leaf area (SLA) was determined from the ratio of S. album total leaf area : total leaf DW.

In this chapter we attempt to determine the effectiveness of hosts by examining the partnerships in the following ways:

- The ratio between the S. album shoot DW and the host shoot DW was termed ‘host use efficiency’ (HUE).

- The total root biomass utilised by the S. album shoot includes its own roots as well as the host
root system. The ratio between this total root DW and the DW of the *S. album* shoot is termed the ‘host root extension’ (HRE).

- The total biomass supported by the host root includes its own shoot as well as the roots and shoot of *S. album*. The ratio between the root DW of the host and the total of the host shoot and *S. album* root and shoot DW is termed the ‘host root support’ (HRS).

Growth data were analysed using ANOVA and Tukey’s pairwise t-test. All analyses were performed using SYSTAT® statistical software (Systat 1992). Regressions were fitted using the regression procedure of SYSTAT®.

### 3.4. Results

Differences in growth of *S. album* as a result of parasitism of different host species became clear after week 13. The increase in *S. album* height and diameter plateaued between week 33 and 38 except for seedlings attached to *A. trachycarpa* (Figures 3.1i and ii). In relative terms seedlings attached to *S. formosa* showed twice the diameter and three times the height of seedlings grown with *E. camaldulensis*. A comparison of *S. album* growth 33 weeks after planting the host and parasite together is shown in Figure 3.2. Unattached seedlings were consistently larger than those attached to *E. camaldulensis*.

Hosts parasitised by *S. album* increased in height and diameter (Figures 3.1iii and iv). *A. trachycarpa* height and diameter were always significantly lower than all other species.

*S. album* seedling leaf DW and leaf area increased irrespective of the host species for 33 weeks then declined with the exception of *S. album* attached to *A. trachycarpa*, which continued to increase (Figure 3.3). Seedling stem DW remained relatively stable between the harvests at 33 and 38 weeks, except for seedlings attached to *A. ampliceps* and *A. trachycarpa*, which decreased and increased, respectively. Unattached seedlings had consistently higher leaf and stem DW and leaf area than seedlings attached to *E. camaldulensis*.

The growth of all parasite and host plant parts was affected by the combination of host and parasite (Table 3.2). The *S. album* seedling root and shoot DW increased irrespective of the host species treatment up to the harvest at 33 weeks.
Figure 3.1: Growth of *Santalum album* (i) mean height and (ii) mean stem diameter of plants whilst attached to (Δ) *Sesbania formosa*, (○) *Acacia ampliceps*, (□) *A. trachycarpa*, (◊) *Eucalyptus camaldulensis* and (▽) no host control. Growth of host species (▲) *Sesbania formosa*, (●) *Acacia ampliceps*, (■) *A. trachycarpa* and (◇) *Eucalyptus camaldulensis* (iii) mean height and (iv) mean stem diameter whilst parasitised by *S. album*. Data are from 8 replicates. See Table 3.2 for statistical data.
Figure 3.2: Growth of the root hemi-parasite *Santalum album* (Sa) after 33 weeks in pot culture with (i) *Sesbania formosa* (Sf), (ii) *Acacia trachycarpa* (At), (iii) *A. ampliceps* (Aa), (iv) *Eucalyptus camaldulensis* (Ec) and (v) no host. Rule is 1 metre.
Figure 3.3: Growth of Santalum album (i) mean leaf dry weight (DW), (ii) mean stem DW and (iii) mean leaf area whilst attached to (Δ) Seshania formosa, (○) Acacia ampliceps, (□) A. trachycarpa, (○) Eucalyptus camaldulensis and (◇) no host. Host and parasite associations were grown as single plant pairings in 25 litre pots under nursery conditions. Data are from 3 replicates. See Table 3.2 for statistical data.
Table 3.2: The effects of intermediate host species on \textit{S. album} growth and the growth of intermediate hosts. \textsuperscript{a} Numbers are the probability of no difference in treatment means. \textsuperscript{b} No measurement (NM) taken. \textsuperscript{c} Treatment means are not significantly (ns) different from each other (p > 0.05).

<table>
<thead>
<tr>
<th>Dependent variable</th>
<th>Weeks after parasite and host association</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>13</td>
</tr>
<tr>
<td><strong>Parasite</strong></td>
<td></td>
</tr>
<tr>
<td>Height</td>
<td>0.004\textsuperscript{a}</td>
</tr>
<tr>
<td>Diameter</td>
<td>0.001</td>
</tr>
<tr>
<td>Leaf DW</td>
<td>NM\textsuperscript{b}</td>
</tr>
<tr>
<td>Stem DW</td>
<td>NM</td>
</tr>
<tr>
<td>Shoot DW</td>
<td>NM</td>
</tr>
<tr>
<td>Root DW</td>
<td>NM</td>
</tr>
<tr>
<td>R:S ratio</td>
<td>NM</td>
</tr>
<tr>
<td>Leaf area</td>
<td>NM</td>
</tr>
<tr>
<td>Host use efficiency</td>
<td>NM</td>
</tr>
<tr>
<td>\textit{S. album} + host root: \textit{S. album} shoot ratio</td>
<td>NM</td>
</tr>
<tr>
<td>Host shoot + \textit{S. album} plant: Host root ratio</td>
<td>NM</td>
</tr>
<tr>
<td>Specific leaf area</td>
<td>NM</td>
</tr>
<tr>
<td><strong>Host</strong></td>
<td></td>
</tr>
<tr>
<td>Height</td>
<td>0.001</td>
</tr>
<tr>
<td>Diameter</td>
<td>0.001</td>
</tr>
<tr>
<td>Leaf DW</td>
<td>NM</td>
</tr>
<tr>
<td>Stem DW</td>
<td>NM</td>
</tr>
<tr>
<td>Shoot DW</td>
<td>NM</td>
</tr>
<tr>
<td>Root DW</td>
<td>NM</td>
</tr>
<tr>
<td>R:S ratio</td>
<td>NM</td>
</tr>
<tr>
<td>Leaf area</td>
<td>NM</td>
</tr>
</tbody>
</table>
Root and shoot DW decreased irrespective of the host species treatment between the harvest at 33 and 38 weeks except for *S. album* attached to *A. trachycarpa*, which increased over time. Unattached *S. album* after weeks 24 showed an increase in root DW and a decrease in shoot DW which resulted in an increasing root : shoot ratio over time. The root : shoot ratio of *S. album* grown with *S. formosa* was lower than all other combinations while *S. album* attached to *E. camaldulensis* had the highest root : shoot ratio over time (Figure 3.4).

The impact on biomass partitioning of parasitism on different host species is most clearly seen in week 38 (Figure 3.5). The proportion of DW in stems was similar for *S. album* attached to *S. formosa* and *E. camaldulensis* or when grown without a host, but the proportion of DW in roots was least when in association to *S. formosa* and greatest with no host. The DW of leaves showed the opposite trend. Due to this relationship the root : shoot ratios of seedlings attached to *E. camaldulensis* and unattached seedlings were more than double that of seedlings attached to *S. formosa* (Figure 3.4iiii).

Leaf and stem DW and leaf area of parasitised *S. formosa* and *E. camaldulensis* declined over time, whereas the leaf and stem DW and leaf area of *A. trachycarpa* and *A. ampliceps* increased slightly (Figure 3.6). The leaf DW, stem DW and leaf area of unparasitised *S. formosa* was reduced by parasitism. Data for unparasitised hosts are only shown for *S. formosa* at week 33.

Root and shoot DW increased for all host species between the 24 and 33 week harvests (Figures 3.7i and ii). Parasitised *S. formosa* and *E. camaldulensis* root and shoot DW declined between the 33 and 38 week harvests whereas *A. trachycarpa* and *A. ampliceps* root and shoot DW continued to increase. *A. trachycarpa* root DW remained constantly lower than the other host species. *S. formosa* root : shoot ratio increased exponentially over time remaining constantly higher than the other host species (Figure 3.7iiii). Unparasitised *S. formosa* root DW, shoot DW and root : shoot ratio was greater than that of parasitised *S. formosa* at the 33 week harvest.

For each *S. album* : host association the shoot DW increments of *S. album* between week 24 and 33 were compared with the total shoot DW of the host at week 33 (Table 3.3). *A. ampliceps* shoot DW was significantly greater than that of the other host species at the 33 week harvest but *S. formosa* promoted the greatest *S. album* shoot DW gain between weeks 24 and 33. On this basis *A. trachycarpa* also supported more growth of *S. album* than did *A. ampliceps*. In contrast *E. camaldulensis* was an extremely poor host as *S. album* shoot DW decreased between the two harvests even though *E. camaldulensis* shoot had a high DW.
Figure 3.4: Growth of *Santalum album* (i) mean root dry weight (DW), (ii) mean shoot DW and (iii) mean root: shoot ratio whilst attached to (Δ) *Sesbania formosa*, (○) *Acacia ampliceps*, (□) *A. trachycarpa*, (◊) *Eucalyptus camaldulensis* and (V) no host. Host and parasite associations were grown as single plant pairings in 25 litre pots under nursery conditions. Data are from 3 replicates. See Table 3.2 for statistical data.
Figure 3.5: The proportion of the total *Santalum album* dry weight from leaf, stem and root whilst attached to (A) *Sesbania formosa*, (B) *Eucalyptus camaldulensis* and (C) no host.
Figure 3.6: Growth of host species (△) Sesbania formosa, (○) Acacia ampliceps, (■) A. trachycarpa, (◇) Eucalyptus camaldulensis (i) mean leaf dry weight (DW), (ii) mean stem DW and (iii) mean leaf area whilst parasitised by Sanicula alba and (Δ) unparasitised S. formosa at the 33 week harvest. Parasitised host and unparasitised S. formosa data are from 3 and 1 replicates, respectively. See Table 3.2 for statistical data.
Figure 3.7: Growth of host species (▲) Sesbania formosa, (●) Acacia ampliceps, (■) A. trachycarpa, (♦) Eucalyptus camaldulensis (i) mean root dry weight (DW), (ii) mean shoot DW and (iii) mean root : shoot ratio whilst parasitised by Santalum album and (Δ) unparasitised S. formosa at the 33 week harvest. Parasitised host and unparasitised S. formosa data are from 3 and 1 replicates, respectively. See Table 3.2 for statistical data.
Table 3.3: Growth of the *Santalum album* and four host species when cultured as single plant pairings under nursery conditions. Values as means ± SE. Treatment means followed by the same letter are not significantly different (p > 0.05) using Tukey’s pairwise t-test. Data are from 3 replicates.

<table>
<thead>
<tr>
<th>Host species</th>
<th>Shoot dry weight of host (g plant(^{-1})) after 33 weeks with the association of <em>S. album</em></th>
<th>Shoot dry weight of <em>S. album</em> (g plant(^{-1})) after 33 weeks with the association with a host</th>
<th>Shoot dry weight increment of <em>S. album</em> (g plant(^{-1})) from week 24 to 33 with the association with a host</th>
<th>Shoot dry weight increment of <em>S. album</em> per unit dry weight of host shoot (g g(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Sesbania formosa</em></td>
<td>59.88 (11.02) c</td>
<td>182.40 (46.98) a</td>
<td>131.07 (44.62) a</td>
<td>2.21 (1.19)</td>
</tr>
<tr>
<td><em>Acacia trachylopa</em></td>
<td>30.15 (26.58) c</td>
<td>57.45 (35.21) b</td>
<td>37.97 (54.22) ab</td>
<td>1.77 (3.52)</td>
</tr>
<tr>
<td><em>Acacia ampliceps</em></td>
<td>277.46 (108.56) a</td>
<td>123.21 (58.37) a</td>
<td>76.60 (62.58) ab</td>
<td>0.35 (0.39)</td>
</tr>
<tr>
<td><em>Eucalyptus camaldulensis</em></td>
<td>169.53 (48.72) b</td>
<td>17.13 (8.85) b</td>
<td>-2.00 (11.95) b</td>
<td>-0.01 (0.09)</td>
</tr>
<tr>
<td>No host</td>
<td>-</td>
<td>35.38 (11.44) b</td>
<td>6.60 (3.47) b</td>
<td>-</td>
</tr>
</tbody>
</table>
Thus the HUE (Figure 3.8i) and the parasites' root : shoot ratios were not correlated to host shoot DW (data not shown).

The combined root biomass of *S. album* and *E. camaldulensis* supported markedly less parasite shoot biomass than the combined root biomass of the other parasite : host associations, as shown by HRE values (Figure 3.8ii).

There was little difference in *S. album* specific leaf area (SLA) (Figure 3.8iii). Between the 33 and 38 week harvests the SLA of *S. album* attached to *E. camaldulensis* continued to increase whereas the SLA of *S. album* attached to the other hosts remained constant or decreased.

The total biomass supported by the host root (HRS) was constantly greater for *A. trachycarpa* than that of the other parasite : host associations (Figure 3.9). While that for *E. camaldulensis* was constantly lower than that for the leguminous hosts.

*S. album* shoot DW was positively correlated to plant leaf area (Figure 3.10) independent of the host species association.

### 3.5. Discussion

#### 3.5.1. Parasite Growth-Response to Attachment

All three N₂-fixing species were better hosts than the non N₂-fixing *E. camaldulensis* in promoting parasite growth and on this basis the hypothesis that *S. album* growth is not enhanced when attached to N₂-fixing woody hosts is rejected. This is consistent with Rai (1990) and Taide *et al.* (1994) who both indicate the N₂-fixing *Casuarina equisetifolia* L. (Casuarinaceae) was a superior *S. album* host from pot culture studies. However these studies did not show conclusively that N₂-fixing species were better *S. album* hosts as several N₂-fixing species, *Albizia lebbeck* (L.) Benth., *Acacia auriculiformis* Cunn. Ex Benth. , *Leucaena leucocephala* (Lam.) De Wit (all Mimosaceae) and *Cassia fistula* L. (Caesalpiniaceae) were poorer hosts than several non N₂-fixing species. A recent pot culture experiment by Tennakoon and Pate (1996a) indicated that the N₂-fixing *Acacia littorea* Maslin was a superior host for the root hemi-parasite *O. phyllanthi* than a range of non N₂-fixing C3 and C4 species. *Phoradendron californicum*, a xylem tapping mistletoe, was recorded with higher growth rates when attached to a N₂-fixing host (Schulze and Ehleringer 1984).
Figure 3.8: Changes in *Santalanum album* (i) host use efficiency (HUE) (*S. album* shoot dry weight (DW) / host shoot DW), (ii) ratio of the combined host and *S. album* root : *S. album* shoot DW (HRE) and (iii) mean specific leaf area (SLA) whilst parasitised to (Δ) *Sesbania formosa*, (○) *Acacia ampliceps*, (□) *A. trachycarpa*, (◇) *Eucalyptus camaldulensis* and (▼) no host. Data are from 3 replicates. See Table 3.2 for statistical data.
Figure 3.9: Changes in the ratio of host shoot DW and *S. album* plant DW : host root DW (HRS) whilst *S. album* was attached to (Δ) *Sesbania formosa*, (○) *Acacia ampliceps*, (□) *A. trachycarpa* and (◇) *Eucalyptus camaldulensis*. Data are from 3 replicates. See Table 3.2 for statistical data.
Figure 3.10: *Santalum album* shoot DW in relation to *S. album* leaf area for *S. album* attached to (Δ) *Sesbania formosa*, (○) *Acacia ampliceps*, (□) *A. trachycarpa*, (⊗) *Eucalyptus camaldulensis* and (▼) no host. Data are from the 33 week harvest.
In terms of promoting overall *S. album* growth, *S. formosa* was the superior N\(_2\)-fixing host. However, an important finding from this study was that total *S. album* leaf, stem, shoot and root DW and leaf area continued to increase over time for plants attached to *A. trachycarpa*, whereas growth of all *S. album* plant parts decreased after week 33 for plants attached to the other hosts. This may indicate that *A. trachycarpa* is a more sustainable host. The selection of a suitable intermediate host should thus be performed over an extended period and different species may be optimal depending on the length of time an intermediate host is required.

Growth of unattached *S. album* was consistently greater than the growth of *S. album* when attached to *E. camaldulensis*. This shows that even though *S. album* is an obligate root hemi-parasite its growth may be reduced by attachment to a poor host. There are other reports of poor growth of root hemi-parasites attached to some hosts, such as *O. phyllanthi* attached to *Amaranthus caudatus* L. and *Portulaca oleracea* L. (Tennakoon and Pate 1996a). Tennakoon and Pate (1996a) suggest this is a result of the minimal uptake of reduced N due to the large proportion of nitrate in the hosts' xylem sap and as a result heterotrophic C uptake is reduced. Our findings also suggest that *S. album* may be a poor competitor for nutrients in nutrient deficient soils; whereas *E. camaldulensis*, which occurs naturally on low nutrient status soils, appears to be an extremely efficient competitor. It has been proposed that hosts with allelopathic properties retard *S. album* growth (Taide *et al.* 1994), but there is no direct evidence of this (Fox *et al.* 1996). Further studies on organic solute transfer from host to *S. album* when grown in association with beneficial and non-beneficial hosts to provide key information on host quality is clearly called for.

The exponential increase in the root : shoot ratio of unattached *S. album* seedlings over time compared to the declining ratio for attached seedlings provides evidence that while in search of a host root system DW is directed to the root system at the expense of the shoot. After attachment, DW partitioning shifts from root to shoot and as a result the root : shoot ratio falls (Radomiljac *et al.* 1998b).

In an earlier study (Radomiljac *et al.* 1998b) it was shown that the *S. album* root : shoot ratio declined when *S. album* was attached to larger *Alternanthera* pot hosts and it was hypothesised that *S. album* used the host root system as an extension of its true root system to support a large shoot biomass. In this study no relationship exists between *S. album* root : shoot ratio and the DW of various host species suggesting that the relationship only applies when *S. album* is attached to plants of different sizes of the same host species.

The host root system may be viewed either as supporting the parasite's total biomass coupled
with supporting its own shoot or as an extension of the parasite's own root system. The HRE value for *S. album* attached to *E. camaldulensis* was far higher than that for *S. album* attached to N\textsubscript{2}-fixing hosts, which were comparatively uniform over time. This suggests that the combined *S. album* and *E. camaldulensis* root system performed poorly, relative to the parasite : N\textsubscript{2}-fixing host associations, by only supporting a small *S. album* shoot. Conversely, the HRS value for *S. album* attached to *E. camaldulensis* was consistently lower than the parasite : N\textsubscript{2}-fixing host associations. An interesting observation is that whilst *A. trachycarpa* had the smallest root system its HRS value was significantly greater than the other parasite : host associations. This suggests there is considerable variation between N\textsubscript{2}-fixing species in ability to support the legume shoot biomass coupled with supporting the whole *S. album* biomass.

*S. album* SLA was similar for *S. album* attached to all hosts however plant leaf area was strongly related to *S. album* shoot DW regardless of host species. This is consistent with non-parasitic plants where a linear relationship exists between tree growth and plant leaf area independent of irrigation and fertilisation (Nambiar 1990; Stoneman and Dell 1993). Stoneman and Dell (1993) indicated that irrigation and fertilisation increase leaf area, which increases radiation interception and leads to an increase in growth. N\textsubscript{2}-fixing hosts increased *S. album* leaf area, which was positively correlated to increased parasite growth. *S. album* leaf area index may be a useful predictor of plantation productivity where host quality influences growth.

### 3.5.2. Host Growth-Response to Parasitism.

*S. album* usually develops into a large tree in its native and naturalised habitats of eastern Indonesia (Harisetijono and Suriawihardja 1993) and southern India (Srinivasan et al. 1992). While most root hemi-parasites such as *Rhinanthus serotinus* Schonh. (Klaren and Jansen 1978), *O. phyllanthis* (Pate et al. 1990a) and *Striga hermonthica* (Del.) Benth. (Musselman 1980) remain smaller than their hosts, *S. album*, other *Santalum* species and *Nutysia floribunda* (Labill.) R. Br. (Hocking 1980) are often larger than their hosts.

In this study, the decline in *S. formosa* biomass (96% decrease in leaf area) is evidence of the substantial drain by *S. album* on the resources of a good host species. This has also been shown for other species (Graves et al. 1990; Graves 1995; Tennakoon and Pate 1996a). *S. album* must therefore be classified as a debilitating hemi-parasite. The deleterious effect on *S. formosa* growth may be attributed to *S. album*’s large size, high growth and metabolic rate and high heterotrophic dependency (Graves 1995). *S. album* may have reduced the growth of *S. formosa* by acting as an additional sink for *S. formosa* carbon and reducing its capacity to fix carbon
(Tuohy et al. 1987; Graves et al. 1990). However this is not consistent with the growth patterns of *A. trachycarpa* and *A. ampliceps* which continued to grow under parasitism. Graves (1995) hypothesises that host size and vigour influences the level of damage by the parasite. Even though *A. trachycarpa* and *S. formosa* possessed similar levels of sink resistance (biomass) the greater size of *S. album* attached to *S. formosa* compared to that attached to *A. trachycarpa* suggests more host assimilates were removed from *S. formosa*, resulting in greater host decline.

3.6. **Conclusion**

This pot study showed that association with N$_2$-fixing woody hosts increased early *S. album* growth. *S. formosa* was the superior N$_2$-fixing host as it promoted greatest *S. album* growth, but *A. trachycarpa* may sustain *S. album* growth for a longer period. Growth of *S. album* without a host was consistently greater than the growth of *S. album* when grown with *E. camaldulensis*, indicating that poor hosts may suppress parasite growth. The large differences in *S. album* growth when grown with different host species in this study highlights the importance of identifying suitable intermediate hosts to promote early *S. album* plantation growth.
XYLEM TRANSFER OF ORGANIC SOLUTES IN SANTALUM ALBUM L. (INDIAN SANDALWOOD) IN ASSOCIATION WITH LEGUME AND NON-LEGUME HOSTS.

4.1. ABSTRACT

Indian sandalwood (*Santulum album*), a commercially important root hemi-parasitic angiosperm, was partnered singly in pot culture with one of three N$_2$ fixing legumes or a eucalypt host. Xylem (tracheal) sap of stems of host and parasite and ethanolic extracts of endophytic tissue of haustoria of the parasite were analysed for amino acids, organic acids and sugars to determine which sets of solutes were available to and obtained by the parasite from different hosts.

There were high concentrations of asparagine, followed by glutamate, aspartate and γ-amino butyrate in the xylem sap solutes of the three legume hosts (*Sesbania formosa*, *Acacia trachycarpa* and *A. ampliceps*) and much lower levels of glutamate, aspartate, glutamine and arginine in the non-legume, *Eucalyptus camaldulensis*. Close resemblance's between *Santulum* and legume hosts in concentration and composition of xylem sap amino acids, and in the amino acid spectra of the corresponding parasite endophytic tissue, indicated substantial direct intake of xylem N by *Santulum* from these hosts. By contrast, low N levels in xylem sap of *E. camaldulensis* and dissimilarities between its amino acid composition and that of partnered *Santulum* indicated that the parasite obtained little N from the xylem sap of this host.

Xylem sap of hosts contained variable amounts of sucrose, glucose and fructose, whereas that of matching parasites was dominated by fructose. Dissimilarities were also evident in the proportional amounts of xylem-borne organic acids between hosts and parasite particularly for the eucalypt : *Santulum* partnership.

Leaf extracts of the host : parasite pairings generally showed substantial differences in sugar and organic acid balance between partner species. Similarly, where amino acid spectra of host and parasite xylem sap and corresponding haustorial endophytes were closely similar, respective leaf compositions were markedly dissimilar. This implied that substantial metabolic
patterns of incoming xylem solutes were highly idiosyncratic of the species in question.

Data are related to previous information showing superior growth performance and higher photosynthetic rates and foliar N concentrations in *Santalum* partnered with the three legumes than with the eucalypt.

### 4.2. Introduction

Species of the genus *Santalum* are xylem-tapping obligate root hemi-parasites, which are widely distributed, occurring in India, across the Pacific to the Juan Fernandez Islands and the Ogasawara Islands, and south to southern Australia (Brennan and Merlin 1993). Most species have been exploited for their aromatic heartwood, known as sandalwood, which has customary, religious and medicinal significance in many eastern cultures (Srinivasan *et al.* 1992).

Despite the commercial and cultural importance of the most valuable species, *S. album* L. (Indian sandalwood), an appropriately detailed understanding of its silvicultural requirements has still to be elucidated (Havel and McKinnell 1993). However it is widely accepted that successful establishment on suitable host species can greatly increase *Santalum* growth both in early pot culture and in the field (Surata 1992a; Fox *et al.* 1996; Radomiljac 1998b; Radomiljac *et al.* 1998b). To maintain high growth rates from a beneficial host *S. album* presumably derives bulk quantities of organic solutes from the host xylem stream, but this has not been demonstrated.

Recent studies have provided considerable information on organic solute xylem transfers between two native Australian root hemi-parasites, *Olax phyllanthi* (Labill) R. Br. (Pate *et al.* 1994; Tennakoon and Pate 1996a) and *Santalum acuminatum* (R. Br.) A. DC. (Tennakoon *et al.* 1997b) and their respective hosts. These investigations have shown that substantial differences in amino acid, sugar and organic acid composition of the xylem stream of these parasites occur when associated with various host species, and such differences have been used alongside parasite growth data to explain why some hosts are markedly superior to others in terms of overall benefit to the parasite. This investigative approach will be followed in this study on *S. album*.

It has already been demonstrated in studies on pot-cultured *S. album* (Radomiljac *et al.* 1998c) that three leguminous woody shrub species are superior intermediate hosts for the parasite compared with the non N_{2}-fixing *Eucalyptus camaldulensis* Dehn. We evaluate this effect
further in the present study by comparing the respective amino acid, organic acid and sugar composition of *S. album* in partnership with the same four intermediate hosts. The hypothesis to be tested is that xylem sap composition of host and *S. album*, especially in respect of nitrogenous solutes, may provide key information on host quality when exploited by the parasite. This will be tested by assessing data on tissue and xylem sap composition against previous information on growth responses, photosynthetic rates and foliar N concentrations of the same four partnerships (Radomiljac 1998a; Radomiljac *et al.*1998b).

### 4.3. Materials and Methods

Plants were grown in a nursery near Kununurra (lat 15° 46’ S. long 128° 44’ E), Western Australia. Intermediate woody host species selected were the N\textsubscript{2}-fixing legumes *Sesbania formosa* (F. Muell.) N. Burk. (Papilionaceae), *Acacia trachycarpa* E. Pritzel (Mimosaceae), *Acacia ampliceps* Maslin (Mimosaceae) and the non N\textsubscript{2}-fixing *E. camaldulensis* (Myrtaceae).

In July 1996, single 6-month old plants of *S. album*, raised from seed in 1.4 litre pots in association with the herbaceous pot host *Alternanthera nana* R. Br. (see Radomiljac 1998b), were transplanted into 25 litre pots. These pots were placed onto benches of weldmesh elevated 10 cm above ground and spaced sufficiently apart to avoid intrusion of roots from one pot into another. Seedlings of potential woody host species grown in 0.3 litre pots were transferred singly into the 25 litre pots, and each positioned about 150 mm from the single partner plant of *S. album*. Leguminous species were inoculated with appropriate strains of *Bradyrhizobium* and this resulted in all plants becoming well nodulated. All nodules examined were found to contain hemoglobin, indicative of effective N\textsubscript{2} fixation. Pots containing a *S. album* seedling were established as controls lacking an intermediate woody host. Controlled release fertiliser, Scotts® Osmocote Plus 8-9M, (N 16.0%, P 3.5%, K 10.0%, S 2.4%, Mg 1.2%, B 0.02%, Cu 0.05%, Fe 0.4%, Mn 0.06%, Mo 0.02%, Zn 0.015%) was applied to the potting media surface at a rate of 10 g / pot. Four weeks following the transplanting, the *Alternanthera* was completely removed from all the pots, including controls, and the solo parasite or parasite plus intermediate host then grown on for a further 10 months in the nursery in full sun. Overhead watering twice daily ensured that potting media were maintained at or near field capacity.

Thirty three weeks after establishing the pot associations, three replicate pots were harvested from each treatment, using a selection protocol in which one harvested plant of the parasite was gauged equal to, another one standard deviation (SD) unit greater, and the third one SD unit less than the mean current stem diameter (2 cm above ground level) of all *Santalum* plants of
the treatment. Mean stem diameter of all_Santalum_plants at 33 weeks partnered with_S. formosa_was 1.82 ± 0.28 cm, _A. trachycarpa_was 1.30 ± 0.39 cm, _A. ampliceps_was 1.72 ± 0.32 cm, _E. camaldulensis_was 0.83 ± 0.21 cm and without a host was 1.21 ± 0.12 cm. The following analyses on the harvested plants were then performed on bulk harvested material:

4.3.1. LEAF TISSUE

Representative leaf material (~ 5 grams) from the_S. album_and the host plants of a parasite: host association was collected from the selected pots and bulked for each treatment. It was immediately extracted three times in 50 mls 80% v/v ethanol, the ethanolic extracts combined, reduced to dryness under vacuum and the residue partitioned between 10 mls of water: petroleum ether (B. P. 60) 1 : 1 (v/v). After two further washings with further 5 ml aliquots of petroleum ether, the aqueous extract was retained by freezing and the petroleum ether fraction containing chlorophyll and other liquid soluble material discarded.

4.3.2. STEM XYLEM TRACHEAL SAP

Stem xylem tracheal sap from plants was collected using a hand operated mini vacuum extraction pump following techniques described by Pate et al. (1994). Xylem sap from each treatment (~ 5 ml) was then bulked and frozen.

4.3.3. ENDOPHYTIC TISSUE

The potting medium was removed carefully from the intermingled root systems of parasite and host to facilitate harvesting of haustoria. Endophytic tissue was then carefully excised from the haustorial sample (see Pate et al. 1994; Tennakoon and Pate 1997) and placed in the equivalent of 10 volumes of 80% v/v ethanol for extraction of solutes. Close to 5% of the haustoria in pots of all parasite: N₂-fixing host associations were found to be directly invading host nodules (Radomiljac 1998a), a finding similar to that recently documented for _O. phyllanthi_ by Tennakoon et al. (1997c). The remaining haustoria were attached in normal fashion to the xylem of major host roots (see study of Tennakoon et al. 1997b).
4.3.4. **ANALYSES OF AMINO ACIDS, ORGANIC ACIDS AND SUGARS**

Xylem sap, and ethanolic extracts of leaves and endophytes were assayed for amino acids, organic acids and sugars using a Waters HPLC system following the techniques described by Pate *et al.* (1994); Tennakoon and Pate (1996a); Tennakoon *et al.* (1997b). Fresh weight : dry weight regressions for endophyte and leaf tissue enabled concentrations of organic solutes to be expressed on the basis of fresh weight of tissue extracted.

4.4. **RESULTS**

4.4.1. **CARBON AND NITROGEN CONCENTRATIONS OF AMINO ACIDS, ORGANIC ACIDS AND SUGARS IN XYLEM SAP OF THE SPECIES.**

Total concentration of carbon and nitrogen in the xylem sap of *S. album* and its hosts were estimated from concentrations and C and N contents of all organic components assayed in the xylem sap samples (Table 4.1). In every instance by far the greatest contribution to xylem carbon came from sugars, with much lesser levels of C as amino acids and organic acids. Organic acids contributed more carbon to the xylem solutes than did amino acids in parasitised *A. ampliceps*, *S. album* attached to *E. camaldulensis* and in unattached *S. album*. In all other cases amino acids far outweighed organic acids in respect of their contributions of carbon to in the xylem sap.

N in xylem sap of corresponding parasites ranked in order of decreasing concentration in the same order as shown for the xylem sap of hosts, despite somewhat lower concentrations in parasite than host in the case of the hosts *S. formosa* and *A. trachycarpa* (Table 4.1). Xylem sap N was somewhat lower in host than parasite in partnerships involving *E. camaldulensis* and *A. ampliceps*.

Table 4.1 summarises data on C : N weight ratios of combined xylem solutes of each species of each relationship. The xylem sap of *Santalum* parasitising *S. formosa* and *A. trachycarpa* turned out to be of much lower C : N balance than xylem sap of *Santalum* associated with the other two hosts or in the absence of a host. The same principal applied to C : N rankings of the first two and last two hosts.
Table 4.1: Carbon and nitrogen concentrations of sugars, amino acids and organic acids in the stem xylem sap of *Santalum album* and its associated hosts grown as single parasite: host pairings in pot culture. Data are from the bulked stem xylem sap from 3 plants.

<table>
<thead>
<tr>
<th>Association</th>
<th>Concentration of C from xylem sap solutes (µg C/ml)</th>
<th>Total conc. of C from xylem sap (µg C/ml)</th>
<th>Total conc. of N from xylem sap amino acid solutes (µg N/ml)</th>
<th>C : N weight ratio of all xylem solutes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sugars</td>
<td>Amino acids</td>
<td>Organic acids</td>
<td></td>
</tr>
<tr>
<td><em>Santalum album</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>attached to <em>S. formosa</em></td>
<td>1150</td>
<td>171</td>
<td>72.3</td>
<td>1390</td>
</tr>
<tr>
<td>attached to <em>A. trachycarpa</em></td>
<td>1440</td>
<td>171</td>
<td>59.8</td>
<td>1670</td>
</tr>
<tr>
<td>attached to <em>A. ampliceps</em></td>
<td>1520</td>
<td>106</td>
<td>94.0</td>
<td>1720</td>
</tr>
<tr>
<td>attached to <em>E. camaldulensis</em></td>
<td>815</td>
<td>41.3</td>
<td>51.4</td>
<td>908</td>
</tr>
<tr>
<td>grown without a host</td>
<td>890</td>
<td>28.6</td>
<td>65.2</td>
<td>984</td>
</tr>
<tr>
<td><em>Parasitised</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>S. formosa</em></td>
<td>3650</td>
<td>314</td>
<td>194</td>
<td>4160</td>
</tr>
<tr>
<td><em>A. trachycarpa</em></td>
<td>1700</td>
<td>317</td>
<td>148</td>
<td>2170</td>
</tr>
<tr>
<td><em>A. ampliceps</em></td>
<td>2400</td>
<td>90.0</td>
<td>222</td>
<td>2710</td>
</tr>
<tr>
<td><em>E. camaldulensis</em></td>
<td>454</td>
<td>19.1</td>
<td>8.9</td>
<td>482</td>
</tr>
</tbody>
</table>
Comparisons within associations showed noticeably lower C : N ratio in parasite than host xylem sap for associations involving *S. formosa* and *A. ampliceps*, suggesting preferential uptake by the parasite of N solutes from the xylem of these hosts. This situation did not apply to the other two partnerships where similar C : N ratios in xylem of host and parasite were ranked.

4.4.2. **AMINO ACID COMPOSITION**

4.4.2.1. **XYLEM SAP OF HOST**

As to be expected of the symbiotically-active legumes, the amino acid composition of xylem sap of *S. formosa*, *A. trachycarpa* and *A. ampliceps* contained asparagine as the major solute translocating N (Figures 4.1A-C). All three legume-species also showed significant but noticeably smaller contributions to their xylem N from aspartate, glutamate and γ-amino butyrate. Xylem sap of *A. trachycarpa* also contained appreciable quantities of threonine and djenkolic acid, the latter having been previously shown to be a regular xylem component of certain species of *Acacia* (Pate et al. 1991b; Pate et al.1994; Tennakoon and Pate 1996a). Glutamate, aspartate, glutamine and arginine comprised the principal N components of tracheal sap of *E. camaldulensis*, with smaller contributions also coming from threonine and serine (Figure 4.1D).

4.4.2.2. **XYLEM SAP OF SANTALUM**

Asparagine was the commonest amino acid of xylem sap of *S. album* when parasitising any of the leguminous hosts (Figure 4.1A-C). Other dominant amino acids in such situations were mostly present in proportions similar to those of the xylem of the attached host. However the xylem amino acid composition of *S. album* attached to *S. formosa* showed serine and arginine at appreciably higher proportional levels than in xylem of this host (Figure 4.1A).

The amino acid composition of *S. album* xylem sap was somewhat different to that of partner *E. camaldulensis*, with aspartate as the principal compounds and lesser contributions from asparagine, glutamate and glutamine. *E. camaldulensis* xylem sap was co-dominated by glutamate, aspartate, glutamine and arginine.
Figure 4.1: The percentage composition (molar basis) of amino acids from host xylem sap and parasite endophyte and xylem sap, from different host: Santalum album associations. S. album parasitic on (A) Sesbania formosa, (B) Acacia trachycarpa, (C) A. ampliceps, (D) Eucalyptus camaldulensis and (E) S. album grown without a host. Host: parasite associations were grown for 33 weeks as single plant pairings in 25 litre pots. (ASN; asparagine, GLU; glutamate, GABA; γ-amino butyrate, ALA; alanine, ASP; aspartate, SER; serine, PRO; proline, VAL; valine, GLN; glutamine, THR; threonine, ARG; arginine, ILEU; isoleucine, GLY; glycine, PIP; pipecolic acid, DJK; djenkolic acid).
There was a very close similarity in amino acid composition between xylem sap of *Santalum* in partnership with the above eucalypt as when *Santalum* had been grown without a host (Figure 4.1E). By contrast, relative proportions of asparagine, γ-amino butyrate, serine and arginine were much less, but that of aspartate greater in xylem sap of unparasitised *Santalum*, when compared to that of *Santalum* attached to any of the three N₂-fixing legume hosts.

4.4.2.3. **ENDOPHYTES OF HAUSTORIA OF SANTALUM**

The endophyte composition of *Santalum* was relatively invariable in respect of proportional amounts of different amino compounds, regardless of the host species on which it had formed. Endophytes autoparasitic on *Santalum* plants grown alone were of closely similar composition to those of *Santalum* partnered with the various hosts (Figure 4.1). When attached to leguminous hosts the endophyte composition of *Santalum* was broadly similar to that of the xylem sap of parasite and host. Amino acid spectra of all endophytes were dominated by asparagine and glutamate, with smaller contributions from γ-amino butyrate, alanine, aspartate, proline and serine. There was a general tendency for endophytes to show lower proportions of asparagine but greater proportions of glutamate, γ-amino butyrate, alanine and proline than in xylem sap of the donor legume. Similarly, endophytes contained somewhat lower proportions of asparagine and aspartate but greater proportions of glutamate, alanine and proline than in corresponding xylem sap of the parasite.

Amino acid composition of *S. album* endophytes differed markedly to that of xylem sap of *E. camaldulensis* or of the parent plant of *S. album*. *S. album* endophytes had about the same proportion of glutamate as *E. camaldulensis* but the proportion of asparagine was more than fifteen times higher. *S. album* xylem sap had more than a four fold greater proportion of aspartate but half the proportion of asparagine and glutamate than the endophyte.

4.4.2.4. **FOLIAGE OF PARASITE AND HOST**

Free amino acid composition of *S. album* foliage differed radically from that of donor xylem sap of hosts and parasite and between host species, suggesting the metabolism of predominant amino acids. The amino acid composition of host foliage differed markedly from that of its own xylem sap and even more dramatically from that of foliage of the partner parasite (data not shown).
4.4.3. **Organic Acid Composition of Parasite and Hosts**

Malate dominated the organic acid fraction of xylem sap of *S. formosa* and *A. ampliceps*, whereas citrate was the major component of xylem sap of *A. trachycarpa* and *E. camaldulensis* (Figure 4.2). Endophytes of the parasite contained mostly malate, whereas xylem sap contained malate with a smaller amount of citrate, regardless of the host species, or presence of absence of a host. Malate was the major organic acid of the endophyte and xylem sap of unattached or attached *S. album* regardless, of which host it was parasitising.

4.4.4. **Sugar Composition of Parasite and Host**

The free sugars in xylem sap of attached and unattached *Santalum* material were consistently dominated by fructose. Corresponding endophytic material also showed fructose as a major compound, but there were appreciable amounts of glucose and sucrose (Figure 4.3). Although *S. formosa* xylem sap contained high levels of fructose, the sugar fraction in these cases consisted principally of sucrose and glucose. The latter situation applied also to xylem sap of the other host species.

4.5. **Discussion**

The principal objective of this chapter was to test whether compositional features of xylem sap of *S. album* and associated hosts might provide a definitive means of assessing the extent and manner by which the parasite is benefiting from presence of a particular host during growth under defined conditions. This was achieved by comparing the data obtained here against previously obtained information on how the same pot cultured set of associations performed in terms of growth, host use efficiency, concentrations of N in parasite leaf dry matter and rates of leaf photosynthesis by the parasite (see Radomiljac 1998a; Radomiljac et al. 1998b). Table 4.2 assembles the results of such a comparison.

Our previous ratings of potential utility of intermediate hosts to *S. album* ranked the three legumes far superior to the eucalypt (see parasite shoot dry weight values Table 4.2), with all legume associations increasing parasite dry weight substantially above that of the eucalypt association and the unparasitised control *Santalum*.
Figure 4.2: The percentage composition (molar basis) of organic acids from host xylem sap and parasite endophyte and xylem sap, from different host: *Santalum album* associations. *S. album* parasitic on (A) *Sesbania formosa*, (B) *Acacia trachycarpa*, (C) *A. ampliceps*, (D) *Eucalyptus camaldulensis* and (E) *S. album* grown without a host. Host: parasite associations were grown for 33 weeks as single plant pairings in 25 litre pots.
Figure 4.3: The percentage composition (molar basis) of sugars from host xylem sap and parasite endophyte and xylem sap, from different host: *Santalum album* associations. *S. album* parasitic on (A) *Sesbania formosa*, (B) *Acacia trachycarpa*, (C) *A. ampliceps*, (D) *Eucalyptus camaldulensis* and (E) *S. album* grown without a host. Host: parasite associations were grown for 33 weeks as single plant pairings in 25 litre pots.
Table 4.2: Effect of *Santalum album*: host associations on parasite biomass growth, foliar N concentrations and C and N xylem sap concentrations. Parasite and host were grown as single plant pairings pot culture. Parasite growth, foliar N and photosynthesis data are from three replicates taken at 33 weeks from commencement of association. Values are means ± SE’s. Treatment means followed by the same letter are not significantly different (p > 0.05).

<table>
<thead>
<tr>
<th>Association</th>
<th><em>S. album</em>: S. formosa</th>
<th><em>S. album</em>: A. trachycarpa</th>
<th><em>S. album</em>: A. ampliceps</th>
<th><em>S. album</em>: E. camaldulensis</th>
<th>Unattached <em>S. album</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Parasite shoot DW (g) a</td>
<td>182 ± 46.9 a</td>
<td>57.5 ± 35.2 b</td>
<td>123 ± 58.4 a</td>
<td>17.1 ± 8.8 b</td>
<td>35.6 ± 11.4 b</td>
</tr>
<tr>
<td>Host use efficiency (Parasite shoot DW / Host shoot DW) a</td>
<td>3.11 ± 0.97 a</td>
<td>2.85 ± 2.21 a</td>
<td>0.52 ± 0.35 b</td>
<td>0.12 ± 0.08 b</td>
<td>-</td>
</tr>
<tr>
<td>Parasite foliar N (%) b</td>
<td>3.76 ± 0.61 ab</td>
<td>4.95 ± 1.32 a</td>
<td>2.79 ± 0.16 bc</td>
<td>1.28 ± 0.16 d</td>
<td>1.58 ± 0.61 cd</td>
</tr>
<tr>
<td>Parasite leaf photosynthesis (μmol m⁻² s⁻¹) b</td>
<td>7.83 ± 0.98 a</td>
<td>6.83 ± 1.17 a</td>
<td>8.83 ± 1.17 a</td>
<td>1.50 ± 1.64 b</td>
<td>3.83 ± 2.48 b</td>
</tr>
</tbody>
</table>

a, Data from Radmiljac et al. (1998c)
b, Data from Radmiljac et al. (1998f).
Using the ratio of parasite shoot dry weight to that of the host as our index of quality of benefit (see host use efficiency ratings Table 4.2), the legumes clearly outperformed the eucalypt by a large margin. *S. formosa* and *A. trachycarpa* supported, in relative terms, more than five times the mass of parasite per unit of their own mass than did the other legume *A. ampliceps*. Extending the analysis further to %N in parasite foliage and associated rates of photosynthesis of the parasite (see values in Table 4.2), the legumes again proved to be considerably better hosts than the eucalypt.

Total N and C in xylem of hosts and parasite, and the corresponding values for C : N ratios of the xylem saps (Table 4.1) indicated that *S. formosa*, the top ranking host in terms of support of parasite growth, carried a xylem stream richest in C and N. The eucalypt, poorest host in all respects, ranked lowest by a considerable margin in terms of both C and N contents of its xylem. The rankings of the *Acacia* species (Table 4.2), showed *A. ampliceps* was clearly better in promoting growth and photosynthesis of the parasite than *A. trachycarpa*, but considerably poorer than the latter species on the basis of host use efficiencies and foliar N. Assessed in terms of xylem composition, the two hosts rank similarly in concentrations of C in their own xylem sap and that of associated *Santalum*, but somewhat surprisingly, in relation to parasite performance, xylem sap of the *A. trachycarpa* is appreciably richer in N than that of *A. ampliceps*. This now raises the question of the relative poor performance of *A. trachycarpa* in terms of promoting *Santalum* growth compared with the other two legume hosts, even though *A. trachycarpa* possessed the highest host use efficiency value and the second richest xylem sap in terms of N concentration. Of the three legume hosts *A. trachycarpa* had the smallest biomass which suggests that a large but less efficient host might be better in terms of promoting parasite growth than a small but highly efficient host.

The pot culture conditions provided only a small supplement of N as slow release fertiliser, so the low levels of foliar N and extremely poor growth of *Santalum* when associated with the eucalypt would indicate both a poor benefit from attachment to this host and an inability of the parasite to compete effectively for the small amount of available N in the rooting medium. *Santalum* on *E. camaldulensis*, competing with its host for soil N, accordingly grew to only half of the extent of single non parasitic control *Santalum* (Table 4.2). In marked contrast, presence of any N₂-fixing hosts greatly improved growth and N status, with evidence of high levels of xylem N in these hosts promoting more or less correspondingly elevated N levels in the recipient xylem stream of the parasite. Effects of this extra N are then abundantly evident in enrichment of foliage, with N flow through benefits expressed in high photosynthetic performance and ultimately as improved dry matter production by the parasite on the hosts in
question. While it is possible that significant heterotrophic inputs of C will result from acquisition of organic solutes from xylem of a host, we would still regard intake of N as the more critical element of response by the parasite. In the present study involving N-deficient rooting substrates, effects of supplementing inputs of fixed N from a host must clearly have been paramount, but this may well also apply to many nutrient poor field situations in which Santalum is likely to be cultivated. Indeed, where indigenous N levels are low, it may be both economically expedient and biologically advantageous to rely on symbiotically fixed N rather than fertiliser N to meet the N demand of the parasite. Extending this principle broadly, one finds many examples in the literature where woody herbaceous root hemi-parasites have been found to associate preferentially with and benefit greatly from N\textsubscript{2}-fixing hosts in natural ecosystems (Walker 1989; Loneragan 1990; Rai 1990; Pate et al. 1990a; Tennakoon and Pate 1996a: Tennakoon et al. 1997a). A recent case study shows that this applies also to Santalum acuminatum in the native coastal sand plain vegetation of south west Western Australia (Tennakoon and Pate 1997; Tennakoon et al. 1997a).

Turning attention in the rest of the discussion to the N relationships of S. album and its hosts, the amino acid compositions of both donor and recipient xylem streams and the endophytes of all parasite : legume associations were dominated by asparagine, glutamate and aspartate – compounds, all of which are well documented as predominating in the translocation products of N\textsubscript{2}-fixing nodules of legumes. These same compounds are suggested here, as in earlier studies (see Tennakoon and Pate 1997), to be absorbed readily by haustoria tissues and to pass unmetabolised to the parasite xylem. Judging from shoot responses and foliar levels of N, these same compounds must clearly comprise ready sources of N for assimilation into shoot tissue protein of the parasite. However, there is also evidence of sufficiently different amino acid composition in leaf soluble pools within and between associations to indicate specific metabolic routings into soluble storage pools highly characteristic of the species involved.

Unusually high aspartate levels and low ratios of amide (glutamine and asparagine) to other amino compounds typify xylem sap of eucalypt-associated or non-parasitised Santalum, both features indicative of N deficiency. By contrast, when associated with legumes, endophyte, xylem sap and soluble pools of leaves of Santalum contain much higher proportional contents of amide and in some cases significant accumulation of the N rich amino compound arginine, which is used in N storage and transport in many other higher plant species.

Our study contrasts markedly with that of Tennakoon et al. (1997b) on S. acuminatum, in that there was essentially no proline in the xylem stream of S. album or its hosts, although sizeable pools of this compound were encountered in foliage of S. album and lesser amounts in respective
haustorial tissue. In *S. acuminatum*, host xylem feeding on a range of inorganic and organic $^{15}$N labeled substrates led to massive incorporation of the label into proline, and this amino acid dominated the soluble N pools of xylem and foliage of the parasite, but mostly did not accumulate in significant quantities in corresponding hosts. Accentuated proline production was considered by Tennakoon *et al.* (1997b) as indicative of the high levels of water stress experienced in the coastal south west Western Australia heath where the study was conducted. The compound was suggested to carry an osmoprotective function and possibly contribute in large measure to a lowering of water potential of the parasite, and thereby facilitate acquisition and retention of host-derived water under stressed conditions. Proline accumulation is well known to be characteristic of plants experiencing salt or water stress (Stewart and Larson 1980; Wyn Jones 1984; Erskine *et al.* 1996), and lack of evidence of major storage of proline in our present study in *S. album* may well reflect the well-watered conditions in the pot culture. Further study of *S. album* in these respects under field conditions with changes in water availability is clearly called for.

Unfortunately the literature on the structure of *S. album* haustoria is relatively limited (see Rao 1942), but, judging from the study by Webb (1984) on *S. spicatum* and recent observations on *S. acuminatum* (Tennakoon *et al.* 1997a), lumen to lumen tracheary continuity between host and parasite is probably lacking. This is in agreement with the situation for other root hemi-parasites (see Pate *et al.* 1990c; Riopel and Timko 1995; Tennakoon and Pate 1996a) and implies that direct apoplastic transfer of xylem solutes from a host will be constrained to highly resistant cell wall pathways through host vessels walls into cell walls of endophyte parenchyma and thence into tracheids of the haustoria (see Coetzee and Fineran 1987; Kuo, Pate and Davidson 1989; Pate *et al.* 1994). Protoplasts of the parenchymatous tissue lining the interface with the host xylem might of course selectively absorb host xylem solutes in their passage through the above apoplastic pathway, and possibly process them before eventually releasing the resulting products to the xylem of the parasite. One would then argue that the greater the differences in the solute pool and the solute concentrations between the xylem stream of the host and parasite, the more likely that the parenchyma is involved in regulating the uptake and initial processing of transferred solutes. The proline-synthesizing potential of *S. acuminatum* haustoria demonstrated in $^{15}$N labeling studies of Tennakoon *et al.* (1997b) and the change in amino acid spectra of haustoria of *O. phyllanthi* on hosts of radically different xylem composition in respect of N solutes (Pate *et al.* 1994) are examples where the solute stream is modified during the transfer process. In these cases haustorial tissue clearly plays a major and highly versatile role in solute processing, ranging from a general ability to utilize nitrate or ammonium to processing of a variety of common and unusual, naturally-occurring solutes endemic to xylem sap of different hosts. In each instance haustoria show an ability to
synthesise from these diverse solutes a remarkably similar set of nitrogenous solutes which it then passes onto the shoot of the parasite via the xylem. Our present study of *S. album* provides a similar but less extreme picture in respect of N transfer into and beyond the haustorium, with the additional feature of a heavy bias towards fructose and malate synthesis from incoming sugars and organic acids as a possible means for efficient uptake and utilisation of the non-nitrogenous solutes. However, the picture in *S. album* in respect of amino acids indicates that when on legume hosts, straight throughput of major solutes from the host such as asparagine, glutamate, aspartate and glutamine comprises the dominant transfer activity. It remains to be seen whether such relatively limited involvement of haustorial tissue in metabolism of host-derived solutes is a general hallmark of compatibility and maximal benefit of *S. album* in respect of a particular host, and alternatively whether patent mismatching of xylem solutes of partners, with associated requirements for extensive processing in haustorial tissue or beyond, invariably carries penalties in terms of growth of the parasite.
CHAPTER 5

GAS EXCHANGE AND WATER RELATIONS OF THE ROOT HEMI-PARASITE SANTALUM ALBUM L. IN ASSOCIATION WITH LEGUME AND NON-LEGUME HOSTS.

5.1. ABSTRACT

This chapter examines foliar N levels, photosynthesis, transpiration, water use efficiency and tissue water relations of the xylem-tapping root hemi-parasite Santalum album in pot culture with various N₂-fixing woody hosts, a non-fixing host (a eucalypt), or in absence of a host.

Foliar N concentrations of Santalum were significantly greater than corresponding hosts and higher when on N₂-fixing hosts than on the eucalypt, or without a host. Strong positive relationships were evident in Santalum between foliar N concentration, rates of net photosynthesis and instantaneous water use efficiencies. Photosynthesis rate and water use efficiency of Santalum were generally lower than in corresponding hosts, but transpiration rates not noticeably different between associations. δ¹³C values of total shoot dry matter of Santalum were poorly correlated with instantaneous water use efficiency, but associations involving the three legumes showed less negative δ¹³C values and better water use efficiencies for hosts, than corresponding parasites. Interpretation of such differences were difficult in view of an earlier demonstration of substantial heterotrophic gain of C from certain hosts. Diurnal profiles of gas exchange and leaf water potential of hosts and parasites indicated closely coordinated diurnal stomatal responses of the parasite water relations to its host, thus resulting in transpiration rates of the parasite generating leaf water potential gradients favouring continuous abstraction of water and nutrients from a host. Tissue water relations of Santalum generally resembled those of water-stress tolerant species. Host-specific effects on relative water content and osmotic adjustment were slight and rated unimportant in regulation of water flow to the parasite, or in protecting it from temporary water stress in a host.
5.2. INTRODUCTION

A number of species of the root hemi-parasite Santalum (Santalaceae) produce highly valued aromatic heartwood known as sandalwood, of which the major aromatic constituents, α- and β-santalol, are widely used in the perfumery and cosmetics industries (Srinivasan et al. 1992). The associated potential for commercial culture of sandalwood is now well recognised, particularly in the case of Indian sandalwood (S. album L.), for which research is now being conducted widely in the tropics (see Hamilton and Conrad 1990; McKinnell 1993; Gjerum et al. 1995) including in northern Western Australia (Radomiljac et al. 1998a). Just as in the case of other root hemiparasites, sandalwood is partly dependent on host species for water and nutrients, with leguminous hosts being generally of better sources of nitrogen than other species (Tennakoon et al. 1997b; Radomiljac et al. 1998d). Proper selection of host species to give maximum benefit to S. album is accordingly proving to be critical for economically effective plantation culture (Rai 1990; Srinivasan et al. 1992; Havel and McKinnell 1993; Fox et al. 1996; Radomiljac et al. 1998a).

The recent demonstration of targeted haustorial initiation on nitrogen fixing hosts, and evidence of greatest benefit in terms of nitrogen from these as opposed to non-fixing species in native habitats (see study on S. acuminatum (R. Br.) A. DC. by Tennakoon et al. 1997b) has raised the issue of the precise physiological basis for such benefit, whether simply in terms of providing nitrogen per se or flow on advantages from such acquisition in terms of improved photosynthesis and water usage. Some of the features involved, such as growth and xylem solute composition when parasitising different hosts, have already been studied for pot cultured S. album (Radomiljac et al. 1998c; Radomiljac et al. 1998d). In the present study we further evaluate host-specific responses of S. album on beneficial (N₂-fixing) and non beneficial hosts or no hosts, specifically examining the impact of host type on net assimilation and transpiration rates and water use efficiencies of host and parasite.

5.3. MATERIALS AND METHODS

5.3.1. POT CULTURE

Santalum and woody hosts were grown as single plant pairings in 25 litre pots in a nursery near Kununurra (lat. 15° 46' S. long. 128° 44' E.), Western Australia as recently described by Radomiljac et al. 1998c). The intermediate woody host species selected were the N₂-fixing
legumes *Sesbania formosa* (F. Muell.) N. Burb. (Papilionaceae), *Acacia trachycarpa* E. Pritzel (Mimosaceae), *Acacia ampliceps* Maslin (Mimosaceae) and the non N₂-fixing *Eucalyptus camaldulensis* Dehn. (Myrtaceae). Seedlings of host species were inoculated with appropriate *Bradyrhizobium*. Pots containing a single *Santalum* seedling were established as controls lacking an intermediate woody host. Controlled release fertiliser, Scotts® Osmocote Plus 8-9M, (N 16.0%, P 3.5%, K 10.0%, S 2.4%, Mg 1.2%, B 0.02%, Cu 0.05%, Fe 0.4%, Mn 0.06%, Mo 0.02%, Zn 0.015%) was applied to the potting media surface at a rate of 10 g / pot at the time of transplanting the parasite and hosts. The associations were grown on for a further 10 months in the nursery in full sun, with overhead watering twice daily to near field capacity.

### 5.3.2. Leaf gas exchange and leaf water potentials of *Santalum* and hosts

Net rates of leaf photosynthesis (*Pₐ*) and transpiration (*Eᵣ*) of foliage of host and parasite were assessed on three replicate pots of each association over a two-day period in high summer (February 1997). Measurements were conducted using a Parkinson broad leaf chamber (PLC-B) coupled to a portable flow through ADC infra red gas analyser (model LCA-3 ADC Instruments, Cambridge, UK). Measurements of *Pₐ* and *Eᵣ* were made on 6.25 cm² enclosed leaf surfaces between 08:00 and 10:45 hr, using the fifth fully expanded leaf on the uppermost main shoot of a plant of host or parasite. Instantaneous water efficiencies (WUE) were then assessed as *Pₐ / Eᵣ*. Using information provided by the cuvette in terms of photosynthetic photon flux density (PPFD), relative humidity and leaf temperature it was possible to target measurements to unshaded conditions of stable PPFD value, ambient humidities between 43-59% and temperatures within the range 29 to 38°C. Under such conditions vapour pressure deficit (VPD) values lay within the range 1.69 – 3.71 (kPa) and CO₂ concentrations and PPFD readings in the cuvette within 329 - 365 μmol mol⁻¹ and 1670 - 2290 μmol m⁻² s⁻¹, respectively.

In a further study also undertaken in summer (1997) diurnal courses of *Pₐ* and *Eᵣ* and WUE of host and parasite leaf material from the same replicated pots of each host : parasite association were examined at 2 hourly intervals between 0400 and 1600 hours on a single day of measurements. Parallel measurements of leaf water potential (*ψ*) were undertaken on sample leafy shoots harvested from the same replicate pot material over the same day, using a standard pressure chamber technique (Scholander *et al.* 1965; Turner 1988).
5.3.3. *NITROGEN AND CHLOROPHYLL CONTENTS OF FOLIAGE*

At the end of the studies of leaf $P_a$ and $E_n$, leaves of hosts and parasite used in the measurements were harvested, oven dried for 48 hours at 80°C, milled and subjected to Kjeldahl analysis for total N content (McKenzie and Wallace 1954).

Mean total chlorophyll concentrations of intact leaves of *Santalum* were determined using a Minolta® SPAD-502 Chlorophyll Meter (Minolta Co., Ltd. Japan) on three replicate leaves of a similar age to those used in the gas exchange studies from each of the five replicate pots of each association, including *Santalum* grown without a host. Calibration of the meter was effected using a selected series of leaves ranging in colour from the lightest yellow (most N deficient) to the deepest green found in the pot cultures. Chlorophyll (a+b) was extracted in 90% acetone from 3 discs of 1.2 cm diameter (combined weight $\equiv 50$ mg) from the above range of leaves and measured spectrophotometrically as detailed by Lichtenthaler and Wellburn (1983). A regression equation was then fitted to convert readings from the SPAD-502 to chlorophyll (a+b) content per unit leaf area (Systat 1992).

5.3.4. *CARBON ISOTOPE DISCRIMINATION ($\delta^{13}C$) OF SHOOT DRY MATTER*

A selective harvest of hosts and parasite from three pots from each parasite : host combination was made at the end of the experiment in late February (1997), using our previously published protocol (see Radomiljac et al. 1998c) in which one plant of the three *Santalum* plants selected was approximately equal to, the second one standard deviation (SD) unit greater, and the third one SD less than the current mean current stem diameter (2 cm above ground level) of all plants in a treatment. Shoots of the selected plants were cut at soil level and sub samples of their whole milled dry matter combusted in an Isoprep 13 apparatus (VG Isogas, Cheshire, UK) and the resulting emissions analysed for the $^{13}C/^{12}C$ using a SIRA9 mass spectrometer (VG Isogas, Cheshire, UK) as described by Tennakoon et al. (1997a).

5.3.5. *TISSUE WATER RELATIONS OF SANTALUM*

Terminal shoot segments of *S. album*, each including 6 – 8 pairs of leaves were harvested in March 1997 from the *Santalum* and immediately cut under water before transfer for 15 hours in a dark humid chamber to restore full leaf turgidity. Each branch tip was then recut and the four youngest leaf pairs on a shoot retained. This material was quickly wrapped in plastic film, its $\psi$
measured using a pressure bomb and quickly weighed before being left to dry for 4 to 6 hours on the laboratory bench. Further measurements of $\psi$ and mass were then undertaken 9 - 12 times during this drying phase (Hinckley et al. 1980; Turner 1988). The shoot was finally dried for 48 hours at 80°C to assess its DW. Pressure volume curves, total water content, dry weight : turgid weight ratio (DW:TW), turgid weight : dry weight ratio (TW:DW), relative water content (RWC), osmotic pressure at full turgor ($\pi_{100}$), osmotic pressure at zero turgor ($\pi_0$) and relative water content at zero turgor (RWC$_0$) of all samples were then assessed using the standard techniques described by Melkonian et al. (1982); Turner (1988) and Stoneman (1992).

5.4. RESULTS

5.4.1. LEAF NITROGEN AND CHLOROPHYLL CONTENTS

As expected due to the higher xylem N concentrations in N$_2$-fixing than non-fixing hosts (Radomiljac et al. 1998d), foliar N concentrations in the parasite were significantly higher when associating with the three legumes than when on E. camaldulensis (Figure 5.1). The N concentration of foliage of Santalum grown on its own was slightly greater than on E. camaldulensis, reinforcing earlier conclusions suggesting minimal benefit, if not a negative response in terms of competition for N when the parasite associates with the latter host (Radomiljac et al. 1998d). Foliage of Santalum consistently had higher N concentrations than corresponding host foliage, this effect being most noticeable in partnerships with A. trachycarpa. A positive relationship ($r^2 = 0.555$, $p = 0.248$) was evident between foliar N concentration of parasite and associated host.

A significant positive relationship was evident between leaf chlorophyll, measured with the SPAD-502 chlorophyll metre, and the foliar N concentration of parasite ($r^2 = 0.739$, $p = 0.000$) and chlorophyll (a+b) concentration of parasite foliage ($r^2 = 0.824$, $p = 0.005$) (data not shown). Chlorophyll (a+b) concentrations in foliage of Santalum were strongly related to N concentrations the relationship fitting the equation :-

$\text{foliar chlorophyll conc. (g m}^{-2}\text{)} = -0.348 + 0.914 \times \text{foliar N conc. (% of dry weight)}$
Figure 5.1: Mean foliar nitrogen concentration of the root hemi-parasite *Santalum album* and host plant when grown in association with four different host species (1 – 4) or without a host (5). *Santalum* foliar N concentration means followed by the same letter are not significantly different from one another using Tukey’s pairwise t-test (p > 0.05). Mean host foliar N concentrations are significantly different from one another (F-value = 78.1, p < 0.000), pairwise comparison not shown. SE bars are shown. n = 6.
5.4.2. Relationship between %N, $P_s$, $E_t$, and WUE of parasite foliage

Mean $P_s$ values for the parasite (Figure 5.2i) are seen to increase as foliar N increases up to about 4%, with essentially no further increase up to 8% N. The corresponding rise in $E_t$ (Figure 5.2ii) with rising foliar N was less pronounced, resulting in a significant, almost three fold, increase in WUE ($P_s / E_t$, Figure 5.2iii) over the non-saturating part of the range of foliar N values.

Commenting on the effects of host on the above relationships, $P_s$ of Santalum was significantly higher when on the three legumes than on the eucalypt or no host and the same applied to $E_t$ and WUE in respect of A. ampliceps and S. formosa versus E. camaldulensis or no host.

Interesting relationships emerge from comparisons of $P_s$, $E_t$ and WUE of parasite and host of each partnership and ranking respective performances in terms of apparent N use efficiency in photosynthesis i.e. mean $P_s$ per unit foliar N (Table 5.1). Host $P_s$ values are always appreciably greater than for corresponding parasites, the effect being most noticeable in respect of the Santalum : S. formosa and Santalum : E. camaldulensis associations. Differences between host and parasite in respect of $E_t$ are slight and insignificant and the same applies to WUE, except for the over two fold greater WUE of host than parasite in the case of Sesbania and the eucalypt. With foliar N concentrations always greater in parasite than host (Figure 1A) but with the opposite true for $P_s$ ratings, values for photosynthetic N use efficiency (Table 5.1) are in all cases less in the parasite than the host.

5.4.3. $\delta^{13}C$ values for shoot dry matter and instantaneous WUE

In keeping with the well-watered unstressed conditions experienced by the species in pot culture, $\delta^{13}C$ values of young shoot dry matter (Figure 5.3) varied between host species and Santalum : host associations within a relatively narrow range of just over 2‰ (-27.4 to -29.7‰). The $\delta^{13}C$ values for host plants were not consistently less negative than the partner parasite, but data for the three beneficial legume : parasite partnerships bore evidence of better WUE of hosts being associated with less negative $\delta^{13}C$ values for host than partner parasite. The reverse of this trend applied to the essentially non-beneficial relationship between Santalum and the eucalypt.
Figure 5.2: Relationship between Santalum album foliar nitrogen and (i) leaf photosynthesis \( y = -1.87 + 4.224x - 0.414x^2 \) over increasing part of response from 0 to 5.2, \( r^2 = 0.637, p = 0.000 \), (ii) leaf transpiration \( y = 6.779 + 0.905x; r^2 = 0.201, p = 0.013 \) and (iii) instantaneous water use efficiency \( y = 0.06 + 0.254x - 0.019x^2 \) over increasing part of response from 0 to 6.5, \( r^2 = 0.479, p = 0.000 \). Associated hosts were Sesbania formosa (A), Acacia trachycarp (\( □ \)), A. ampliceps (\( ♦ \)) and Eucalyptus camaldulensis (\( ♣ \)) and Santalum without a host (O).
Table 5.1: Mean leaf gas exchange and growth data of the root hemiparasite *Santalum album* when grown in pot culture as single plant pairings with *Serbonia formosa*, *Acacia trachycarpa*, *A. amplexcs* and *Eucalyptus camaldulensis* hosts or without a host. Values as means ± SE’s. Means followed by the same letter are not significantly different using Tukey’s pairwise t-test (p > 0.05). Lower case letters are for *S. album* data. Upper case letters are for host data.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>S. album</em></td>
<td><em>S. formosa</em></td>
<td><em>S. album</em></td>
<td><em>A. trachycarpa</em></td>
<td><em>S. album</em></td>
<td><em>A. amplexcs</em></td>
<td><em>S. album</em></td>
<td><em>E. camaldulensis</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leaf photosynthesis (μmol m⁻² s⁻¹)</td>
<td>7.83 ± 0.98 a</td>
<td>19.16 ± 6.46 A</td>
<td>6.83 ± 1.17 a</td>
<td>9.50 ± 2.43 B</td>
<td>8.83 ± 1.17 a</td>
<td>14.33 ± 6.35 AB</td>
<td>1.50 ± 1.64 b</td>
<td>7.67 ± 5.24 B</td>
<td>3.83 ± 2.48 b</td>
<td></td>
</tr>
<tr>
<td>Leaf transpiration (mmol m⁻² s⁻¹)</td>
<td>11.50 ± 2.16 ab</td>
<td>11.67 ± 2.07 bc</td>
<td>9.33 ± 1.97 bc</td>
<td>11.00 ± 2.10 A</td>
<td>12.67 ± 2.34 a</td>
<td>11.17 ± 3.31 A</td>
<td>6.50 ± 1.05 c</td>
<td>9.33 ± 2.66 A</td>
<td>6.83 ± 1.94 c</td>
<td></td>
</tr>
<tr>
<td>Instantaneous water use efficiency (mmol mol⁻¹) (^a)</td>
<td>0.71 ± 0.18 a</td>
<td>1.68 ± 0.71 A</td>
<td>0.74 ± 0.10 a</td>
<td>0.86 ± 0.13 B</td>
<td>0.73 ± 0.20 a</td>
<td>1.26 ± 0.56 AB</td>
<td>0.21 ± 0.24 b</td>
<td>0.78 ± 0.41 B</td>
<td>0.52 ± 0.22 ab</td>
<td></td>
</tr>
<tr>
<td>N use efficiency in (P_s) (μmol s⁻¹ mg⁻¹ N) (^b)</td>
<td>0.021</td>
<td>0.072</td>
<td>0.014</td>
<td>0.045</td>
<td>0.032</td>
<td>0.062</td>
<td>0.012</td>
<td>0.115</td>
<td>0.024</td>
<td></td>
</tr>
<tr>
<td>Leaf N conc (%)</td>
<td>3.8 ± 0.6</td>
<td>2.7 ± 0.3</td>
<td>4.9 ± 1.3</td>
<td>2.1 ± 0.2</td>
<td>2.8 ± 0.2</td>
<td>2.2 ± 0.3</td>
<td>1.3 ± 0.3</td>
<td>0.7 ± 0.1</td>
<td>1.6 ± 0.6</td>
<td></td>
</tr>
<tr>
<td>Total conc of N from xylem sap amino acid solutes (μgN ml⁻¹) (^c)</td>
<td>77</td>
<td>-</td>
<td>78</td>
<td>-</td>
<td>41</td>
<td>-</td>
<td>14</td>
<td>-</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>Shoot DW (g) (^d)</td>
<td>182 ± 47 a</td>
<td>-</td>
<td>57 ± 30 b</td>
<td>-</td>
<td>123 ± 58 a</td>
<td>-</td>
<td>17 ± 9 b</td>
<td>-</td>
<td>36 ± 11 b</td>
<td></td>
</tr>
<tr>
<td>Leaf area (cm²) (^d)</td>
<td>7473 ± 2255 a</td>
<td>773.5 ± 520 B</td>
<td>2203 ± 1612 b</td>
<td>985.2 ± 1202 B</td>
<td>5296 ± 2691 a</td>
<td>489 ± 173 b</td>
<td>120.3 ± 36.6</td>
<td>46.86</td>
<td>101.9 ± 11.3</td>
<td></td>
</tr>
<tr>
<td>Specific leaf area (cm² g⁻¹) (^d)</td>
<td>109.4 ± 4.1</td>
<td>122.3</td>
<td>99.4 ± 3.1</td>
<td>65.0</td>
<td>90.3 ± 11.5</td>
<td>44.9</td>
<td>46.86</td>
<td>101.9 ± 11.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Air temp. (°C)</td>
<td>33.3</td>
<td>33.2</td>
<td>34.2</td>
<td>34.2</td>
<td>33.7</td>
<td>34.5</td>
<td>36.8</td>
<td>35.7</td>
<td>36.8</td>
<td></td>
</tr>
<tr>
<td>Relative hum. (%)</td>
<td>49.2</td>
<td>48.8</td>
<td>48.0</td>
<td>47.0</td>
<td>46.7</td>
<td>46.8</td>
<td>47.2</td>
<td>47.0</td>
<td>45.8</td>
<td></td>
</tr>
<tr>
<td>VPD (KPa)</td>
<td>2.5</td>
<td>2.6</td>
<td>2.7</td>
<td>2.8</td>
<td>2.8</td>
<td>3.0</td>
<td>3.2</td>
<td>3.0</td>
<td>3.3</td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) Instantaneous water use efficiency (mmol mol⁻¹) = Leaf photosynthesis (μmol m⁻² s⁻¹) / Leaf transpiration (mmol m⁻² s⁻¹).

\(^b\) N use efficiency = Leaf photosynthesis (μmol m⁻² s⁻¹) / foliar nitrogen concentration (mg g⁻¹);

\(^c\) data from Radomiljac et al. (1998d);

\(^d\) data from Radomiljac et al. (1998c).
Figure 5.3: Relationship between instantaneous water use efficiency and δ^{13}C values of Sanicula album partnered singly with various associated hosts. Host codings: Sesbania formosa (□ Sf), Acacia trachycarpa (▲ At), A. ampliceps (○ Aa), Eucalyptus camaldulensis (◇ Ec). Sanicula parasitising S. formosa (■ P on Sf), parasitising A. trachycarpa (▲ P on At), parasitising A. ampliceps (● P on Aa), parasitising E. camaldulensis (◆ P on Ec) and in absence of a host (▼ P no H). Dotted lines connect values for the parasite with that of the relevant host, enabling one to visualise how δ^{13}C and water use efficiency values compare for each partnership.
5.4.4. Matched diurnal profiles of $P_n$, $E_t$, WUE and leaf $\psi$ for different host : parasite pairings and parasite without a host.

This analysis, following changes in all four quantities from dawn to dusk of a single day, produced the data sets depicted in Figure 5.4. Note that the two hosts $S. formosa$ and $A. ampliceps$ were highly beneficial to Santalum, Santalum shoot dry weight being 182 and 123 g plant $^{-1}$ and corresponding leaf areas 7473 and 5296 cm$^2$ plant $^{-1}$ respectively (see Table 5.1). By contrast the much poorer responses by the parasite, namely 51 g plant $^{-1}$ and 2203 cm$^2$ leaf area plant $^{-1}$ for $A. trachycarpa$, 36 g and 940 cm$^2$ plant $^{-1}$ for the unpartnered Santalum and only 17 g and 489 cm$^2$ plant $^{-1}$ for parasitism on $E. camaldulensis$. Changes in PAR, VPD and air temperature for the day of study are in Figure 5.4(F).

There was a general tendency for $P_n$ values of host and parasite to achieve peak values in early morning (Figure 5.4), coinciding with sharply increasing PAR but still low temperature and VPD values. As shown earlier for the mean data of Figure 5.2, $P_n$ values throughout the day were higher in Santalum on leguminous than on the non-legume host or no host, and corresponding data for hosts segregated $S. formosa$ and $A. ampliceps$ as exhibiting higher $P_n$ than other hosts. With the exception of $A. trachycarpa$ peak values for $P_n$ of all hosts exceeded those of the partner parasite by a considerable margin.

As expected of the higher temperatures and VPD late than early in the day, diurnal courses of $E_t$ showed maxima later in the day than for corresponding $P_n$. Differences between host and parasite of the partnerships tended to be less than for $P_n$. In some cases host $E_t$ exceeded slightly that of the parasite for a period of the day, in others the reverse situation applied.

Values for WUE also showed relatively small differences between parasite and associated hosts, but there was some evidence of better WUE of the host than the parasite in mid morning to just after noon. This was not so for the Santalum : $A. trachycarpa$ relationship.
Figure 5.4a: Diurnal courses of changes in net rate of leaf photosynthesis, leaf transpiration, water use efficiency and leaf water potential of the (A) *Santalum album* (P - ∙) and *Sesbania formosa* (H - □) association. Changes in photosynthetically active radiation (PAR), air vapour pressure deficit (VPD) and air temperature are given for the day of measurement (Kununurra, January 1997) in Figure 5.4(F).
Figure 5.4b: Diurnal courses of changes in net rate of leaf photosynthesis, leaf transpiration, water use efficiency and leaf water potential of (B) the *Santalum album* (P - •) and *Acacia ampliceps* (H - □) association. Changes in photosynthetically active radiation (PAR), air vapour pressure deficit (VPD) and air temperature are given for the day of measurement (Kununurra, January 1997) in Figure 5.4(F).
Figure 5.4c: Diurnal courses of changes in net rate of leaf photosynthesis, leaf transpiration, water use efficiency and leaf water potential of (C) the Santalum album (P - ●) and Acacia trachycarpa (H - □) association. Changes in photosynthetically active radiation (PAR), air vapour pressure deficit (VPD) and air temperature are given for the day of measurement (Kununurra, January 1997) Figure 5.4(F).
Figure 5.4d: Diurnal courses of changes in net rate of leaf photosynthesis, leaf transpiration, water use efficiency and leaf water potential of (D) the *Santalum album* (P - •) and *Eucalyptus camaldulensis* (H - □) association. Changes in photosynthetically active radiation (PAR), air vapour pressure deficit (VPD) and air temperature are given for the day of measurement (Kununurra, January 1997) Figure 5.4(F).
Figure 5.4e: Diurnal courses of changes in net rate of leaf photosynthesis, leaf transpiration, water use efficiency and leaf water potential of (E) the *Santalum album* grown without a host (F - •). Changes in photosynthetically active radiation (PAR), air vapour pressure deficit (VPD) and air temperature are given for the day of measurement (Kununurra, January 1997) Figure 5.4(F).
Figure 5.4f: Diurnal courses of changes in photosynthetically active radiation (PAR), air vapour pressure deficit (VPD) and air temperature are given for the day of measurement (Kununurra, January 1997).
Despite differences between partnerships in absolute values and diurnal amplitudes of values for leaf $\psi$ of respective partner species, all associations exhibited relatively unstressed $\psi$ values (-0.5 MPa or less) at dawn and consistently with slightly lower (more negative) values for parasite than host. In the following morning $\psi$ values of hosts and parasite became more negative but with the parasite values more negative than the hosts. By late morning or shortly after, maximum differences in $\psi$ between parasite and host were recorded, after which $\psi$ values showed some recovery more or less in parallel until the end of measurements at 1600 h. The diurnal course of changes in $\psi$ of parasite without a host followed essentially that shown when a host was present.

5.4.5. TISSUE WATER RELATIONS OF SANTALUM ON DIFFERENT HOSTS OR WITHOUT A HOST.

As shown in Table 5.2, values for the various measures of tissue water relations of Santalum showed only slight differences when on different hosts or in absence of a host. The differences involved proved to be insignificant implying relatively little effect of a host on directing osmotic adjustment or other attributes of the parasite. However, shown in Figure 5.5, relative water content (RWC) of leaves varied in a highly predictable fashion in relation to leaf $\psi$, with some evidence of differences in species responses in this respect (eg see plots for Santalum on A. trachycarpa versus Santalum on E. camaldulensis). In general terms RWC showed a change of 20-30% as $\psi$ of the parasite changed over approximately 3 MPa.

5.5. DISCUSSION

When grown in association with N$_2$-fixing hosts, Santalum shoot growth (Table 5.1) was markedly greater than when grown with E. camaldulensis or without a host. This difference has been directly attributed to the substantial benefit in terms of nitrogen gain accruing from the intake of xylem solutes from a well nodulated legume host (Radomiljac et al. 1998d). Flowing from this effect would be increased foliar N concentration, leaf chlorophyll contents and leaf photosynthesis and eventually large increases in leaf area. Following in turn from improved leaf photosynthesis on N$_2$-fixing hosts one finds increase in the instantaneous water use efficiency of the parasite, presumably reflecting an adaptive response to the greater N benefit derived per unit of transpirational loss from the xylem stream of a legume, as opposed to a non-legume host or no host.
Table 5.2: Tissue water relations of shoots of the root hemi-parasite Santalum album when grown in pot culture as single plant pairings with Sesbania formosa, Acacia trachycarpa, A. ampliceps, Eucalyptus camaldulensis hosts or without a host. Values as means ± SE’s. Data are from three replicates.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Turgid mass / dry mass ratio</td>
<td>3.47 ± 0.18</td>
<td>3.74 ± 0.58</td>
<td>3.22 ± 0.04</td>
<td>3.97 ± 0.93</td>
<td>3.69 ± 0.17</td>
<td>0.968</td>
<td>0.466</td>
</tr>
<tr>
<td>Dry weight / turgid weight ratio</td>
<td>0.29 ± 0.01</td>
<td>0.28 ± 0.04</td>
<td>0.30 ± 0.01</td>
<td>0.26 ± 0.05</td>
<td>0.27 ± 0.01</td>
<td>1.216</td>
<td>0.363</td>
</tr>
<tr>
<td>Osmotic pressure at full turgor (MPa)</td>
<td>-2.70 ± 0.28</td>
<td>-2.56 ± 0.65</td>
<td>-2.70 ± 0.07</td>
<td>-2.78 ± 0.16</td>
<td>-2.73 ± 0.09</td>
<td>0.788</td>
<td>0.559</td>
</tr>
<tr>
<td>Osmotic pressure at zero turgor (MPa)</td>
<td>-3.10 ± 0.19</td>
<td>-3.13 ± 0.09</td>
<td>-3.09 ± 0.05</td>
<td>-3.20 ± 0.15</td>
<td>-3.13 ± 0.10</td>
<td>0.800</td>
<td>0.552</td>
</tr>
<tr>
<td>Relative water content at zero turgor (%)</td>
<td>75.67 ± 3.79</td>
<td>72.83 ± 5.53</td>
<td>75.67 ± 2.52</td>
<td>83.33 ± 3.22</td>
<td>78.33 ± 4.73</td>
<td>2.800</td>
<td>0.085</td>
</tr>
</tbody>
</table>
Figure 5.5: Representative pressure-volume curves (relationship between relative water content and leaf water potential) for detached shoots of Santalum album previously growing in association with Sesbania formosa (Δ), Acacia trachycarpa (□), A. ampliceps (●), Eucalyptus camaldulensis (♦) and without a host (O).
These findings are fully consistent with data for xylem-tapping mistletoes (Marshall et al. 1994; Schulze and Ehleringer 1984), but contrast with the findings of Seel et al. (1993) who found no consistent relationship between host foliar N and water use efficiency in the herbaceous root hemi-parasite *Rhinanthus minor* L. on various hosts.

It is now widely recorded that parasitic angiosperms have generally lower water use efficiency values than their associated hosts (Schulze et al. 1984; Press et al. 1987; Shah et al. 1987; Press et al. 1988; Davidson et al. 1989), but notable exceptions to this rule have been reported for *S. acuminatum* by Tennakoon et al. (1997a) and *O. phyllanthis* by Pate et al. (1990b). In the present study values for water use efficiency of *Santalum* attached to *A. trachycarpa* and *A. ampluscips* were 0.74 and 0.73 mmol mol$^{-1}$, and those of corresponding hosts 0.86 and 1.23 mmol mol$^{-1}$, respectively. In comparable studies on *S. acuminatum* attached to *A. rostellifera* Benth. Tennakoon et al. (1997a) recorded values for the parasite in the range 1.34 to 3.72 mmol mol$^{-1}$, again not noticeably different from the host (2.40 to 4.98 mmol mol$^{-1}$). In light of these findings δ$^{13}$C values for shoot dry matter of *Santalum* would be expected to become less negative (more water use efficient) as a result of parasitising N$_2$-fixing hosts. This proved to be the case for all associations except the poorly performing *Santalum* on eucalypt. One must however be cautious when interpreting the significance of the δ$^{13}$C values. Firstly, whole shoot material rather than discrete phenologically equivalent foliar samples were compared for each host and parasite. The values thus represent long-term integrated measures of isotope discrimination, which may have been compromised by differing phenologies of growth. Secondly, the differences in δ$^{13}$C values concerned in certain cases were relatively small (eg. less than 1% for all but one of the partnerships) and compositional differences in dry matter in respect, say, of relative amounts of lignin might have had a relatively large impact in determining the differences concerned. Thirdly, and perhaps most importantly, *S. album* effects large heterotrophic gains of C from certain hosts (Radomiljac 1998) and such acquisition would be expected to change δ$^{13}$C values of the parasite significantly towards those of the donor host. Use of δ$^{13}$C values to assess instantaneous WUE of the parasite would clearly be unwarranted in such circumstances.

Arguing more generally, Hogberg et al. (1993) and Hogberg et al. (1995) have suggested that higher levels of foliar N in any species should tend to promote higher rates of leaf photosynthesis and, by lowering p/p$_{a}$, sponsor generation of leaf dry matter with less negative δ$^{13}$C values than one would expect of low N foliage. It is important note that δ$^{13}$C values are not simply a reflection of p/p$_{a}$ but could also include variation in the resistance to CO$_2$ diffusion within leaves. Unfortunately, differences in δ$^{13}$C values recorded in the present study
between hosts and parasite were relatively slight, and probably inconclusive in view of the well-watered pot conditions used. Studies of δ¹³C signals of foliage of host and parasite under stressed field conditions is clearly called for.

Diurnal variations in transpiration of Santalum proved to be closely similar to those of the host it was parasitising. This is in agreement with data reported for S. acuminatum by Tennakoon et al. (1997a) but contrast with those reported for several herbaceous root hemi-parasites (Press et al. 1987; Shah et al. 1987; Press et al. 1988). It is widely accepted that mistletoe transpiration rates are generally much greater than those of their hosts on the grounds that high transpiration would be required to take up sufficient xylem nitrogen from a host to produce the highly succulent predominantly leafy shoot biomass typical of this class of parasite (Glatzel 1983; Hollinger 1983; Schulze et al. 1984; Ehleringer et al. 1985; Davidson et al. 1989; Stewart and Press 1990). Schulze and Ehleringer (1984) extended this concept by suggesting that mistletoes should increase transpiration in adaptive response to situations where N levels in a host transpiration stream were low, but this supposition has been contested by Givnish (1986). In our study S. album transpiration was found to be significantly lower when attached to the low N yielding host E. camaldulensis than when attached to N₂-fixing hosts suggesting essentially no capacity to adapt to hosts with low N concentrations in their xylem stream. A similar conclusion has been drawn from studies with certain herbaceous Scrophulariacean root hemi-parasites by Press et al. (1993) and Seel et al. (1993).

It seems more likely that the close similarities in transpiration of Santalum and host reflects principally an ability of the parasite to follow closely the stomatal responses of its associated host and thereby maintain coordinated responses in leaf water potential continuously promoting uptake of xylem fluid from a host. This is in accord with the many demonstrations of similar responses in mistletoes (Pate 1995b) but contrasts with data for Striga showing limited capacity for control of transpiration as water stress develops in a host (Press et al. 1987; Shah et al. 1987).

Santalum associations examined in this study showed the midday leaf water potential some 2 MPa more negative than in the corresponding host, precisely as has been reported for a whole range of root parasites and mistletoes (Glatzel 1983; Schulze et al. 1984; Ullman et al. 1985; Ehleringer et al. 1986; Davidson et al. 1989; Davidson and Pate 1992; Veenendaal et al. 1996). Although the well watered pot conditions of the study were unlikely to have stressed the parasite to limits of its functioning, it was shown to continue transpiring at high rates (10-15 mmol m⁻² s⁻¹) when leaf water potential indicated maximum daily stress at or close to −3 MPa.
Relative water content of foliage of *Santalum* decreased slowly but consistently as more negative leaf water potential values were recorded for leaf xylem water potential. Bearing in mind that these values again relate to well watered pot conditions, relative water content values of 70 – 80% for *Santalum* at a leaf water potential value of −3 MPa would still come close to the sort of values which one would associate with sclerophytic species such as eucalypts; e.g. *E. viminalis* Labill. (67%), *E. melliodora* A. Cunn. Ex Schauer (81%), *E. microcarpa* (Maiden) Maiden (86%), *E. behriana* F. Muell. (87%), (Ladiges 1975; Clayton-Greene 1983; Myers and Neales 1984) or the xerophytic species of *Acacia*; *A. aneura* F. Muell. Ex Benth. (85%) (Connor and Tunstall 1968). Furthermore, water-stress tolerant species are regarded by Cowan (1981) as typically showing lesser proportional decreases in relative water content with decreasing ψ. Our study suggests this applies also to *S. album*, despite it being indigenous to the monsoonal tropics where exposure to drought may be limited due to a relatively cool and unstressed dry winter season. Interestingly the data of Figure 5.5 suggests slight differences in response in this respect for *Santalum* on certain hosts compared to others. The reasons for this are not clear.

Under conditions reported here *Santalum* exhibited little capacity for osmotic adjustment when associating with different hosts. Thus, when attached to *A. trachycarpa* an osmotic pressure at full turgor of -2.56 MPa was recorded for *Santalum* versus -2.78 MPa when attached to *E. camaldulensis*, host-specific osmotic adjustment of only 0.2 MPa. Similarly osmotic pressure at zero turgor values of − 3.09 and − 3.20 MPa were recorded for *Santalum* attached to *A. ampliceps* and *E. camaldulensis* respectively indicative of an adjustment of a mere 0.11 MPa. These findings suggest that the bulk tissue water relations of *S. album* are not appreciably compromised when associated with high or low quality host species.

Our study reports closely similar values for osmotic potential at full turgor and at turgor loss point, relative water content at turgor loss point and dry weight to turgid weight ratio for *Santalum* regardless of whether grown with a N₂ or non-N₂ fixing host or when grown without a host. In a similar vein, ratios of dry weight : turgid weight of *Santalum* ranged narrowly from 0.26 to 0.30 between treatments indicating similar leaf reserves of water for accommodating stress control irrespective of type of host being parasitised. Findings are thus consistent with those recorded for the mistletoe *Amyena* (Davidson *et al.* 1989; Davidson and Pate 1992) where the relatively small differences in tissue water relations observed would carry little impact in establishing or maintaining the observed potential gradients between host and parasite. This reinforces our earlier conclusion that *Santalum* transpiration rate perpetuates a favourable water potential gradient from its host.
HETEROTROPHIC CARBON GAIN AND MINERAL NUTRITION OF THE ROOT HEMI-
PARASITE SANTALUM ALBUM L. IN POT CULTURE WITH DIFFERENT HOSTS.

6.1. ABSTRACT

This chapter examines heterotrophic gain of carbon and mineral composition of Santalum album partnered singly in pot culture with three beneficial woody N₂-fixing hosts and a non-
beneficial eucalypt host. Based on dry matter gains of the parasite at 33 weeks, Sesbania formosa proved the best host followed by Acacia ampliceps and A. trachycarpa while no improvement in growth was seen with Eucalyptus camaldulensis as a host in comparison with Santalum grown without a host. Numbers of haustoria formed by Santalum on roots of different hosts were poorly correlated with host quality. Small proportions of haustoria on legume hosts were attached to root nodules. Santalum partnered with any host or grown alone exhibited self-
parasitism where haustoria attached to its own root system.

Based on net C and N gains of Santalum and the C : N ratios of xylem solutes of Santalum the heterotrophic gains of C from xylem of the three beneficial legume hosts over a nine week period were equivalent to 57.9% of total carbon (35.9 g C plant⁻¹) on A. ampliceps, 45.5% (12.7 g C plant⁻¹) on A. trachycarpa and 34.6% (29.9 g C plant⁻¹) on S. formosa.

Assays of leaf, stem, bark and root tissue of Santalum and its hosts and net increases in mineral contents of Santalum over the first nine weeks of the study showed that parasitism on beneficial hosts increased the mineral contents of the parasite, with evidence of net gains in certain elements (e.g. Ca, K, P, Na) being greatest when associated with hosts richest in the corresponding element. Foliage of Santalum was extraordinarily rich in Na and in some cases also in P and N in comparison with associated hosts. Net losses or only small gains of P, K, Ca and Na over the study interval in Santalum grown alone or associated with the eucalypt indicated poor ability for nutrient uptake through its own root system.

Regression analysis showed incremental gains of N, C and Na, leaf area, amount of K, N and
Na in foliage of the parasite and root: shoot ratio to be excellent predictors of growth benefit from different hosts. Examples of stepwise regression analysis are provided indicating how such data might be employed for monitoring growth and host benefit under future plantation cultures of the parasite.

6.2. INTRODUCTION

Santalum album L., commonly known as Indian sandalwood or chandana, is commercially and culturally a most important root hemi-parasite, subject to considerable exploitation since at least 1000 BC (Srinivasan et al. 1992). Strong interest now exists in cultivating the species in mixed species plantations throughout the tropics (Hamilton and Conrad 1990; McKinnell 1993; Havel and McKinnell 1993; Gjerum et al. 1995; Radomiljac et al. 1998a). There are considerable uncertainties regarding the best culture and management practices in relation to the best host species, and how to achieve the highest volume and quality of sandalwood in a particular set of environmental and edaphic circumstances.

Our current projects aimed at defining best protocols for growth of S. album under irrigation culture in the Ord River region of North West Australia, have utilised a native herbaceous perennial, Alternanthera nana R. Br., as a host during pot culture with seedlings of the parasite, followed by use of various fast growing but relatively short lived species as ‘intermediate hosts’ once plants are transferred to the field. Ultimately it is hoped that the Santalum will parasitise long-lived valuable timber trees such as the leguminous Dalbergia (Papilionoideae) until it achieves harvestable status. While the system has yet to be trialed in the field beyond the 4 year stage, it is already apparent in both pot and plantation culture that in contrast to a eucalypt host, N₂-fixing hosts promote much greater dry weight gain, leaf N concentrations, photosynthetic rates and water use efficiencies in the parasite, but lower root: shoot ratio and C: N ratio of xylem sap organic solutes of the parasite (Radomiljac et al. 1998c, d, f). These findings concur with the conclusions from a number of other studies that nitrogen fixing hosts are more effective than non N₂-fixing hosts in promoting growth of angiosperm parasites, presumably or demonstrably as a result of greater enrichment of transport streams with N in the N₂-fixing species (Schulze and Ehleringer 1984; Rai 1990; Seel and Press 1993; Seel et al. 1993; Chechin and Press 1993; Taide et al. 1994; Tennakoon and Pate 1996a).

In this chapter we build further upon our earlier pot culture studies on Santalum (Radomiljac et al. 1998c, d, f) to determine the heterotrophic inputs of carbon to Santalum through uptake of xylem solutes from different hosts, and also how the mineral nutrition of Santalum is modified
though attachment to different hosts. Then, using this information together with earlier measured attributes of performance on different hosts we evaluate the relative usefulness of different growth and compositional features of parasite and host in ranking growth benefit to the parasite from different hosts under a specific set of cultured conditions.

6.3. MATERIALS AND METHODS

The effect of beneficial and non-beneficial woody hosts on the heterotrophic carbon gain and mineral composition of pot cultured *S. album* seedlings was studied in a nursery near Kununurra (lat 15° 46' S. long 128° 44' E), Western Australia. The four intermediate hosts selected for matching with *Santalum* were the N$_2$-fixing *Sesbania formosa* (F. Muell.) N. Burb, *Acacia trachycarpa* E. Pritzel, *A. ampliceps* Maslin, the non N$_2$-fixing *Eucalyptus camaldulensis* Dehnh. *Santalum* grown singly without a host comprised a control.

In July 1996, 40 uniform 6-month old *S. album* seedlings (mean height ± std error; 40.5 ± 2.98 cm and diameter; at 2 cm, 3.9 ± 0.58 mm) were selected. Each had been cultured singly in 1.4 litre pots with the native herbaceous *Alternanthera nana* as an initial pot host. The *Alternanthera* was then cut to soil level and the *Santalum* transplanted to 25 litre pots of coarse river sand : peat : perlite at 3 : 2 : 2. A single seedling of one of the intermediate hosts previously propagated in 0.3 litre pots, and in the case of legume inoculated with appropriate *Bradyrhizobium* (see Radomiljac et al. 1998c), was introduced into each pot at about 150 mm from the single *Santalum* seedling. Remaining host seedlings formed a series of control unparasitised plants. A 10 g dressing of slow release fertiliser, Scotts® Osmocote Plus (8-9M) (N 16.0%, P 3.5%, K 10.0%, S 2.4%, Mg 1.2%, B 0.02%, Cu 0.05%, Fe 0.4%, Mn 0.06%, Mo 0.02% and Zn 0.015%) was applied to the surface of each pot. Pots were placed on mesh benches 10 cm above ground to avoid possible intrusion of roots from one pot to neighbouring treatments. Four weeks following transplanting as much as possible of the remaining *Alternanthera* was removed and all host : parasite pairings cultured in the nursery for a further 10 months in full sun with overhead watering twice daily, for approximately 15 minutes, to near field capacity. Using these culture conditions it was hoped that cultures would become moderately nutrient deficient, thereby accentuating the dependence of the parasite on host xylem solutes for its growth.

Five pots per treatment, as single pot plots, were randomised within each of eight replicates. The position of the replicate and pots within each replicate were rearranged in random fashion

109
twice during the course of the study.

At each of three harvest times (12 December 1996 and 17 February and 24 March 1997) three pots were selected for harvesting from each treatment. Harvested plants were chosen carefully, with one of the selected plants of *Santalum* approximately equal to, the second one Standard Deviation (SD) unit greater, and the third one SD less than the current mean current stem diameter (2 cm above ground level) of all plants of that treatment. This ensured that each harvest was representative of all the plants in each treatment. *Santalum* and host plants were harvested by cutting the stem at soil level and the shoot partitioned into stem, bark and leaf material. Pots with intermingled root systems of *Santalum* and host root systems still intact were then cut horizontally into three layers, 0-8 cm, 8-16 cm and 16-24 cm from top to bottom of the pot. The rooting medium was then carefully vacuumed away from the intermingled roots, taking care not to lose fine root material in the process. This procedure was preferred to washing and sieving as roots of host and parasite tended to remain better intact with each other, thus facilitating counting of haustoria and determining whether haustoria were (a) connected to host root xylem tissue, (b) connected to nodules in the case of leguminous hosts, or (c) connected to other *Santalum* roots (auto-parasitic).

The morphology and anatomy of haustoria of *Santalum* were studied in partnership with three of the legume hosts, using mature haustoria attached either to host roots or to symbiotically active nodules. Haustoria were fixed in 2.5% glutaraldehyde in 0.025M phosphate buffer (pH 7.0). After ethanol dehydration and embedding in wax, sections were cut at 10 μm through the haustoria – nodule plane and stained in 1% aqueous toluidine blue. Alternatively specimens were dehydrated in acetone, embedded in Spurr’s resin, cut at 1 μm and stained in 1% methylene blue and 1% azure II in 1% borax.

Harvested leaf, stem, bark and root material of parasite and hosts were oven dried at 80°C for 48 hours and finely milled for chemical analysis. Total N in dry matter of plant samples was determined by the Kjeldahl digestion method (McKenzie and Wallace 1954), and K, Ca, Mg, Na, Fe, Cu, Mn and Zn assayed using a nitric/perchloric acid digestion procedure followed by atomic absorption spectrophotometry of appropriately diluted digests (see Pate et al. 1991a). Total P was estimated separately on the digests using the colourimetric molybdenum blue method (Kitson and Mellon 1944).

Data for haustorial frequencies or mineral nutrient amounts in plant dry matter were analysed using ANOVA and Tukey’s pairwise t-test. All analyses were performed using SYSTAT® statistical software (Systat 1992). Linear regression and stepwise multiple regression
procedures for predicting *Santalum* dry weight were performed using the regression procedure of SYSTAT®.

The formula used for estimating the heterotrophic gain of carbon (H) by *Santalum* when grown as single pairwise plantings with a particular N₂-fixing host was as follows:

\[ H = C:Nxs \times (TN^1 - TN^2) \]

Where C:Nxs is the C : N ratio of the total organic solutes of xylem sap of *Santalum* grown with N₂-fixing host, TN^1 is the incremental gain of N of *Santalum* when grown on the same host and TN^2 is the incremental gain of N of *Santalum* cultured without a host. The following assumptions are made: (a) N uptake from by the parasite from the potting medium was the same when growing with a host and when grown alone, (b) all N in parasitic *Santalum* additional to that accumulated when grown alone represents fixed or medium derived N abstracted by the parasite from host xylem (Press *et al.* 1987; Tennakoon and Pate 1996a), (c) organic solutes recovered from xylem sap of *Santalum* were representative of the proportional amounts of C and N currently being gained from the host (Tennakoon and Pate 1996a), (d) C derived from host and that from parasite photosynthesis mix and are in equilibrium so that they are proportionally respired and used as C-skeletons and (e) the quantity of C and N in the xylem recycling within the parasite, the respiratory loss from below ground parts of the parasite and the extent of dark fixation of CO₂ by haustoria are negligible. The analysis used C and N contents of dry matter and C : N ratios obtained earlier from analysis of xylem solutes (Radomiljac *et al.* 1998d) of the same *Santalum* cultures used in this study.

**6.4. Results**

**6.4.1. Haustorial Numbers and Sittings of Attachments**

The number of mature haustoria attached to host roots and presumably penetrating to xylem tissue varied considerably with the host species which was being parasitised (Figure 6.1) but a very strong correlative relationship \( r^2 = 0.99, \ p = 0.066, \ n = 12 \) existed between haustorial number on a host and root dry weight of that host. However somewhat surprisingly, a poor and non-significant correlation was found between haustorial number and the extent of benefit to *Santalum*, as gauged by its shoot or root DW at the time of harvest. This finding contrasts with that of Tennakoon *et al.* (1997c) who reported a strong positive relationship of this nature for
Olax phyllanthi (Labill.) R. Br pot cultured with single hosts. Figure 6.1 shows relatively small proportional numbers of haustoria attached to nodules on leguminous hosts compared with the number on the same hosts. However there was a strong positive, although non-significant, relationship between the number of haustoria connected to nodules and Santalum shoot DW ($r^2 = 0.87$, $p = 0.238$, $n = 9$) and a significant positive correlation to Santalum root DW ($r^2 = 0.99$, $p = 0.031$, $n = 9$). Autoparasitism, involving haustorial connections of Santalum onto its own roots, was appreciable in all associations, but never exceeded more than 27% of the total number of haustorial connections recovered from a pot. Self-parasitism was also prevalent on plants of Santalum grown without a host (Figure 6.2b).

Endophytes of haustoria penetrating nodules were examined in the case of all three legume hosts. The point of entry of the endophyte was apparently random in relation to the gross morphology of the nodule and in all cases failed to connect to the peripheral vasculature of the nodule (Figures 6.2a and c). Endophytes within nodules were bulbous, in comparison the typical flattened endophytes against the xylem of host roots (Figure 6.2d and e). There was usually a marked gap between the absorbing face of the endophyte and bacterial tissue of the nodule and disintegration of bacterioids and collapse of nodule cells were evident in older nodules (Figure 6.2d). There appeared to be less development of vascular tissue in the endophytes of haustoria on nodules than in those attached to roots (Figures 6.2d and f). Haustorial structures were found penetrating the xylem tissue of the eucalypt in a normal manner (data shown not )

6.4.2. C AND N CONTENT OF SANTALUM AND PARASITISED AND UNPARASITISED HOSTS

C and N contents of Santalum shoots grown in association with S. formosa were much greater than those of any other association. Santalum grown in association with E. camaldulensis showed considerably less C and N content than in any other association and less also than in Santalum grown without a host. As to be expected from the drain made on host resources by the presence of the parasite, growth and associated C and N contents of unparasitised hosts were consistently much greater than in those parasitised, the largest differences in these respects being recorded for S. formosa (Table 6.1).
Figure 6.1: Number of haustorial attachments made by *Santalum album* on host roots, N₂-fixing nodules and on its own roots when cultured as single plant pairings with four host species or without a host. Values are means with standard error bars shown. Treatment means of haustoria attachments on N₂-fixing nodules followed by the same letter are not significantly different (p > 0.05) using Tukey’s pairwise t-test. Treatment means for haustoria on roots of different hosts were not significantly different, neither were data on haustorial formation by the parasite on its own roots. Data are from 3 replicates.
Figure 6.2: *Santalum album* haustoria on (A) *Acacia trachyacarpa* nodule, bar = 1 mm; (B) *Santalum* roots (self-parasitism), bar = 1 cm; (C) *A. trachyacarpa* nodule. An immature haustorium on a functional nodule, section through fresh material, bar = 1 mm; (D) *A. trachyacarpa* nodule, bacteroids in this nodule have degenerated and there is a zone of tissue disintegration at the face of the endophyte, section wax embedded, cut at 10 μm and stained in toluidine blue, bar = 1 mm; (E) *Sesbania formosa* root, bar = 1 mm; (F) *S. formosa* root in TS, section resin embedded, cut at 1 μm and stained in methylene blue and azure II, bar = 0.1 mm. S, *S. album* root. H, *S. album* haustorium. N, *A. trachyacarpa* root nodule. E, endophyte of haustorium. Sf, *S. formosa* root.
Table 6.1: Total carbon and nitrogen content of shoots of *Santalum* and associated host species, cultured as single plant pairings. Corresponding data for non-parasitised hosts are included for each host species. Data are means ± std errors. Data for *Santalum* and parasitised hosts are from 3 replicates, data for unparasitised hosts are from 1 replicate.

<table>
<thead>
<tr>
<th>Parasite : Host association</th>
<th>Parasitised shoot DW (g) a</th>
<th>Parasitised host shoot DW (g) a</th>
<th>Non-parasitised host shoot DW (g) a</th>
<th>C content of <em>Santalum</em> shoot (%) b</th>
<th>C content of parasitised host shoot (%) b</th>
<th>C content of non-parasitised host shoot (%) b</th>
<th>Total C of <em>Santalum</em> shoot DW (g)</th>
<th>Total C parasitised host shoot DW (g)</th>
<th>Total C non-parasitised host shoot DW (g)</th>
<th>N content of <em>Santalum</em> shoot (%) b</th>
<th>N content of parasitised host shoot (%) b</th>
<th>N content of non-parasitised host shoot (%) b</th>
<th>Total N of <em>Santalum</em> shoot DW (g)</th>
<th>Total N parasitised host shoot DW (g)</th>
<th>Total N non-parasitised host shoot DW (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>182 ± 46.9</td>
<td>123 ± 58.4</td>
<td>57.5 ± 35.2</td>
<td>17.1 ± 8.8</td>
<td>35.6 ± 11.4</td>
<td></td>
<td>86.8</td>
<td>29.7</td>
<td>184</td>
<td>1.41 ± 0.2</td>
<td>1.22 ± 0.1</td>
<td>1.20 ± 0.1</td>
<td>0.59 ± 0.04</td>
<td>0.55 ± 0.01</td>
<td></td>
</tr>
<tr>
<td><em>S. formosa</em></td>
<td>59.9 ± 11.0</td>
<td>277 ± 108</td>
<td>30.2 ± 26.6</td>
<td>169 ± 48.7</td>
<td>-</td>
<td></td>
<td>50.0 ± 0.7</td>
<td>49.0 ± 1.3</td>
<td>46.7 ± 0.4</td>
<td>47.7 ± 1.24</td>
<td>-</td>
<td></td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>A. ampliceps</em></td>
<td>394</td>
<td>ND b</td>
<td>100</td>
<td>239</td>
<td>-</td>
<td></td>
<td>43.7 ± 0.6</td>
<td>46.7 ± 0.4</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>S. trachycarpa</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>50.3 ± 0.7</td>
<td>47.0</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>E. camaldulensis</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>47.7 ± 1.24</td>
<td>74.9</td>
<td>17.0</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>Santalum</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>86.8</td>
<td>29.7</td>
<td>184</td>
<td>1.41 ± 0.2</td>
<td>1.22 ± 0.1</td>
<td>1.20 ± 0.1</td>
<td>0.59 ± 0.04</td>
<td>0.55 ± 0.01</td>
<td></td>
</tr>
<tr>
<td><em>Santalum</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1.08 ± 0.02</td>
<td>1.35 ± 0.1</td>
<td>0.32 ± 0.02</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>No host <em>Santalum</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2.57</td>
<td>0.69</td>
<td>0.10</td>
<td>0.19</td>
<td>-</td>
<td>-</td>
<td></td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

a, Data from Radmiljic et al. (1998c); b, Radmiljic et al. (1998d); ND, No data.
6.4.3. ESTIMATES OF PROPORTIONAL HETEROTROPHIC GAIN (H) OF CARBON BY SANTALUM ON N₂-FIXING HOSTS

Santalum grown with S. formosa had the greatest increment of C and N over a nine week period, whereas C and N increment of Santalum grown with E. camaldulensis was considerably lower than when grown with any other host (Table 6.2). Estimates of H based on calculations from the C : N ratios of total organic solutes in xylem sap and N increments of parasitised Santalum over those of Santalum alone showed C gain from S. formosa (29.9 g C plant⁻¹), followed by A. ampliceps (about 35.9 g C plant⁻¹) and A. trachycarpa (12.7 g C plant⁻¹). The C gains are equivalent to 34.6%, 57.9% and 45.5% of net C increments in Santalum.

6.4.4. MINERAL NUTRIENT DISTRIBUTION OF PARASITE AND HOST

N, P, K, Ca, Mg and Na amounts in dry matter and N : P and K : Na ratios for various plant parts of all Santalum : host associations harvested at 33 weeks are shown in Figure 6.3 (Data for Fe, Mn, Zn and Cu are not shown). Mineral nutrient amounts of Santalum plant parts when grown in partnership with E. camaldulensis differed little from those of Santalum grown without a host, consistent with little or no dry matter benefit to Santalum resulting from association with this host (Table 6.1). By contrast Santalum attached to any of the leguminous hosts showed amounts of N and K in plant parts which were significantly greater than in corresponding parts of Santalum growing without a host. The reverse applied to P, Na and Mg. As expected, levels of all nutrients were higher in bark than in stem tissue as recorded earlier for a mistletoe by Tennakoon and Pate (1996b).

Analysis of all mineral nutrient data for Santalum indicated that amounts of N, P, K, Ca, Mg, Mn, Na and Fe in leaves, of K and Zn in stem wood and K and Ca roots were all significantly affected by the host to which Santalum was attached (Table 6.3).
Table 6.2: Proportional heterotrophic gain of carbon by the root hemi-parasite *Santalum* calculated from the C : N ratio of total organic solutes in *Santalum* xylem sap and the difference between the incremental gain of N of *Santalum* when grown with or without a N$_2$-fixing host.

<table>
<thead>
<tr>
<th>Parasite : Host association</th>
<th><em>S. formosa</em></th>
<th><em>A. ampliceps</em></th>
<th><em>A. trachycarpa</em></th>
<th>No host <em>Santalum</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>C : N ratio of total organic solutes from <em>Santalum</em> xylem sap</td>
<td>18.0</td>
<td>28.3</td>
<td>21.5</td>
<td>102</td>
</tr>
<tr>
<td><em>Santalum</em> DW 24 weeks after parasite : host association (g plant$^{-1}$)</td>
<td>110</td>
<td>52.9</td>
<td>50.8</td>
<td>35.7</td>
</tr>
<tr>
<td><em>Santalum</em> DW 33 weeks after parasite : host association (g plant$^{-1}$)</td>
<td>292</td>
<td>180</td>
<td>106</td>
<td>60.9</td>
</tr>
<tr>
<td><em>Santalum</em> DW increment over a 9 week period (g plant$^{-1}$)</td>
<td>181</td>
<td>127</td>
<td>55.7</td>
<td>25.2</td>
</tr>
<tr>
<td>Total C of <em>Santalum</em> at 24 weeks (g plant$^{-1}$)</td>
<td>52.4</td>
<td>25.7</td>
<td>25.3</td>
<td>17.0</td>
</tr>
<tr>
<td>Total N of <em>Santalum</em> at 24 weeks (g plant$^{-1}$)</td>
<td>1.55</td>
<td>0.64</td>
<td>0.61</td>
<td>0.19</td>
</tr>
<tr>
<td>Total C of <em>Santalum</em> at 33 weeks (g plant$^{-1}$)</td>
<td>138</td>
<td>87.6</td>
<td>53.1</td>
<td>29.0</td>
</tr>
<tr>
<td>Total N of <em>Santalum</em> at 33 weeks (g plant$^{-1}$)</td>
<td>4.11</td>
<td>2.20</td>
<td>1.27</td>
<td>0.33</td>
</tr>
<tr>
<td>ΔC of <em>Santalum</em> dry matter from 24 to 33 weeks (g plant$^{-1}$)</td>
<td>86.6</td>
<td>62.0</td>
<td>27.8</td>
<td>12.0</td>
</tr>
<tr>
<td>ΔN of <em>Santalum</em> dry matter from 24 to 33 weeks (g plant$^{-1}$)</td>
<td>2.56</td>
<td>1.6</td>
<td>0.66</td>
<td>0.14</td>
</tr>
<tr>
<td>C : N ratio of <em>Santalum</em> dry matter increment</td>
<td>33.7</td>
<td>39.8</td>
<td>41.6</td>
<td>86.7</td>
</tr>
<tr>
<td>Difference between incremental N of <em>Santalum</em> when grown with N$_2$-fixing hosts and without a host over a 9 week period (g plant$^{-1}$)</td>
<td>1.66</td>
<td>1.27</td>
<td>0.59</td>
<td>-</td>
</tr>
<tr>
<td>Heterotrophic gain of C of <em>Santalum</em> from host based on C : N ratio of total organic solutes in <em>Santalum</em> xylem sap and incremental gain of N of <em>Santalum</em> when grown with N$_2$-fixing hosts and without a host over a 9 week period (g plant$^{-1}$)</td>
<td>29.9</td>
<td>35.9</td>
<td>12.7</td>
<td>-</td>
</tr>
<tr>
<td>Values for H as % of total C gain of parasite from 24 to 33 weeks</td>
<td>34.6</td>
<td>57.9</td>
<td>45.5</td>
<td>-</td>
</tr>
</tbody>
</table>

$^a$, Data from Radomiljac *et al.* (1998d)

$^b$, Data from Radomiljac *et al.* (1998c)

$^c$, Data from Table 1
Figure 6.3a: The nitrogen concentrations in dry matter of leaf, stem, bark and root tissue at final harvest at 33 weeks in Santalum and its hosts (A) Sesbania formosa, (B) Acacia trachycarpa, (C) A. ampliceps or (D) Eucalyptus camaldulensis or when grown without a host (E). Data are from 3 replicates from assessment at week 33 of parasite: host association. See Table 6.3 for statistical data.
Figure 6.3b: The phosphorus concentrations in dry matter of leaf, stem, bark and root tissue at final harvest at 33 weeks in *Santalum* and its hosts (A) *Sesbania formosa*, (B) *Acacia trachyacarpa*, (C) *A. ampliceps* or (D) *Eucalyptus camaldulensis* or when grown without a host (E). Data are from 3 replicates from assessment at week 33 of parasite: host association. See Table 6.3 for statistical data.
Figure 6.3c: The N : P ratios of leaf, stem, bark and root tissue at final harvest at 33 weeks in *Santalum* and its hosts (A) *Sesbania formosa*, (B) *Acacia trachycarpa*, (C) *A. ampliceps* or (D) *Eucalyptus camaldulensis* or when grown without a host (E). Data are from 3 replicates from assessment at week 33 of parasite : host association. See Table 6.3 for statistical data.
Figure 6.3d: The potassium concentrations in dry matter of leaf, stem, bark and root tissue at final harvest at 33 weeks in *Santalum* and its hosts (A) *Sesbania formosa*, (B) *Acacia trachycarpa*, (C) *A. ampliceps* or (D) *Eucalyptus camaldulensis* or when grown without a host (E). Data are from 3 replicates from assessment at week 33 of parasite : host association. See Table 6.3 for statistical data.
Figure 6.3e: The sodium concentrations in dry matter of leaf, stem, bark and root tissue at final harvest at 33 weeks in Santalum and its hosts (A) Sesbania formosa, (B) Acacia trachycarpa, (C) A. ampliceps or (D) Eucalyptus camaldulensis or when grown without a host (E). Data are from 3 replicates from assessment at week 33 of parasite: host association. See Table 6.3 for statistical data.
Figure 6.3f: The K : Na ratios of leaf, stem, bark and root tissue at final harvest at 33 weeks in *Santalum* and its hosts (A) *Sesbania formosa*, (B) *Acacia trachyacarpa*, (C) *A. ampliceps* or (D) *Eucalyptus camaldulensis* or when grown without a host (E). Data are from 3 replicates from assessment at week 33 of parasite : host association. See Table 6.3 for statistical data.
Figure 6.3g: The calcium concentrations in dry matter of leaf, stem, bark and root tissue at final harvest at 33 weeks in Santalum and its hosts (A) Sesbania formosa, (B) Acacia trachycarpa, (C) A. ampliceps or (D) Eucalyptus camaldulensis or when grown without a host (E). Data are from 3 replicates from assessment at week 33 of parasite: host association. See Table 6.3 for statistical data.
Figure 6.3h: The magnesium concentrations in dry matter of leaf, stem, bark and root tissue at final harvest at 33 weeks in Santalum and its hosts (A) Sesbania formosa, (B) Acacia trachycarpa, (C) A. ampliceps or (D) Eucalyptus camaldulensis or when grown without a host (E). Data are from 3 replicates from assessment at week 33 of parasite: host association. See Table 6.3 for statistical data.
Table 6.3: The effects of intermediate host species on *Santalum* and host mineral distribution 33 weeks after commencement of parasite-host association as single plant pairings. *a*, Numbers are the probability of accepting the null hypothesis of no difference in treatment means. *b*, Treatment means are not significantly (ns) different from each other (p > 0.05).

<table>
<thead>
<tr>
<th>Dependent variable</th>
<th>N</th>
<th>P</th>
<th>K</th>
<th>Ca</th>
<th>Mg</th>
<th>Zn</th>
<th>Cu</th>
<th>Mn</th>
<th>Na</th>
<th>Fe</th>
<th>N : P ratio</th>
<th>K : Na ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Santalum</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leaf</td>
<td>0.000&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.001</td>
<td>0.001</td>
<td>0.008</td>
<td>0.000</td>
<td>ns&lt;sup&gt;b&lt;/sup&gt;</td>
<td>ns</td>
<td>0.000</td>
<td>0.000</td>
<td>0.001</td>
<td>0.000</td>
<td>0.000</td>
</tr>
<tr>
<td>Stem</td>
<td>ns</td>
<td>ns</td>
<td>0.001</td>
<td>ns</td>
<td>ns</td>
<td>0.002</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>0.004</td>
</tr>
<tr>
<td>Bark</td>
<td>ns</td>
<td>0.005</td>
<td>ns</td>
<td>0.000</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>0.029</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>Root</td>
<td>ns</td>
<td>ns</td>
<td>0.041</td>
<td>0.026</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td><em>Host</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leaf</td>
<td>0.000</td>
<td>ns</td>
<td>0.000</td>
<td>ns</td>
<td>0.001</td>
<td>ns</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
<td>ns</td>
<td>0.000</td>
<td>0.000</td>
</tr>
<tr>
<td>Stem</td>
<td>ns</td>
<td>0.029</td>
<td>ns</td>
<td>0.000</td>
<td>0.038</td>
<td>0.002</td>
<td>ns</td>
<td>0.001</td>
<td>0.000</td>
<td>ns</td>
<td>0.000</td>
<td>0.000</td>
</tr>
<tr>
<td>Bark</td>
<td>0.000</td>
<td>0.036</td>
<td>ns</td>
<td>0.001</td>
<td>0.012</td>
<td>0.001</td>
<td>0.001</td>
<td>0.004</td>
<td>0.010</td>
<td>0.001</td>
<td>ns</td>
<td>0.000</td>
</tr>
<tr>
<td>Root</td>
<td>ns</td>
<td>ns</td>
<td>0.048</td>
<td>0.027</td>
<td>0.003</td>
<td>ns</td>
<td>ns</td>
<td>0.000</td>
<td>0.009</td>
<td>ns</td>
<td>0.037</td>
<td></td>
</tr>
</tbody>
</table>
6.4.5. **Increments in dry weight, N, P, K, Ca and Na of Santalum on different hosts**

Changes in total contents of N, P, K, Ca and Na in *Santalum* from week 24 to week 33 indicated that relative to dry weight increment there was disproportionately greater benefit in N when grown on any legume host; in Na when on *S. formosa*, in P and K when on *A. ampluseps* and Ca when on *A. trachycarpa* (Figure 6.4). Net decreases or only slight increases in N, P, K, Ca and Na content in *Santalum* grown on *E. camaldulensis* or without a host were generally indicative of poor growth of such cultures in the latter part of the growth period. Strong positive correlation's were demonstrated between *Santalum* dry weight increment and total N ($r^2 = 0.97$, $p = 0.002, n = 15$) and Na increment ($r^2 = 0.88, p = 0.019, n = 15$).

6.5 **Discussion**

Despite extensive studies on the carbon and water economies and mineral nutrition of root hemiparasitic plants and their hosts, in both pot culture and native habitat (Hocking 1980; Lamont and Southall 1982; Glatzel 1983; Schulze and Ehleringer 1984; Struthers *et al.* 1986; Pate *et al.* 1991a; Seel and Press 1993; Tennakoon and Pate 1996b; Veenendaal *et al.* 1996; review by Pate 1995a), limited definitive information exists on mechanisms and extents of benefit in terms of heterotrophic gains of carbon and acquisition of specific mineral elements from hosts. Using *Santalum* partnered singly with leguminous and a non-leguminous host, an attempt is made in this chapter to quantify benefits and thus rank hosts in terms of suitability as donors of organic and inorganic solutes to the parasite.

Our study confirms for *S. album* the general conclusion that xylem-tapping root hemi-parasites grow best when associated with N$_2$-fixing legume hosts, apparently as a result of greater N concentrations in xylem of legumes compared to non legumes (Subbarao *et al.* 1990; Cechin and Press 1993; Seel and Press 1993; Tennakoon and Pate 1996a; Tennakoon *et al.* 1997a, b, c; Radomiljac *et al.* 1998c, d). Since the pot culture conditions in this study provided only a small initial dressing of N in the form of slow release fertiliser the unsurpassed benefit from nodulated legume hosts to *Santalum* comes from the abstraction of fixed N. Conversely, *Santalum* partnered with *E. camaldulensis* had to compete with its host for the fertiliser N applied and not surprisingly grew extremely poorly compared to those plants on legume hosts and amassed only half the dry weight of plants grown without hosts (Table 6.1).
Figure 6.4a: Increment in the root hemi-parasite *Santalum* (A) dry weight, (B) nitrogen and (C) phosphorous over a nine week period of effective attachment (week 24 to 33 of association) when partnered singly with *Sesbania formosa, Acacia trachycarpa, A. ampliceps* or *Eucalyptus camaldulensis* or when grown without a host.
Figure 6.4b: Increment in the root hemi-parasite Santalum (D) potassium, (E) calcium and (F) sodium over a nine week period of effective attachment (week 24 to 33 of association) when partnered singly with Sesbania formosa, Acacia trachycarpa, A. amplätzeps or Eucalyptus camaldulensis or when grown without a host.

Pot cultured partnerships
As further evidence of competition, as opposed to benefit to *Santalum*, the root biomass of the eucalypt was much greater than that of other hosts while root mass of the *Santalum* was less than in any other treatment. At the other extreme, *A. trachycarpa*, the host with the smallest root biomass, promoted growth of a root system in associated *Santalum* four times larger than when parasitising *E. camaldulensis* (Radomiljac *et al.* 1998c).

Somewhat surprisingly this study showed a very poor relationship between haustorial number and resulting growth benefit of *Santalum*. For example *Santalum* parasitising *A. trachycarpa* established only half the number of haustoria as the *Santalum : E. camaldulensis* association, yet benefit from the former host was very much greater. Of course counts or even weights of haustoria offer no direct evidence of benefit and without sectioning and proof of uptake from host xylem, their efficacy remains unproven. Indeed, Santalacean root hemi-parasites can attach to inanimate objects such as small stones, decaying plant material, surfaces of pots and particularly to slow-release fertilizer pellets (A. M. Radomiljac unpubl. obs.; Hocking and Fineran 1983; Pate *et al.* 1990a). In the present study we report also on direct parasitism of the nodules of legume hosts, as described for *Olax phyllanthi* haustoria on nodules of *Acacia littorea* Maslin (Tennakoon *et al.* 1997c). In both cases haustoria penetrating nodules represented very small proportions of the total contacts made with a host and quickly resulted in degeneration of nodule bacterial tissue and therefore, presumably, cessation of fixation. On these grounds attachment to nodules is likely to yield minimal benefit compared with longer term and more prolific contacts between haustoria and root xylem.

It has been long accepted for plants generally that N deficiencies results in abnormally greater partitioning of assimilates to the root as opposed to shoots. This results in higher root : shoot ratios and may be viewed as a mechanism for exploiting greater possible volumes of soil towards improvement of N uptake (Linder and Rook 1984; Nambiar 1990; Stoneman and Dell 1993). In a similar manner young as yet unattached seedlings of *Santalum* show preferential partitioning of biomass to their root system (Radomiljac *et al.* 1998b), but following attachment to a beneficial host, biomass partitioning shifts progressively in favour of the shoot. High N : P ratio of *Santalum* foliage when grown on legume hosts (10.5 – 17.2) compared with that when grown on *E. camaldulensis* (3.8) or without a host (4.4) provides confirmation of the extent to which a legume host alleviates N-deficiency of the parasite. However, N : P ratios for *S. album* recorded on N₂-fixing hosts in this study were 2 to 3 times lower than those reported for *S. spicatum* (R. Br.) A. DC. when attached to *Acacia acuminatum* Benth. in native habitat (Struthers *et al.* 1986). Presumably P limitation applied to the ecosystem where *S. spicatum* was growing, whereas the slow release fertiliser used in this study was likely to have provided
non-limiting amounts of P but not of N.

In this chapter we employ a novel technique for estimating heterotrophic gains (H) of C by *Santalum*, by matching the C : N ratio of the organic solutes of its xylem sap when grown on a N₂-fixing host with the difference between the incremental gain of N of *Santalum* grown with the same host to that when grown without a host. Estimates of H were assessed in terms of total carbon gain by the parasite over a 9-week interval and these indicated that *A. ampliceps* was the best provider of C (35.9g), followed closely by *S. formosa* (29.9g) and then *A. trachycarpa* (12.7g). Expressed in terms of proportional benefit, that is the percentage of the net C gain in dry matter of the parasite afforded by C flow from xylem of the host, *A. ampliceps* again turned out to better provider of C (57.9% of net C gain of parasite) compared to values of 45.5% and 34.6% for *A. trachycarpa* and *S. formosa*, respectively. It should be noted that the last mentioned host elicited much greater N gain than the other two, despite the above low H rating on a percentage basis.

It is almost universally true that both aerial and root hemi-parasites have substantially higher K amounts than their hosts (Lamont and Southall 1982; Glatzel 1983; Schulze and Ehleringer 1984; Struthers et al. 1986; Seel and Press 1993; Pate 1995a) and was so for the *Santalum* : N₂-fixing host associations of this study (Figure 6.1), but, overtly not so for *Santalum* grown with the non-beneficial host *E. camaldulensis*. Barrett and Fox (1997) have suggested that *S. spicatum* may be capable of substantial independent K uptake through its own roots, but our results suggest that *S. album* competes ineffectively for K when grown with *E. camaldulensis* in a confined rooting medium. According to Glatzel (1983) and Seel and Press (1993), accumulation of K in xylem tapping hemi-parasites is a passive progressive enrichment process, with increases in the element proportional to transpiration rate of the parasite (Schulze and Ehrlinger 1984; Kuppers et al. 1992). In our study transpiration rates per unit leaf area of *Santalum* grown with *E. camaldulensis* were just over half of those of when grown on N₂-fixing hosts, a result in keeping with the conclusion suggested above (see Radomiljac et al. 1998f).

Consistent with data reported for *S. spicatum* by Struthers et al. (1986), amount of Na in foliage of *S. album* were very high compared with all other parts of the parasite and generally much greater than in leaves and other parts of the associated host. High leaf Na amounts compared with those of a host might be viewed as contributing to osmotic gradients (Struthers et al. 1986), which coupled with high transpiration rates (Table 5.1), would ensure the efficient capture of water from the host. Fer et al. (1994) suggests that *Thesium humile* Vahl. (Santalaceae) has a high ability to take up Na direct from the soil and our study suggests the
Santalum also has this ability. The K : Na ratios in foliage of Santalum plants grown on N₂-fixing hosts (0.55 – 1.19) in comparison with the low value of 0.27 when grown on *E. camaldulensis* and 0.13 when without a host, suggests K discrimination over Na is improved when grown in association with N₂-fixing hosts. The mechanism whereby this occurs would be worthy of further study.

Our study provides convincing evidence that certain hosts provide Santalum with minerals as well as N, and that the degree of benefit in respect of specific elements may be idiosyncratic of the host involved. This is indicated, for example, by the greater amount of Ca in all parts of Santalum when in association with *A. trachycarpa*, a species that accumulated Ca more than 10 times the amounts shown in other hosts. Ca amounts in hemi-parasites have been used to distinguish between phloem and xylem feeding parasites (Ziegler 1976; Tsivion 1978; Lamont and Southall 1982; Struthers *et al.* 1986) due to Ca being relatively mobile in the xylem. As there is no lumen to lumen tracheary continuity between host and parasite, in the haustoria of most root hemi-parasites (Rao 1942; Webb 1984; Pate *et al.* 1990c; Riopel and Timko 1995; Tennakoon and Pate 1996a) xylem transfer from host to parasite must involve the apoplastic pathway through haustoria cell walls (Coetzee and Fineran 1987; Kuo *et al.* 1989; Pate *et al.* 1994), with the additional possibility that parenchymatous cells at the haustoria interface with the host xylem might facilitate transfer of selectively absorbing host xylem solutes and then releasing them and derived solutes to the xylem of the parasite (Radomiljac *et al.* 1998d). The low Ca amount in all Santalum plant parts when grown with *E. camaldulensis* is consistent with there being little such xylem-derived benefit in terms of Ca uptake from this host. This hypothesis is confirmed by the findings that amounts in Santalum plant tissue of the phloem mobile P, K, Mg and Na showed little resemblance to that of its associated host for all associations.

During the course of the studies reported on in this and earlier chapters (3, 4 and 5), we have employed a wide range of indirect criteria as possible mechanisms for assessing potential benefit to the parasite. A selection of those is listed in Table 6.4 and ranked in order of relative accuracy as predictors of biomass gain of the parasite by the final harvest of the study. The analysis ascribes very high $r^2$ ratings (0.87 – 0.99) to N and C increments of the parasite over the last 9 weeks of growth, N amounts in shoot and leaf dry matter of the parasite and its final leaf area. All of these parameters are likely to be highly correlated with growth performance. Near equal ratings also apply to final K amount of foliage ($r^2 = 0.90$) and somewhat surprisingly to Na increment of Santalum over the final 9 weeks of growth ($r^2 = 0.94$). All other criteria listed in Table 6.4 carry $r^2$ ratings in a much lower range (0.03 – 0.60) and are
accordingly discarded in terms of yield prediction.

Using various combinations of the highly significant predictive attributes listed above, the stepwise regression procedure was tested for predicting final dry weight of Santalum in the various associations. Four such regressions are shown in Table 6.5, one (A) achieving an $r^2$ rating of 1.0 after two steps, one (B) after three, and the other two (C and D) after four. While obviously highly successful, the regression approach of B and D carries the major disadvantage of using information from destructive harvests of the parasite and, in the cases of increments of C and N, a requirement for two such sequential harvests. This would clearly limit the practical value of procedure in the field. An almost equally useful procedure would be to restrict parameters used in the regression to minimally destructive assays of foliage or xylem sap as in the case for A and C for specific elements such as N, K and possibly Na, since all of these were particularly well correlated with Santalum yield. With the prospect of large scale commercial enterprises cultivating S. album in north Western Australia, using the primary and some of the intermediate hosts suggested in this series of chapters, effective procedures for monitoring growth and models for predicting yield and quality of wood will become of great value, particularly since protocols suggested for plantation management have so far been tested only for relatively short time spans in early life of hosts and parasite.
Table 6.4: Growth and compositional criteria relative to single pot cultured associations of *Santalum album* with leguminous and a eucalypt host and the relative usefulness of these in prediction of final dry weight of the parasite. The model $y = b_0 + b_1x$. $r^2$ is used to calculate respective coefficients of determination.

<table>
<thead>
<tr>
<th>Factor</th>
<th>$b_0$</th>
<th>$b_1$</th>
<th>$r^2$</th>
<th>$p$</th>
</tr>
</thead>
<tbody>
<tr>
<td>N concentration of <em>Santalum</em> shoot dry matter (%) $^b$</td>
<td>24.8</td>
<td>104.5</td>
<td>0.99</td>
<td>0.002</td>
</tr>
<tr>
<td><em>Santalum</em> root : shoot ratio $^a$</td>
<td>673.5</td>
<td>-722.1</td>
<td>0.99</td>
<td>0.006</td>
</tr>
<tr>
<td>Increment in total N of <em>Santalum</em> over 9 week period (24 – 33 weeks) (g plant$^{-1}$) $^c$</td>
<td>31.9</td>
<td>102.2</td>
<td>0.99</td>
<td>0.002</td>
</tr>
<tr>
<td><em>Santalum</em> leaf area (cm$^2$) $^a$</td>
<td>14.6</td>
<td>0.03</td>
<td>0.98</td>
<td>0.011</td>
</tr>
<tr>
<td>Increment in total C of <em>Santalum</em> over 9 week period (g plant$^{-1}$) $^c$</td>
<td>23.7</td>
<td>2.9</td>
<td>0.98</td>
<td>0.012</td>
</tr>
<tr>
<td>Increment in total Na of <em>Santalum</em> over 9 week period (g plant$^{-1}$) $^d$</td>
<td>76.7</td>
<td>461.8</td>
<td>0.94</td>
<td>0.033</td>
</tr>
<tr>
<td>K concentration of foliage of <em>Santalum</em> (%) $^e$</td>
<td>-236.1</td>
<td>578.9</td>
<td>0.90</td>
<td>0.050</td>
</tr>
<tr>
<td>N concentration of foliage of <em>Santalum</em> (%) $^e$</td>
<td>-145.2</td>
<td>162.6</td>
<td>0.87</td>
<td>0.065</td>
</tr>
<tr>
<td>C:N ratio of organic solutes of <em>Santalum</em> xylem sap $^f$</td>
<td>285</td>
<td>-4.04</td>
<td>0.60</td>
<td>0.224</td>
</tr>
<tr>
<td>N concentration of <em>Santalum</em> xylem sap (µgN/ml) $^f$</td>
<td>-7.89</td>
<td>2.674</td>
<td>0.51</td>
<td>0.283</td>
</tr>
<tr>
<td>Increment in total K of <em>Santalum</em> over 9 week period (g plant$^{-1}$) $^d$</td>
<td>99.1</td>
<td>295.7</td>
<td>0.42</td>
<td>0.350</td>
</tr>
<tr>
<td>Total number of haustorial attachments to host $^e$</td>
<td>45.2</td>
<td>2.03</td>
<td>0.41</td>
<td>0.363</td>
</tr>
<tr>
<td>Host use efficiency (parasite DW / host DW) $^a$</td>
<td>81.6</td>
<td>42.5</td>
<td>0.35</td>
<td>0.412</td>
</tr>
<tr>
<td>N use efficiency in photosynthesis (µmol s$^{-1}$ mg$^{-1}$ N) $^b$</td>
<td>11.7</td>
<td>7091</td>
<td>0.33</td>
<td>0.428</td>
</tr>
<tr>
<td><em>Santalum</em> shoot : root ratio $^a$</td>
<td>-55.22</td>
<td>130.594</td>
<td>0.24</td>
<td>0.515</td>
</tr>
<tr>
<td>Na concentration of foliage of <em>Santalum</em> (%) $^e$</td>
<td>285.9</td>
<td>-99.5</td>
<td>0.21</td>
<td>0.539</td>
</tr>
<tr>
<td>Increment in total P of <em>Santalum</em> over 9 week period (g plant$^{-1}$) $^d$</td>
<td>125.9</td>
<td>2431</td>
<td>0.19</td>
<td>0.563</td>
</tr>
<tr>
<td>Total dry weight of host root (g plant$^{-1}$) $^a$</td>
<td>203.7</td>
<td>-0.73</td>
<td>0.12</td>
<td>0.645</td>
</tr>
<tr>
<td>N concentration of host shoot dry matter (%) $^b$</td>
<td>91.5</td>
<td>68.9</td>
<td>0.07</td>
<td>0.732</td>
</tr>
<tr>
<td>Total dry weight of host shoot (g plant$^{-1}$) $^a$</td>
<td>172.7</td>
<td>-0.15</td>
<td>0.03</td>
<td>0.843</td>
</tr>
<tr>
<td>C:N ratio of organic solutes of host xylem sap $^f$</td>
<td>180.5</td>
<td>-0.62</td>
<td>0.03</td>
<td>0.843</td>
</tr>
</tbody>
</table>

$^a$, Data from Radomiljac et al. (1998c); $^b$, Table 6.1; $^c$, Table 6.2; $^d$, Figure 6.4; $^e$, Figure 6.2; $^f$, Radomiljac et al. (1998d); $^g$, Figure 6.1; $^h$, Radomiljac et al. (1998f)
Table 6.5: Examples of a stepwise regression procedure for accurately predicting *Santalum* dry weight.

<table>
<thead>
<tr>
<th>Regression Model</th>
<th>b0</th>
<th>b1</th>
<th>b2</th>
<th>b3</th>
<th>r²</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>(A)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Santalum</em> DW = b0 + b1Nxs(^a)</td>
<td>-1.789</td>
<td>2.674</td>
<td>-</td>
<td>-</td>
<td>0.51</td>
<td>0.283</td>
</tr>
<tr>
<td><em>Santalum</em> DW = b0 + b1Nxs + b2N%sdm(^b)</td>
<td>14.787</td>
<td>0.291</td>
<td>98.952</td>
<td>-</td>
<td>1.00</td>
<td>0.005</td>
</tr>
<tr>
<td>(B)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Santalum</em> DW = b0 + b1Ninc(^c)</td>
<td>31.9</td>
<td>102.2</td>
<td>-</td>
<td>-</td>
<td>0.99</td>
<td>0.004</td>
</tr>
<tr>
<td><em>Santalum</em> DW = b0 + b1Ninc + b2Cinc(^d)</td>
<td>35.9</td>
<td>142.3</td>
<td>-1.16</td>
<td>-</td>
<td>0.99</td>
<td>0.054</td>
</tr>
<tr>
<td><em>Santalum</em> DW = b0 + b1Ninc + b2LA(^e)</td>
<td>47.6</td>
<td>185.9</td>
<td>-0.03</td>
<td>-</td>
<td>1.00</td>
<td>0.004</td>
</tr>
<tr>
<td><em>Santalum</em> DW = b0 + b1Ninc + b2Cinc + b3LA</td>
<td>47.9</td>
<td>184.8</td>
<td>0.16</td>
<td>-0.03</td>
<td>1.00</td>
<td>-</td>
</tr>
<tr>
<td>(C)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Santalum</em> DW = b0 + b1Nxs</td>
<td>-1.789</td>
<td>2.674</td>
<td>-</td>
<td>-</td>
<td>0.51</td>
<td>0.283</td>
</tr>
<tr>
<td><em>Santalum</em> DW = b0 + b1Nxs + b2K%sl(^f)</td>
<td>-288.007</td>
<td>0.765</td>
<td>501.207</td>
<td>-</td>
<td>0.93</td>
<td>0.267</td>
</tr>
<tr>
<td><em>Santalum</em> DW = b0 + b1Nxs + b2N%sl(^g)</td>
<td>-205.132</td>
<td>-2.974</td>
<td>288.948</td>
<td>-</td>
<td>0.98</td>
<td>0.134</td>
</tr>
<tr>
<td><em>Santalum</em> DW = b0 + b1Nxs + b2K%sl + b3N%sl</td>
<td>-230.254</td>
<td>-2.027</td>
<td>188.473</td>
<td>202.767</td>
<td>1.00</td>
<td>-</td>
</tr>
<tr>
<td>(D)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Santalum</em> DW = b0 + b1Rs ratio(^h)</td>
<td>673.5</td>
<td>-722.1</td>
<td>-</td>
<td>-</td>
<td>0.99</td>
<td>0.002</td>
</tr>
<tr>
<td><em>Santalum</em> DW = b0 + b1Rs ratio + b2N%(^i)</td>
<td>38.2</td>
<td>-14.9</td>
<td>102.3</td>
<td>-</td>
<td>0.99</td>
<td>0.058</td>
</tr>
<tr>
<td><em>Santalum</em> DW = b0 + b1LA + b2N%</td>
<td>25.4</td>
<td>-0.002</td>
<td>109.5</td>
<td>-</td>
<td>0.99</td>
<td>0.004</td>
</tr>
<tr>
<td><em>Santalum</em> DW = b0 + b1Rs ratio + b2N% + b3LA</td>
<td>2753.3</td>
<td>-2999.9</td>
<td>-8.9</td>
<td>-0.11</td>
<td>1.00</td>
<td>-</td>
</tr>
</tbody>
</table>

\(^a\) N concentration of *Santalum* xylem sap (µgN/ml)
\(^b\) N concentration of *Santalum* shoot dry matter (%)
\(^c\) Increment in total N of *Santalum* from week 24 to 33 of parasite : host association (g plant\(^{-1}\))
\(^d\) Increment in total C of *Santalum* from week 24 to 33 of parasite : host association (g plant\(^{-1}\))
\(^e\) *Santalum* leaf area (cm\(^2\))
\(^f\) K concentration of foliage of *Santalum* (%)
\(^g\) N concentration of foliage of *Santalum* (%)
\(^h\) *Santalum* root : shoot ratio
\(^i\) N concentration of *Santalum* shoot dry matter (%)
~ CHAPTER 7 ~

ROOT HEMI-PARASITE : HOST RELATIONS - INFLUENCE OF HOST QUALITY AND PROXIMITY ON SANTALUM ALBUM L. GROWTH IN PLANTATION CULTURE.

7.1. ABSTRACT

A plantation of Santalum album L. with three leguminous host species Cathormion umbellatum (Vahl) Kosterm. (Mimosaceae), Sesbania formosa (F. Muell.) N. Burb. (Fabaceae) and Acacia anuera F. Muell. ex. Benth. (Mimosaceae) showed marked decline during its third to fifth years in the earlier health and vigour of parasite and hosts. Various forms of assessment of host and parasite performance were tested as possible predictors of crown health of Santalum. A measure termed ‘plantation host index’, derived by combining host quality with proximity of host from parasite emerged as the best predictor of growth and health status of Santalum. Santalum health and vigour increased as host quality increased, but decreased as distance of Santalum from that host decreased, indicating interaction of these opposing effects on Santalum productivity. Crown health and leaf area of Santalum were strongly correlated with extent of growth between year three and five, thereby providing non-destructive means of monitoring performance of Santalum within plantations and between plantations at different sites.

7.2. INTRODUCTION

Despite the considerable cultural and commercial significance of the root hemi-parasitic tree Santalum album L. (Indian sandalwood), a degree of uncertainty still remains on how its silvicultural requirements should be formulated in a manner realising full potential in plantation culture. Particularly deficient in these respects is an understanding of how the hemi-parasite interacts with primary, intermediate and final hosts under field conditions (Havel and McKinnell 1993; Radomiljac 1998; Radomiljac et al. 1998a, b). Nevertheless, it is widely supposed, though by no means conclusively proven that Santalum, as is the case of other root hemi-parasites, can exercise appreciable deleterious influences on host trees and shrubs which it is parasitising, whether simply by abstraction of water and organic solutes from the
transpiration stream of a host, or more subtly by reducing host photosynthesis and thus changing dry matter allocation in favour of the parasite. Where such effects apply, they are likely to result in marked reductions in overall biomass and health of a host and ultimately, on the parasite which is feeding from it (Press and Stewart 1987; Press et al. 1990; review by Graves 1995; Tennakoon et al. 1997c; Radomiljac et al. 1998c). There is now evidence that plantation cultures of S. album may experience decline in vigour or even demise of hosts, due to deleterious effects of the parasite or other agencies and that such a situation can in turn lead to a dramatic lowering of the contemporary vigour of the parasite (Radomiljac 1998). Long term carryover effects on ultimate productivity of plantation will then accrue. In this chapter we coin this general phenomenon ‘plantation decline’.

Visual assessments of tree or stand vigour are widely used as non-destructive measures for estimating plantation productivity under different silvicultural practices (Munster-Swendsen 1987), but in cases where a parasitic species such as Santalum are also involved, it is obviously desirable to conduct joint assessments of vigour of both parasite and hosts. Factors which might be important in this connection would be (a) the intrinsic qualities of a host in supporting growth of Santalum, (b) the distance of the parasite from roots of the host species and (c) the impact of the respective growth and longevity of primary, intermediate and final members of a sequence of hosts in setting the progress of productivity in the parasite. Several studies have identified the especially beneficial influence which N2-fixing species may exercise on Santalum growth when grown as single plant pairings in pot or field culture (Ananthapadmanabha et al. 1988; Fox et al. 1996; Radomiljac 1998; Surata 1992a; Shinde et al. 1993), but no concerted effort has been made so far to reconcile such findings to observed long term benefits of such hosts on productivity of Santalum under field conditions.

In the course of our investigations on Santalum culture in the Ord River Irrigation Area, near Kununurra we observed that both Santalum and three associated native legume tree hosts exhibited signs of declining vigour in relatively young plantations. This conclusion was based on high growth rates and continued good health of all participants for the first three years of growth, but marked lowering of growth rate and signs of poor health over the subsequent two years. This circumstance, with its abundant evidence of variable deleterious effects through the plantation, therefore provided an ideal opportunity for quantifying reciprocal relationships between Santalum and its host under conditions clearly sub-optimal for the parasite. To accomplish such an analysis we developed a modeling procedure based on a number of defined non-destructive measurements to monitor retrospectively and prospectively likely interactions of hosts and parasites during growth.
7.3. METHODS

7.3.1. SITE AND STAND DESCRIPTION

The study site was located at the Frank Wise Institute for Tropical Agricultural Research, 10 km north of Kununurra (15° 40' S, 128° 44' E), Ord River Irrigation Area of northern Western Australia at an elevation of 45 m.a.s.l. and a mean annual rainfall of 750 mm.

Soil at the study site is Kununurra clay (alkaline phase - pH 7.8), largely derived from recently deposited river alluvia. It is typically dark brown colour (10YR 4/3), self mulching, very plastic and poorly draining when wet, and hard with seasonal cracking when dry (Aldrick et al. 1990). The site (comprising a total of 1.43 hectares) was intensively cultivated, leveled and mounded following normal Ord River Irrigation Area agricultural techniques for flood irrigation in the area. In June 1991 nursery grown seedlings of *S. album* were transplanted at the site together with three native northern Australian leguminous host tree species *Cathormion umbellatum* (Vahl) Kosterm. (Mimosoaceae), *Acacia anuera* F. Muell. ex. Benth. (Mulga) (Mimosoaceae) and *Sesbania formosa* (F. Muell.) N. Burb. (Corkwood) (Fabaceae). The planting format in the southern half the site comprised an alternating row pattern (3.6 metres apart) of seedlings of *Santalum*, *A. anuera* and *S. formosa*, with each plant species at 3 metres distance from neighbours of other species. The northern part of the site was planted in similar row fashion to *Santalum, A. anuera, S. formosa* and *C. umbellatum*. Each seedling received an initial dressing of 100 grams NPK Blue Special fertiliser (11.8% N, 6.0% P, 15.8% K, 0.05% Cu, 0.05% Zn, 0.13% Mn, 1.0% Mg, 8.3% S) soon after planting. Once seedlings had established, the site was flood irrigated successively on sixteen occasions through the dry season from July up to December 1991, ten times the following dry season between March to December 1992 and thereafter in similar fashion over the next four years. Each episode of irrigation lasted 24 hours. Herbaceous annuals recruiting between rows were mechanically slashed twice per year, but despite such management a sparse ground cover of annual grass and broadleaf species persisted at the site, mostly made up of *Vigna radiata* (L.) Wilczek (Mung bean), *Digitaria bicorns* (Lam.) Roemer and Schultes (Finger grass), *Chloris gayana* Kunth. (Rhodes grass), *Tridax procumbens* L. and *Passiflora foetida* L. (Wild passionfruit). However, because of their small collective biomass these weed species were regarded as insignificant supporters of growth of *Santalum* relative to the likely benefits to be obtained from large tree hosts.

Poor and non-uniform initial survival of *Santalum* radically corrupted the systematic planting format so that by 1994 stand densities of *Santalum* had been reduced from an initial 462 to a
patchily distributed 102 stems ha\(^{-1}\) amidst a still relatively even distribution of *S. formosa* and *A. amhuera*. Remaining *Santalum* trees were pruned once a year to establish a uniform and upright bole. By 1996 growth rates of surviving *Santalum* proved to be generally lower than in the previous two years, and both the *Santalum* and its various hosts had declined in health to the extent of marked loss of foliage in many specimens.

**7.3.2. Assessment**

In May 1994, at age 3 years, the basal diameters at 20 cm above ground level and heights of all *Santalum* trees were recorded across the entire study site. The same trees were remeasured at 5 years of age (May 1996), when linear distances between the nearest three host species and each remaining *Santalum* were also recorded. Each host plant was then assigned a quality classification based on canopy size, crown health and an estimation of leaf area. A comparative semi-quantitative ranking of 1 to 5 was employed, recording as a score of 1 for a host in poor health with a very low leaf area, through to a score of 5 for a host in excellent health with large and healthy leaf area (see Figure 7.1). Concurrently each *Santalum* was also assigned a crown health classification, again based on an semi-quantitative ranking of 1 to 5, with 1 signifying a dead tree up to a score of 5 for one in full and healthy canopy (see Figure 7.2). Host plants were also scored qualitatively as still healthy or whether being in ‘decline’ as evident from appreciable leaf loss and death of branches. Data from year 3 was then correlated using corresponding data at a later time of assessment in year 5.

In a preliminary series of analyses various models were proposed and tested for possible use as a newly coined term ‘plantation host index (\(V_h\))’ for quantifying the interaction of host plants and neighbouring *Santalum*. The relatively simple model finally selected assumed that growth of *Santalum* was best described in terms of quality of the host (Hq) compounded with distance of the parasite from suitable hosts (Hd). Variables used in assessing \(V_h\) were mean distance of the parasite to closest three host trees (a, b and c), the mean quality of these three hosts, it being assumed that benefit from a host would be inversely related to distance between that host and the parasite. The composite index for \(V_h\) tested was thus assessed as follows:

\[
V_h = \sum \left\{ \frac{Hq_a}{Hd_a} + \frac{Hq_b}{Hd_b} + \frac{Hq_c}{Hd_c} \right\}
\]

Where Hq (a-c) is the semi-quantitative ranking of quality of hosts a, b and c based on leaf area (see Figure 7.1), and Hd (a-c) is the distance in metres between the parasite in question and the three closest hosts a, b and c.
Figure 7.1: Illustrations of the three native leguminous host species, (a) *Cathormion umbellatum*, (b and d) *Sesbania formosa* and (c and e) *Acacia anuera*, used in this study showing varying degrees of leaf biomass. Each host plant was assigned a host quality classification based on canopy size, crown health and an estimation of leaf area using semi-quantitative ranking of 1 to 5, where 1 was a host in poor health with a very low leaf area and 5 was a host in very good health with a high leaf area.
Figure 7.2: *Santalum* crown health categories; (a) crown health and leaf area score of 5; very high, (b) 4; high, (c) 3; moderate, (d) 2; low and (e) 1; dead. Rule is 1 metre.
7.3.3. **Statistical Analysis.**

*Santalum* height and diameter increment data allotted to different *Santalum* crown health classes were analysed by ANOVA, and a linear regression calculated using the Systat statistical package (Systat 1992). A log transformation was applied to plantation host vigour indices to normalise distribution of residuals and thereby linearise responses.

Diameter increment (Dinc) at 20 cm above ground level assessed between measurements in 1994 to 1996 was selected in a stepwise multi regression procedure for predicting *Santalum* crown health at age 5 years. At this age trunks of *Santalum* trees may be safely assumed to consist solely of sapwood since the mean age for start of heartwood development in the species is not until 23 years (Haffner 1993). At these early stages of growth one would therefore anticipate that progressive increases in stem diameter would proceed directly in line with increases in sapwood area and leaf area, that is essentially as predicted from the ‘pipe model theory’ proposed generally for trees by Shinozaki *et al.* (1964).

7.4. **Results**

7.4.1. **Crown Health Classification of Host Plants.**

*C. umbellatum* ranked consistently highest in terms of these quality criteria, *S. formosa* ranked ranging from 4 to 2 and all *A. anuera* from 3 to 1 (see Figure 7.1).

7.4.2. **Relationship between Santalum Crown Health and Plantation Host Index.**

Single regression equations using either mean host quality or the mean linear distance of host to *Santalum* were relatively poor indicators when used alone. Log transformed variations in each of these quantities were essentially poor to moderate predictors ($r^2$ ranging from 0.40 to 0.53) (Table 7.1).
Table 7.1. Prediction of projected *Santalum* crown health for trees; using the model $y = b_0 + b_1 \times \text{factor}$. Log transformed variables are preceded by L. Values in parentheses are standard errors of the estimate. $n = 159$ with the exception of LVh where $n = 154$. All equations are significant at $p < 0.0001$.

<table>
<thead>
<tr>
<th>Factor</th>
<th>$b_0$</th>
<th>$b_1$</th>
<th>$r^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>LVh</td>
<td>2.022 (0.066)</td>
<td>3.649 (0.162)</td>
<td>0.769</td>
</tr>
<tr>
<td>MHd</td>
<td>4.820 (0.170)</td>
<td>-0.351 (0.031)</td>
<td>0.450</td>
</tr>
<tr>
<td>MHq</td>
<td>0.505 (0.209)</td>
<td>0.853 (0.066)</td>
<td>0.515</td>
</tr>
<tr>
<td>LMHd</td>
<td>6.040 (0.231)</td>
<td>-4.535 (0.338)</td>
<td>0.534</td>
</tr>
<tr>
<td>LMHq</td>
<td>0.768 (0.219)</td>
<td>5.091 (0.457)</td>
<td>0.441</td>
</tr>
<tr>
<td>$\Sigma$LHd $^d$</td>
<td>6.004 (0.232)</td>
<td>-1.527 (0.116)</td>
<td>0.526</td>
</tr>
<tr>
<td>$\Sigma$LHq $^e$</td>
<td>1.059 (0.210)</td>
<td>1.569 (0.154)</td>
<td>0.399</td>
</tr>
</tbody>
</table>

$^a$ plantation host vigour index  
$^b$ mean linear distance of three closest hosts to *Santalum*  
$^c$ mean host quality of three closest hosts to *Santalum*  
$^d$ sum of the log transformed linear distance of three closest to *Santalum* ($\Sigma \log a + \log b + \log c$).  
$^e$ sum of the log transformed host quality of three closest hosts to *Santalum* ($\Sigma \log a + \log b + \log c$).
Multiple regression equations using a combination of linear distance to host and host quality improved the coefficients of determination to 0.75 (Table 7.2). This stressed the marked synergism between host quality and proximity (Figure 7.4). Prediction of the health of Santalum crowns was improved slightly further when log values for mean host quality and Santalum basal diameter increment from 1994 to 1996 (Dinc) were combined with log mean linear distance of host to Santalum (Table 7.2). Thus, using all these three variables, the coefficients of determination ranked at 0.76.

The final favoured analysis (see Tables 7.1 and 7.2) using plantation host index ($V_h$) as predictor of Santalum crown health proved superior to any other model using both single and multiple regression parameters individually using mean linear distance of hosts to Santalum (Hd), or mean host quality (Hq) of the three closest hosts (or the log values of these parameters) alone (Table 7.1 and Figure 7.3). Models using higher order functions of Hd were also tested, but did not improve the prediction of crown health (data not shown).

Crown health, the index ($V_h$), mean linear distance of host to Santalum and their log transformed variations were all shown to be significantly correlated to Santalum height increment from 1994 to 1996 (Htinc), mean annual increment of Santalum height at age 5 years (Htmai), Santalum basal diameter increment from 1994 to 1996 (Dinc) and mean annual increment of Santalum basal diameter at age 5 years (Dmai), although the coefficients of determination were relatively low (Table 7.3).

7.4.3. RELATIONSHIP BETWEEN SANTALUM HEIGHT AND DIAMETER GROWTH AND PLANTATION HOST INDEX

The logarithmic expressions of mean annual increment in height of Santalum at age 5 years (Htmai) was influenced by plantation host index ($V_h$), with a slope of 0.156, a finding to be expected from the association that increase in Santalum height would become less as plantation host index values decreased (Figure 7.5). A similar relationship existed with annual increment in diameter of Santalum (Dmai) (data not shown).
Table 7.2. Stepwise regression for predicting *Santalum* crown health. Values in brackets are standard errors of the estimate. All equations are significant at p < 0.0001. Log transformed variables are preceded by L. n = 159 with the exception of Dbinc where n = 132.

<table>
<thead>
<tr>
<th>Equation</th>
<th>b0</th>
<th>b1</th>
<th>b2</th>
<th>b3</th>
<th>r²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crown health = b0 + b1LMHq ^a</td>
<td>0.768</td>
<td>5.091</td>
<td>-</td>
<td>-</td>
<td>0.441</td>
</tr>
<tr>
<td>Crown health = b0 + b1LMHq + b2LDinc</td>
<td>0.773</td>
<td>4.141</td>
<td>1.352</td>
<td>0.252</td>
<td>0.564</td>
</tr>
<tr>
<td>Crown health = b0 + b1LMHq + b2LMHd ^b</td>
<td>3.751</td>
<td>3.711</td>
<td>-3.602</td>
<td>0.264</td>
<td>0.746</td>
</tr>
<tr>
<td>Crown health = b0 + b1LMHq + b2LMHd + b3LDinc</td>
<td>3.322</td>
<td>3.654</td>
<td>-3.149</td>
<td>0.305</td>
<td>0.498</td>
</tr>
</tbody>
</table>

^a^ mean host quality of three closest hosts to *Santalum*

^b^ mean linear distance of three closest hosts to *Santalum*

^c^ change in *Santalum* basal diameter (at 20cm above ground) from May 1994 to May 1996
Figure 7.3. Relationship between *Santalum* crown health and projected plantation host index (n=154).
Figure 7.4. Regression surface for *Santalum* crown health as a function of log transformed mean linear distance of host to *Santalum* and mean host quality (n = 159).
Table 7.3: Significance of various factors for the prediction of *Santalum* basal diameter (at 20cm above ground level) increment from age 3 years to 5 years (Dinc), *Santalum* mean annual basal diameter increment at age 5 years (Dmai), *Santalum* height increment from age 3 years to 5 years (Htinc), *Santalum* mean annual height increment at age 5 years (Htmai) using linear regression. *Santalum* growth and host health in 1994 was uniformly high across the study site whereas in 1996 *Santalum* growth was lower and some trees of both *Santalum* and hosts had declined in health. All equations are significant at p < 0.0001.

<table>
<thead>
<tr>
<th>Factor</th>
<th>Dinc</th>
<th>Dmai</th>
<th>Htinc</th>
<th>Htmai</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>slope</td>
<td>r²</td>
<td>n</td>
<td>r²</td>
</tr>
<tr>
<td>Crown health</td>
<td>+</td>
<td>0.450</td>
<td>142</td>
<td>0.280</td>
</tr>
<tr>
<td>Vh a</td>
<td>+</td>
<td>0.374</td>
<td>141</td>
<td>0.207</td>
</tr>
<tr>
<td>LVh b</td>
<td>+</td>
<td>0.405</td>
<td>151</td>
<td>0.178</td>
</tr>
<tr>
<td>MHd c</td>
<td>-</td>
<td>0.181</td>
<td>153</td>
<td>0.111</td>
</tr>
<tr>
<td>GMHd d</td>
<td>-</td>
<td>0.272</td>
<td>142</td>
<td>0.106</td>
</tr>
</tbody>
</table>

a: plantation host vigour index
b: log transformed plantation host index
c: mean linear distance of three closest hosts to *Santalum*
d: geometric mean of the linear distance of three closest hosts to *Santalum* \( \sqrt[3]{(\Sigma Hda + Hdb + Hdc)} \)
Figure 7.5. Relationship between projected plantation host index and log transformed mean annual increment of *Santalum* height. Data points (*) are for individual trees at 5 years of age.
7.4.4. **Relationship between height and diameter growth and Santalum crown health**

Mean annual increments of *Santalum* heights and diameters (Htmai and Dmai) recorded in the third year of growth were consistently greater than those recorded later for trees in the fifth year and, unlike the more uniform situation in the plantation in the third year, had become strongly correlated with *Santalum* crown health at five years. Thus a tree with a crown health score of 5 would be expected to show a significantly faster growth rate than one with a crown health value of 1 or 2 (Table 7.4).

7.5. **Discussion**

Our study showed that young three year old plantations contained *Santalum* of relatively uniform health and growth, but that two years later the *Santalum* and hosts showed loss of vigour with great variability between trees in health and growth performance. We define this phenomenon as plantation decline and use a term ‘plantation host index (Vh)’ in such situations to predict reliably *Santalum* health and future productivity. This decline of *Santalum* was assessed primarily on the basis of inadequacy of host quality and deleterious effects of distance of the parasite from nearest suitable host. It is suggested that this relatively simple model might be further refined in the future by incorporating other possibly more reliable measures of host quality, such as the xylem nitrogen benefit to the parasite, along the lines discussed in earlier work in single host pairings with *Santalum* in pot culture (Radomiljac *et al.* 1998d).

Deleterious influences of agriculturally-important herbaceous root hemi-parasites such as Striga and Orobanche on economically-important annual crops have been widely highlighted and chronicled in a number of recent studies and suggestions have been made as to how such effects of parasitism might be controlled (Press and Stewart 1987; Press *et al.* 1990; Seel *et al.* 1992; Press 1995a; Ehleringer and Marshall 1995; Press 1995b). The reverse objective of course applies where one is attempting to maximise growth of a commercially valuable parasite like *Santalum*. This situation, requiring careful and balanced nurturing of host and parasite, requires very different treatment. Indeed, a great paucity of long term studies exists on effects of differing *Santalum* : host stand densities under field conditions and on how one might optimise long term host species density.
Table 7.4: Mean annual increment of *Santalum* height and stem diameter at 20cm above ground level based on *Santalum* crown health at age 5 years. *Santalum* growth and host health in 1994 was uniformly high across the study site whereas in 1996 *Santalum* growth was lower and some trees of both *Santalum* and hosts had died. Figures in brackets are standard errors. Different letters indicate a significant difference at $p < 0.05$, using Tukey's HSD multiple comparison test.

<table>
<thead>
<tr>
<th>Crown health</th>
<th>Mean annual increment of <em>Santalum</em> height (cm year$^{-1}$)</th>
<th>Mean annual increment of <em>Santalum</em> diameter (cm year$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>111.3 (17.5) a</td>
<td>2.75 (0.58) a</td>
</tr>
<tr>
<td>4</td>
<td>95.1 (18.9) ab</td>
<td>2.27 (0.51) b</td>
</tr>
<tr>
<td>3</td>
<td>90.2 (15.9) ab</td>
<td>2.19 (0.49) b</td>
</tr>
<tr>
<td>2</td>
<td>87.3 (16.2) b</td>
<td>2.03 (0.37) bc</td>
</tr>
<tr>
<td>1</td>
<td>71.7 (15.0) c</td>
<td>1.58 (0.35) c</td>
</tr>
<tr>
<td>Mean value for all <em>Santalum</em> in 1994 (age 3 years)</td>
<td>130.4 (24.1)</td>
<td>3.02 (0.77)</td>
</tr>
</tbody>
</table>
So far we have had to rely principally on pot culture studies (Surata 1992a; Taide 1994; Fox et al. 1995; Radomiljac 1998; Radomiljac et al. 1998c) and selection of beneficial long term host species has yet to reach a stage where one can forecast yield under plantation conditions with any degree of certainty (Radomiljac et al. 1998a). However it is already clearly evident from causal observations that *Santalum* will fail to thrive under plantation culture unless highly beneficial primary host species are employed initially and if long term host species also selected of proven quality to withstand long term parasitism. However, much better defined research needs to be undertaken to determine what *Santalum* : long term host plantings ratios provide sufficient host resource for maximal growth of the parasite while not overly restricting the possible long term value of hosts, should they be valuable timber species. Of particular importance, the temptation must be avoided of over-planting of the parasite with the mistaken idea that greater yield increases will eventuate at inappropriately high ratios of parasite to host.

In this study higher plantation host index values were closely correlated with *Santalum* crown health and the latter in turn translated into better *Santalum* growth. Thus *Santalum* productivity is dependent on good host health being maintained through appropriately high host stand density. This applies especially after *Santalum* trees have increased to a size where hosts will be subjected to escalating abstraction of their water and nutrients. Whatever the interaction, growth of hosts measured, say, as dry matter gain per unit leaf area will always decline with increasing tree size and age, since old large trees will inevitably incur higher maintenance costs relative to photosynthetic gain than in younger trees (Kaufmann and Ryan 1986; Waring and Schlesinger 1985). One would therefore argue that plantation densities of the parasite relative to its hosts should be suitably conservative at initial establishment. Benefit might also be obtained by planting *Santalum* seedlings close to short lived fast growing intermediate host species but suitably distant from the final long lived hosts. Before adopting a particular planting scheme it is essential to evaluate from previous trial experimentation in the ‘carrying capacity’ of each host species employed in a particular study region, while also acquiring foreknowledge of the potency of the influences which parasitism may have on vigour and longevity of the various hosts selected for the planting.

Even though host vigour and *Santalum* crown health declined dramatically in the three to five year plantation age at our study site, there was little mortality during this period, presumably because of drastic unexpected natural attrition of *Santalum* in early stages of plantation growth and that the decline of hosts may not have progressed to a stage causing widespread *Santalum* mortality. Early natural attrition of *Santalum* may have been due to hosts being at too low density and therefore mostly out of range of the parasite. Survival of the parasite at this early stage may have involved exploitation of herbaceous and semi-woody weed species, but because
of its tree habit, *Santalum* is unlikely to attain prolonged sustenance and associated high growth rates when solely dependent on such ephemeral species for water and solutes. Indeed, other observations have shown *S. album* to remain small and stunted in open field conditions in absence of perennial host species (Barrett and Fox 1994; Radomiljac *et al.* 1998b). Then, once parasitic connections are successfully made, *Santalum* quickly establishes a large shoot mass relative to root mass, thus rendering it highly susceptible to decline in growth of hosts and resulting deficiencies in water and nutrient supply to the parasite. Even then, if shoot growth of the *Santalum* were to decrease sharply and its root : shoot ratio to increase, water and nutrient deficits might still reach proportions causing death of the plant (Begg and Turner 1976; Ericsson 1981; Cannell 1985; Nadelhoffer *et al.* 1985; Cromer and Jarvis 1990; Kirschbaum *et al.* 1992; Raison and Myers 1992; Cromer *et al.* 1993; Stoneman and Dell 1993; Radomiljac *et al.* 1998b).

Our plantation host vigour model indicated that planting distance between *Santalum* and long-term, high quality hosts should be reasonably close, even if at a larger distance than from intermediate hosts. In southern India, for example, the long term host employed (*Casuarina equisetifolia* L.) is planted very closely adjacent to *Santalum* (Ananthapadmanabha *et al.* 1984; Rai and Kulkarni 1986), but in Kununurra we have found that such a close planting arrangement can be counterproductive, particularly where a fast growing tree host obstructs early growth of the parasite through shading and stem abrasion, causing poor configuration of the parasite (A. M. Radomiljac unpubl. obs.).

The time required for merchantable heartwood to be achieved in plantation grown *Santalum*, is perceived as being lengthy in the Ord River Irrigation Area (Radomiljac *et al.* 1998a). This is also suggested by Haffner (1993) for natural stands in Timor where heartwood development does not commence until 14 – 46 years of age or at 10 – 13 years under Indian conditions as reported by Rai (1990). In any event our observations on substantial plantation decline at 5 years under north west Australian conditions are patently not the result of tress switching to lower rates of growth as they lay down heartwood, but the data of course predict cumulative later penalties which would eventually compromise heartwood yield. It is therefore paramount that every effort should be made to maintain high plantation host vigour over this early period of potentially rapid growth.
~ Chapter 8 ~

The influence of paraquat (1:1-dimethyl-4,4'-bipyridinium dichloride) and ethrel (2-chloroethylphosphonic acid) on the induction of heartwood in young plantation grown Santalum album L. (Indian sandalwood).

8.1. Abstract

In Santalum album L. (Indian sandalwood) heartwood forms at about 10-15 years of age but commercial quantities only exist in much older trees. Five year old Santalum trees received stem injections of paraquat or ethrel or combinations of the two at two different concentrations (0.25% or 1%), at 15 cm above ground level. Eight months after stem injections of paraquat, 10-15% of the cross-sectional area of disks at the treatment site consisted of induced heartwood. Wood at the site of treatment showed the presence of high lipid concentration and no or relatively little starch and polysaccharide content compared to unaffected sapwood. The total volatile oil and santalol oil concentrations was equal to or greater than that of naturally formed heartwood and greater than that of the sapwood. The main volatile oils formed were alpha and beta santalol. Induced heartwood had a significantly lower moisture content and contained significantly lower concentrations of K and Mg, and in some treatments Ca than that of the sapwood. The radial and vertical extension of induced heartwood in all paraquat treatments was significantly greater than treatments of ethrel alone or distilled water.

8.2. Introduction

Sandalwood is the highly valued fragrant heartwood of most species of Santalum and the wood and the oil derived from it are among the oldest known perfumery materials (Srinivasan et al. 1992). Heartwood is the central part of the secondary xylem in woody plants containing non-functional tracheary elements, which is derived from sapwood as it deteriorates due to age and/or damage. It is usually infiltrated with organic compounds such as resins, tannins, gums, pigments and aromatic substances, which make it more valuable than sapwood (Hillis 1987).
Heartwood formation is a complicated phenomenon and sapwood parenchyma cell death is a result and not the cause of heartwood formation (Bamber 1976). During heartwood formation the metabolic activities of the parenchyma cells are altered and phenolic compounds are produced (Holl 1994). Changes in parenchyma cells associated with heartwood formation are regarded as regulatory processes as serving to keep the amount of sapwood at an optimum level (Bamber 1976) and may involve the formation of ethylene, activation of certain enzymes and increased respiration (Hillis 1987). Heartwood formation in *Santalum* involves the deposition of complex aromatic benzene-ring compounds (α- and β-santalol and α- and β-santalene) into the non-functional xylem. Oil content decreases markedly along the length of the tree, being highest in the root system of the tree (Srinivasan *et al.* 1992).

The commercial value of *Santalum* depends entirely on the santalol content of the heartwood and the quantity of heartwood per tree rather than total wood biomass. Therefore in plantations it is important to maximise heartwood production. Due to the relatively slow growth rate of *Santalum* and the considerable time required to facilitate heartwood development, the rotation length of plantation grown *Santalum* is perceived to be lengthy, more than 20 years (Radomiljac *et al.* 1998a). Thus there are clear economic benefits in inducing early heartwood formation in young *Santalum*.

Scant literature exists on heartwood induction in *Santalum*, with the exception of Kadambi (1954) and Li YingLan *et al.* (1994). Kadambi (1954) showed Seradix A®, zinc sulphate and copper sulphate treatments induced heartwood formation in 12 year old *S. album* trees but this study did not analyse the oil content of induced heartwood or quantify the extent of heartwood formation within the tree. Li YingLan *et al.* (1994) found that an unspecified plant growth regulator had a marked effect on forcing heartwood formation and increasing total and santalol oil content in 2 year old trees of *S. album*.

The induction of ‘lightwood’ and the increased yield of oleoresin in the southern USA softwood species, *Pinus taeda* L. (loblolly pine) and *P. elliottii* Englem. var. *elliottii* (slash pine) using the herbicide paraquat and plant growth regulator ethrel to increase the production of oleoresin is well documented (Stubbs *et al.* 1984). Paraquat administered to sapwood tissue acts as a catalyst for the formation of hydrogen peroxidise, which may peroxidise lipids within the cell leading to ultimate cell disruption (Hillis 1987). Simultaneously starch, sugars, fatty acids, amino acids and enzymes are released leading to the synthesis of oleoresin and other heartwood constituents (Stubbs *et al.* 1984). Paraquat administered to the stem of *Azadirachta indica* A. Juss. induced heartwood and increased the activity of enzymes in the axial and ray parenchyma cells at the periphery of the heartwood (Nair and Shah 1983). These enzymes
include acid phosphatase, which is involved in the hydrolysis of starch and its transport (Hillis 1971), and succinic dehydrogenase, which is indicative of high respiration and metabolism (Varner 1961). Ethylene administered to the stem of a number of Pinus species increased oleoresin production (Peters and Roberts 1977; Wolter 1977; Hillis 1987) and other heartwood constituents (Wolter and Zinkel 1984). Ethylene production is a common early cell response in trees following injury (Shain 1979) and is indicative of increased enzyme activity (Hillis 1987). In *P. elliottii* oleoresin production was enhanced with combined ethrel and paraquat application, suggesting the two chemicals were synergistically linked in their mode of action (Peters and Roberts 1977; Peters *et al.* 1978; Hillis 1987).

This chapter tests the hypothesis that 1:1-dimethyl-4,4'-bipyridinium dichloride (paraquat) and 2-chloroethylphosphonic acid (ethrel) injected into the sapwood of young *Santalum* trees will initiate ‘artificial heartwood’ formation.

### 8.3. MATERIALS AND METHODS

#### 8.3.1. SITE AND STAND DESCRIPTION

The study site was located at Frank Wise Institute for Tropical Agricultural Research, 10 km north of Kununurra (15° 40' S, 128° 44' E), Ord River Irrigation Area (ORIA) of northern Western Australia at an elevation of 45 m.a.s.l. and a mean annual rainfall of 750 mm.

The study site soil type is Kununurra clay (alkaline phase - pH 7.8), derived from recent river alluvia. It is dark brown (10YR 4/3), self-mulching and very plastic with poor drainage when wet, and hard with seasonal cracking when dry (Aldrick *et al.* 1990). The site was intensively cultivated, leveled and mounded following normal ORIA agricultural techniques for flood irrigation. In June 1991 the site was planted with seedlings of the root hemi-parasite *S. album* and host species *Cathormion umbellatum* (Vahl) Kosterm., *Acacia anuera* F. Muell. ex. Bent. (Mulga) and *Sesbania formosa* (F. Muell.) N. Burb. (Corkwood). Establishment design consisted of an alternating pattern along the row of *Santalum*, *Ac. anuera* and *S. formosa* seedlings. *C. umbellatum* was mostly planted in the northern half of the study site. Each seedling received a spot application of 100 grams NPK Blue Special fertiliser (11.8% N, 6.0% P, 15.8% K, 0.05% Cu, 0.05% Zn, 0.13% Mn, 1.0% Mg, 8.3% S) soon after planting. Following establishment, the site was flood irrigated 16 times from July to December 1991. Following the 1991/92 monsoon rain season the site was flood irrigated ten times between
March to December for all subsequent years with an irrigation period consisting of 24 hours. Due to the absence of appropriate long term hosts, the high *Santalum* : host ratio and the short life-span of the existing 'intermediate' hosts the plantation was exhibiting signs of decline as described by (Radomiljac et al. 1998e) at the time the experiment was initiated.

In October 1995 *Santalum* basal diameter at 10 cm above soil surface, diameter at breast height, total height, clear bole height, form and presence or absence of heartwood were recorded. Heartwood presence or absence was determined by drilling into the tree centre on a southern aspect at 5 cm above soil surface and recording the colour and aroma of the drill shavings. Natural heartwood was detected in six trees of *Santalum*. Following assessment 45 trees were selected on the basis that heartwood was not present, trees had a clear bole height greater than 115 cm, basal diameter greater than 7.1 cm and a diameter at breast height greater than 4.5 cm. Trees exhibiting poor form or vigour were not selected.

Over a period of three days (7 - 9 December 1995) trees were injected with a single dose of commercially available paraquat as Gramoxone® ICI™ (a.i. paraquat 250 g/l) and ethrel as Ethrel® Rhone-Poulenc (a.i. ethrel 480 g/l) or a combination of both chemicals (Table 8.1). Chemicals were diluted to 0.25% or 1.0% concentration with distilled water (Table 8.1). Each chemical treatment was injected into one tree in each of five replicates, randomly selected across the site, using 10 ml syringe and a 38 mm needle. A control treatment consisted of 3 ml of distilled water. Injections were performed in December (monsoon wet season). At this time of year physiological activity is high and it was hoped that increased sap flow would enable efficient longitudinal translocation of the treatment solution within the stem.

Treatments were injected into a pre-drilled hole on the northern side of the stem, 15 cm above the soil surface on a 45° downward angle. The hole was made using a 5.95 mm wide bit drilled 70 mm into the sapwood creating a drill hole volume of 1.94 cm³. About half of the treatment solution was injected into the stem filling the drill hole. This solution was absorbed into the stem after 3 minutes and the remainder of the treatment solution was then injected.
Table 8.1: Chemical treatments of 5 year old *Santalum album* trees. There were five trees in each treatment and the mean diameters and heights are also given.

<table>
<thead>
<tr>
<th>Chemical treatment</th>
<th>Basal diameter (cm)</th>
<th>Diameter at 1.3 m (cm)</th>
<th>Total height (cm)</th>
<th>Clear bole height (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3ml 1% paraquat</td>
<td>12.54 ± 1.03</td>
<td>9.04 ± 1.57</td>
<td>481.6 ± 24.16</td>
<td>199.6 ± 21.00</td>
</tr>
<tr>
<td>3ml 0.25% paraquat</td>
<td>12.46 ± 1.16</td>
<td>8.20 ± 1.27</td>
<td>437.4 ± 23.25</td>
<td>203.8 ± 29.80</td>
</tr>
<tr>
<td>3ml 1% ethrel</td>
<td>14.14 ± 1.45</td>
<td>8.26 ± 0.95</td>
<td>468.2 ± 51.98</td>
<td>209.4 ± 19.19</td>
</tr>
<tr>
<td>3ml 0.25% ethrel</td>
<td>9.36 ± 0.88</td>
<td>6.34 ± 0.71</td>
<td>392.8 ± 18.68</td>
<td>182.8 ± 26.51</td>
</tr>
<tr>
<td>3ml 1% paraquat + 3ml 1% ethrel</td>
<td>11.54 ± 0.84</td>
<td>7.16 ± 0.87</td>
<td>431.2 ± 38.87</td>
<td>163.0 ± 16.34</td>
</tr>
<tr>
<td>3ml 1% paraquat + 3ml 0.25% ethrel</td>
<td>10.32 ± 0.75</td>
<td>7.44 ± 0.75</td>
<td>429.8 ± 22.45</td>
<td>169.8 ± 28.03</td>
</tr>
<tr>
<td>3ml 0.25% paraquat + 3ml 1% ethrel</td>
<td>10.76 ± 0.71</td>
<td>6.78 ± 0.50</td>
<td>486.0 ± 15.89</td>
<td>228.2 ± 18.00</td>
</tr>
<tr>
<td>3ml 0.25% paraquat + 3ml 0.25% ethrel</td>
<td>11.08 ± 1.01</td>
<td>8.10 ± 0.45</td>
<td>463.8 ± 24.60</td>
<td>173.6 ± 16.01</td>
</tr>
<tr>
<td>3ml distilled water</td>
<td>11.84 ± 0.54</td>
<td>8.06 ± 0.51</td>
<td>420.6 ± 21.85</td>
<td>170.4 ± 15.76</td>
</tr>
</tbody>
</table>
8.3.2. ASSESSMENT

Harvesting and assessment was performed in August 1996, the end of the cool-dry season (the dormancy period), for two reasons. Firstly, the amount of starch stored in sapwood varies with season (Hillis 1987) and is usually greatest during the cold periods (Holl 1975). High concentrations of starch in the sapwood may assist in identifying the extent of heartwood development through staining starch black with IKI solution. Secondly, heartwood formation mainly occurs during the dormant season (Shain and Hillis 1973). On 14 and 15 August 1996 (8 months after stem injections) the northern aspect of the tree bole of all 45 trees was marked with white paint and the tree then felled as close as possible to ground level.

8.3.3. DESTRUCTIVE SAMPLING

Immediately after harvesting the branches and foliage were removed from the bole. One disk (2 cm in width) was cut from the base of the bole (ground level), then four disks were cut near the treatment site (15 cm above ground level and referred to as 15-1 to 15-4). Further disks were cut at 30, 60 and 90 cm above ground level. From each tree the 15-1 disk was used for volatile oil analysis, 15-2 for histochemical analysis and 15-4 for mineral nutrient concentration and moisture content analysis. The base, 15-3, 30, 60 and 90 disks were used to determine the radial extension of induced heartwood.

8.3.4. RADIAL EXTENSION OF INDUCED HEARTWOOD AND SAPWOOD CROSS SECTIONAL AREA

All disks used for assessment of radial extension of induced heartwood were soaked in IKI reagent (0.2g iodine dissolved in 100ml of saturated potassium iodide aqueous solution) on one side to stain starch black and then the other side was soaked in tetrazolium chloride solution (1g tetrazolium dissolved in 100ml of 2 parts 0.91% KH₂PO₄ solution and 3 parts 0.95% Na₂HPO₄ solution to stain living tissue pink. The area of the disk and the area of induced heartwood were then determined. A transition zone was observed on the periphery of the induced heartwood area. This area did not stain black following the IKI treatment, but together with the sapwood it stained pink following tetrazolium chloride treatment. The width of the transition zone was also recorded.

159
8.3.5. **Yield of volatile oil by SC CO₂ extraction**

Disks used for volatile oil analysis were vacuumed sealed and kept at 4°C. A sample of induced heartwood, transition zone wood and sapwood were cut from each disk, ground to a fine powder in a Waring blender in the presence of dry ice. Yields of volatile oil recoverable by supercritical fluid extraction (SC CO₂) were determined using a HP 7680T supercritical fluid extraction module equipped with a 7 ml extraction thimble fitted with a Filter Floes MN2101 (Pigott *et al.* 1997). Gas chromatograph analysis was conducted using a HP 5890 series II gas chromatograph equipped with a HP Innowax column (Pigott *et al.* 1997). Identification of dominant sesquiterpene alcohols were determined by comparison with authentic (Z)-α- and (Z)-β- santalol and 2(E), 6(E)- farnesol standards samples, obtained from DRAGOCO Aust. Pty. Ltd. and Aldrich, respectively.

8.3.6. **Analysis of naturally formed heartwood**

Two *Santalum* trees from the same plantation with naturally formed heartwood were harvested. One disk was taken at 15 cm above ground level and the total volatile oil concentration determined.

8.3.7. **Histochemical analysis**

Disks used for histochemical analysis were vacuumed sealed and kept at 4°C. Representative blocks (10mm sides) were cut from the induced heartwood, transition zone and sapwood from each disk. Blocks were fixed in 3% glutaraldehyde in 0.025 M phosphate buffer at 4°C for histochemical analysis. Sections (15 – 20µm thick) were prepared using a Leitz sledge microtome. Sections were stained with IKI to detect starch granules, Sudan Black (saturated 70% ethanol solution) to detect lipids (oil droplets) and Periodic Acid – Schiff’s reagent (PAS) to detect polysaccharides.

Some smaller blocks (2mm sides) were embedded in Spurrs’ resin and cut at 2µm thick using a Sorvall VB4 microtome using glass knives and stained with 1% methylene blue, 1% azur II in 1% borax for 10 seconds. Sections of sapwood were also treated with a number of stains in attempt to visualise the nuclei in the ray cells. They included ethidium bromide (Hough *et al.* 1985), DAPI (Coleman *et al.* 1981) and Feulgen’s reagent. Some sections were also stained for phenolics using Ferric chloride, or Xanilin/HCl (Ling-Lee *et al.* 1977), and general structure
observed in section stained with Toluidine-Blue O (O’Brien et al. 1964).

8.3.8. Mineral nutrient concentration and moisture content

Disks used for mineral nutrient concentration and moisture content analysis were immediately separated into sapwood and induced heartwood components and the fresh weight (FW) recorded. Samples were then oven dried at 70°C for 48 hours, the dry weight (DW) recorded and the moisture content determined using the following formula:

\[ \text{Moisture content} = \frac{\text{FW} - \text{DW}}{\text{DW}} \times 100 \]

Induced heartwood and sapwood samples were finely milled for chemical analysis. Total N in the sample was determined by the Kjeldahl digestion method (McKenzie and Wallace 1954). Concentrations of K, Ca, Mg, Na, Fe, Cu, Mn and Zn in dry matter were assayed using a nitric/perchloric acid digestion procedure followed by atomic absorption spectrophotometry of appropriately diluted digests (Pate et al. 1991a). Total P was estimated separately on the digests using the colourimetric molybdenum blue method (Kitson and Mellon 1944).

8.4. Results

8.4.1. Heartwood induction

Trees treated with paraquat or a combination of paraquat and ethrel showed significant areas of induced heartwood (Figure 8.1). Trees injected with combinations of 1% paraquat and ethrel had a greater proportion of induced heartwood at 15 and 30 cm above ground than all other treatments. Treatments with ethrel alone did not induce significant heartwood. Both these treatments, and injections with distilled water resulted in a small zone of wound wood around the drill hole.

The disk at 15 cm, which included the drill hole and the site of injection of treatment solution, showed some induced heartwood in all trees, with the exception of the 0.25% ethrel treatment, which had induced heartwood in only 80% of the trees. The induced heartwood extended to ground level in 40% of trees treated with paraquat alone or 1% paraquat and 1% ethrel. The highest proportion of trees in which the induced heartwood extended vertically to 60 cm above
ground level were those treated with 1% paraquat or 1% paraquat and 0.25% ethrel (Table 8.2). 

Trees injected with 0.25% paraquat and combinations of 1% paraquat and 1% ethrel had a significantly greater transition zone width than the distilled water treatment at 15 cm above ground level (Figure 8.2A). Those injected with paraquat alone and both combinations of 1% paraquat and ethrel had significantly greater mean induced heartwood dry weight at 15 cm above ground level (Figure 8.2B). A strong positive relationship exists between induced heartwood dry weight and induced heartwood area at 15 cm above ground level (Figure 8.3).

**8.4.2. MOISTURE CONTENT**

The moisture content of induced heartwood was consistently lower than the mean sapwood moisture content value, irrespective of the treatment (Figure 8.4).

**8.4.3. MINERAL NUTRITION**

The concentrations of potassium and magnesium in induced heartwood of all treatments were significantly lower than the mean concentrations in sapwood, with the exception of K in the 1% ethrel treatment (Table 8.3). Induced heartwood from the 0.25% paraquat and 1% paraquat and 1% ethrel treatments had significantly lower Ca concentrations than the sapwood. The concentration of remaining mineral elements (Na, Fe, Cu, Mn and Zn) within the induced heartwood did not differ significantly from the mean concentration of the sapwood (data not shown).

**8.4.4. COMPONENTS OF VOLATILE OILS**

The components of the volatile oils extracted from induced heartwood were similar to those in natural heartwood. The yield of volatile oils from the induced heartwood from all treatments was higher than the mean yield from sapwood. However, the yield of oils from the induced heartwood regions was not uniform across treatments and was highly variable within treatments. The yield of volatile oils in induced heartwood was higher than for natural heartwood with the exception of 1% paraquat, 0.25% ethrel and 1% paraquat + 1% ethrel treatments. The oil content in the transition zone was variable being higher than sapwood in the two paraquat treatments but lower than sapwood in the 1% ethrel, and 0.25% paraquat + 1% ethrel treatments (Table 8.4).
Figure 8.1: Induced heartwood as a proportion of the total area of disks taken at 15 and 30 cm above ground level in *Santalum album* trees eight months after stem injections of paraquat and ethrel solutions. Treatments with significantly greater proportional area of induced heartwood than that of the control treatment are marked with *, ** or *** using Dunnett's pairwise t-test. Significant values of F are denoted by * P < 0.05, ** P < 0.01 or *** P < 0.001.
Table 8.2: Presence of induced heartwood within the stem of *Santalum* eight months after stem injections of paraquat and ethrel at 15 cm above ground level. Disks were taken at ground level, and 15, 30, 60 and 90 cm above ground level.

<table>
<thead>
<tr>
<th>Chemical treatment</th>
<th>Ground level</th>
<th>Treatment site - 15 cm above ground level</th>
<th>30 cm above ground level</th>
<th>60 cm above ground level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Paraquat 1%</td>
<td>40</td>
<td>100</td>
<td>60</td>
<td>40</td>
</tr>
<tr>
<td>Paraquat 0.25%</td>
<td>40</td>
<td>100</td>
<td>40</td>
<td>20</td>
</tr>
<tr>
<td>Ethrel 1%</td>
<td>-</td>
<td>100</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Ethrel 0.25%</td>
<td>-</td>
<td>80</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Paraquat 1% + Ethrel 1%</td>
<td>40</td>
<td>100</td>
<td>80</td>
<td>20</td>
</tr>
<tr>
<td>Paraquat 1% + Ethrel 0.25%</td>
<td>-</td>
<td>100</td>
<td>100</td>
<td>60</td>
</tr>
<tr>
<td>Paraquat 0.25% + Ethrel 1%</td>
<td>-</td>
<td>100</td>
<td>40</td>
<td>-</td>
</tr>
<tr>
<td>Paraquat 0.25% + Ethrel 0.25%</td>
<td>-</td>
<td>100</td>
<td>100</td>
<td>-</td>
</tr>
<tr>
<td>Distilled water</td>
<td>-</td>
<td>100</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
Figure 8.2: (A) Mean transition zone width and (B) induced heartwood dry weight from disks taken at 15 cm above ground level in *Sampium album* trees eight months after stem injections of paraquat and ethrel solutions. Treatments with significantly greater transition wood width or heartwood dry weight than that of the control treatment are marked with * or ** using Dunnett's pairwise t-test. Significant values of F are denoted by * P < 0.05 or ** P < 0.01.
Figure 8.3: Relationship between induced heartwood dry weight and induced heartwood area on disks taken at 15 cm above ground level in Samalum album trees eight months after stem injections of paraquat and ethrel solutions.
Figure 8.4: Mean moisture content of sapwood and induced heartwood tissue at 15 cm above ground level in *Santalum album* trees eight months after stem injections of paraquat and ethrel solutions. Moisture content values for all induced heartwood tissue was significantly lower than the mean sapwood moisture content ($p < 0.05$).
Table 8.3: Concentration of potassium, calcium and magnesium in sapwood (SW) and induced heartwood (HW) of *Santalum*. Values are means with standard errors. Values for mineral concentration of heartwood marked with * are significantly less than that of the mean mineral concentration of the sapwood (p < 0.05). ND is no data.

<table>
<thead>
<tr>
<th>Chemical treatment</th>
<th>K (mg g⁻¹) SW</th>
<th>K (mg g⁻¹) HW</th>
<th>Ca (mg g⁻¹) SW</th>
<th>Ca (mg g⁻¹) HW</th>
<th>Mg (mg g⁻¹) SW</th>
<th>Mg (mg g⁻¹) HW</th>
</tr>
</thead>
<tbody>
<tr>
<td>Paraquat 1%</td>
<td>1.36 ± 0.27</td>
<td>0.52 ± 0.19 *</td>
<td>0.82 ± 0.24</td>
<td>0.66 ± 0.27</td>
<td>0.14 ± 0.07</td>
<td>0.05 ± 0.05 *</td>
</tr>
<tr>
<td>Paraquat 0.25%</td>
<td>1.48 ± 0.18</td>
<td>0.82 ± 0.40 *</td>
<td>0.80 ± 0.12</td>
<td>0.56 ± 0.18 *</td>
<td>0.14 ± 0.06</td>
<td>0.04 ± 0.02 *</td>
</tr>
<tr>
<td>Ethrel 1%</td>
<td>1.40 ± 0.20</td>
<td>1.25 ± 0.13</td>
<td>0.72 ± 0.16</td>
<td>0.73 ± 0.32</td>
<td>0.16 ± 0.03</td>
<td>0.06 ± 0.04 *</td>
</tr>
<tr>
<td>Ethrel 0.25%</td>
<td>1.14 ± 0.35</td>
<td>ND</td>
<td>1.20 ± 0.47</td>
<td>ND</td>
<td>0.19 ± 0.10</td>
<td>ND</td>
</tr>
<tr>
<td>Paraquat 1% + Ethrel 1%</td>
<td>1.22 ± 0.19</td>
<td>0.64 ± 0.22 *</td>
<td>0.82 ± 0.28</td>
<td>0.50 ± 0.07 *</td>
<td>0.15 ± 0.10</td>
<td>0.05 ± 0.03 *</td>
</tr>
<tr>
<td>Paraquat 1% + Ethrel 0.25%</td>
<td>1.35 ± 0.29</td>
<td>0.45 ± 0.16 *</td>
<td>1.08 ± 0.24</td>
<td>0.70 ± 0.15</td>
<td>0.14 ± 0.06</td>
<td>0.04 ± 0.03 *</td>
</tr>
<tr>
<td>Paraquat 0.25% + Ethrel 1%</td>
<td>1.23 ± 0.38</td>
<td>0.50 ± 0.20 *</td>
<td>1.00 ± 0.49</td>
<td>0.80 ± 0.47</td>
<td>0.19 ± 0.09</td>
<td>0.08 ± 0.08 *</td>
</tr>
<tr>
<td>Paraquat 0.25% + Ethrel 0.25%</td>
<td>1.26 ± 0.30</td>
<td>0.67 ± 0.35 *</td>
<td>0.66 ± 0.11</td>
<td>0.63 ± 0.15</td>
<td>0.14 ± 0.08</td>
<td>0.06 ± 0.09 *</td>
</tr>
<tr>
<td>Distilled water</td>
<td>1.28 ± 0.21</td>
<td>ND</td>
<td>0.75 ± 0.21</td>
<td>ND</td>
<td>0.18 ± 0.09</td>
<td>ND</td>
</tr>
</tbody>
</table>

Mean concentration of sapwood: 1.30 ± 0.26, 0.88 ± 0.31, 0.16 ± 0.07
Table 8.4: Volatile oils recovered from natural *Santalum* heartwood, and sapwood, transition zone and induced heartwood from trees injected with paraquat and/or ethrel. Figures in brackets are standard errors. Mean values marked * are significantly greater than that of the mean value for sapwood (p < 0.05). Data from transition zone wood are from one sample. ND is no data.

<table>
<thead>
<tr>
<th>Chemical treatment</th>
<th>Sapwood</th>
<th>Transition wood</th>
<th>Induced heartwood</th>
<th>Naturally formed heartwood</th>
</tr>
</thead>
<tbody>
<tr>
<td>Paraquat 1%</td>
<td>0.17 (0.06)</td>
<td>4.52 *</td>
<td>0.49 (0.25) *</td>
<td></td>
</tr>
<tr>
<td>Paraquat 0.25%</td>
<td>0.23 (0.05)</td>
<td>1.75 *</td>
<td>1.28 (0.72) *</td>
<td></td>
</tr>
<tr>
<td>Ethrel 1%</td>
<td>0.26</td>
<td>0.14</td>
<td>1.74 (1.22) *</td>
<td></td>
</tr>
<tr>
<td>Ethrel 0.25%</td>
<td>ND</td>
<td>ND</td>
<td>0.51 (0.24) *</td>
<td></td>
</tr>
<tr>
<td>Paraquat 1% + Ethrel 1%</td>
<td>0.30</td>
<td>0.00</td>
<td>0.61 (0.27) *</td>
<td></td>
</tr>
<tr>
<td>Paraquat 1% + Ethrel 0.25%</td>
<td>ND</td>
<td>ND</td>
<td>0.62 (0.61)</td>
<td></td>
</tr>
<tr>
<td>Paraquat 0.25% + Ethrel 1%</td>
<td>0.00</td>
<td>0.08</td>
<td>1.23 (0.42) *</td>
<td></td>
</tr>
<tr>
<td>Paraquat 0.25% + Ethrel 0.25%</td>
<td>0.03</td>
<td>ND</td>
<td>2.95 (0.21) *</td>
<td></td>
</tr>
<tr>
<td>Distilled water</td>
<td>ND</td>
<td>ND</td>
<td>1.82 (0.31) *</td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>0.17 (0.38)</td>
<td></td>
<td>0.71 (0.20)</td>
<td></td>
</tr>
</tbody>
</table>
The proportion of $\alpha$- and $\beta$- santalol of the total volatile oils extracted from the sapwood were considerably lower than that of the induced heartwood. The proportion of the total volatile oil extracted from induced heartwood that was $\alpha$- and $\beta$- santalol was in the same range as for naturally formed heartwood, and significantly higher than for sapwood (Table 8.5 and Figure 8.5). The peak at 41.525 was consistently higher in induced heartwood. This peak was shown not to be lanceol and its chemical identity is not known (Figure 8.5).

8.4.5. Staining reactions and histology

Unstained sapwood was often of two distinct colours, the inner rings being deeper red (Figure 8.6 and 8.7), but the entire sapwood of all disks treated with iodine turned brown/black indicating the presence of starch, while the heartwood did not change colour (Figure 8.8A). Between the sapwood and heartwood of most trees with either natural or induced heartwood there was a ring of intermediate wood. This did not contain starch, but together with the sapwood turned pink when stained with tetrazolium indicating that there were living cells in both zones (Figure 8.8B).

The wood anatomy (Figure 8.9) matched descriptions given by Metcalfe and Chalk (1950) and Rao et al. (1998). There were no obvious anatomical or structural differences between natural and induced heartwood. Heartwood induced by paraquat and/or ethrel was similar in appearance and staining reactions. The wound wood formed by drilling and injection of distilled water was also similar to natural heartwood.

Nuclei could not be seen in the ray cells of sap or heartwood using any of the stains. The sapwood ray and vertical parenchyma contained abundant starch grains (Figure 8.10A). The greatest concentration of starch was just under the bark, and starch density decreased towards the heartwood. The density of starch grains also varied with season and was markedly different between trees. There were no or few starch grains in the intermediate wood or the natural or induced heartwood (Figure 8.10B and C). Staining with PAS showed from the purple reaction, that sapwood ray and vertical parenchyma contained polysaccharides. These were less abundant in the intermediate wood and usually absent from the natural or induced heartwood (Figure 8.10D, E and F). Sudan black staining indicated there were small globules of lipids in the sapwood but they were difficult to see being obscured by the starch grains. In most induced and natural heartwood there was a greater density of lipids, with globules present in ray cells as well as tracheids and vessels (Figure 8.10G, H and I).
Table 8.5: Proportion of α- and β- santalol in total volatile oils extracted from Santalum natural heartwood, and sapwood, transition zone and induced heartwood in trees injected with paraquat and/or ethrel. Figures in brackets are standard errors. Data from transition zone wood are from one sample from each treatment. The proportion of α- and β- santalol in total volatile oils extracted from Santalum natural heartwood were not significantly different to the induced heartwood. ND is no data.

<table>
<thead>
<tr>
<th>Chemical treatment</th>
<th>Induced heartwood</th>
<th>Transition zone</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>α-santalol (%)</td>
<td>β-santalol (%)</td>
</tr>
<tr>
<td>Paraquat 1%</td>
<td>40.4 (2.7)</td>
<td>12.4 (1.4)</td>
</tr>
<tr>
<td>Paraquat 0.25%</td>
<td>46.5 (6.3)</td>
<td>14.6 (2.0)</td>
</tr>
<tr>
<td>Ethrel 1%</td>
<td>29.6 (2.9)</td>
<td>10.3 (0.9)</td>
</tr>
<tr>
<td>Ethrel 0.25%</td>
<td>31.3 (15.6)</td>
<td>10.3 (7.1)</td>
</tr>
<tr>
<td>Paraquat 1% + Ethrel 1%</td>
<td>32.1 (9.9)</td>
<td>10.4 (3.7)</td>
</tr>
<tr>
<td>Paraquat 1% + Ethrel 0.25%</td>
<td>43.7 (0.5)</td>
<td>12.6 (1.0)</td>
</tr>
<tr>
<td>Paraquat 0.25% + Ethrel 1%</td>
<td>42.2 (4.2)</td>
<td>13.4 (1.6)</td>
</tr>
<tr>
<td>Paraquat 0.25% + Ethrel 0.25%</td>
<td>41.2 (5.4)</td>
<td>12.2 (1.5)</td>
</tr>
<tr>
<td>Distilled water</td>
<td>40.4 (9.7)</td>
<td>17.2 (4.8)</td>
</tr>
</tbody>
</table>

Mean proportion in sapwood 8.4 (2.7) 2.1 (1.0)  
Mean proportion in natural heartwood 36.0 (35.6) 14.2 (14.1)  

171
Figure 8.5: Gas chromatographs of volatiles extracted using the SC CO$_2$ extraction technique in *Santalum album* (A) sapwood, (B) transition zone, (C) induced heartwood from 1% paraquat treatment and (D) naturally formed heartwood. RT peaks marked with a: (Z)-α- santalol and b: (Z)-β- santalol.
Figure 8.6: Induced heartwood in *Santalum album* at 20cm above ground level. Cross section of a tree treated with 1% paraquat solution. SW, sapwood; IHW, induced heartwood.

Figure 8.7: Cross section of *Santalum album* trunks at 15cm above ground level. (A) Control tree injected with water. Note wound around drill hole, (B) tree treated with 1% paraquat and (C) tree treated with 1% ethrel, ruler = 31cm. [The star shaped mark on each section was a reference point].
Figure 8.8: Staining reaction of *Santalum album* wood from a tree treated with 1% paraquat. (A) Cross section stained with IKI. The black stain of the sapwood (SW) indicates the presence of starch, while the transition zone (TZ) and the induced heartwood (IHW) show no starch, bar = 1cm. (B) The same cross section as in A stained with tetrazolium. The red stain, which indicates living cells, is seen in the sapwood and the transition wood, but not in the induced heartwood.
Figure 8.9: Anatomy of *Santalum album* sapwood (A) transverse, (B) tangential longitudinal and (C) tangential radial sections, scale is same for A, B and C; bar = 0.1mm. Resin sections cut at 2 μm and stained with 1% methylene blue, 1% azur II in 1% borax.
Figure 8.10: Staining reactions of Santalum album wood sectioned in longitudinal radial plane at 15 μm. Top row is sapwood; middle row is natural heartwood; bottom row is induced heartwood in trees treated with 1% paraquat. A, B and C stained with iodine for starch which from the black stain can be seen to be abundant in ray and vertical parenchyma in A (sapwood) but absent in from B and C (heartwood). D, E and F stained with PAS. The red stain in the ray cells of A (sapwood) indicate the presence of polysaccharides. These are almost completely depleted in B (natural heartwood) and absent from C (induced heartwood). G, H and I stained with Sudan Black. A (sapwood) shows no lipids while both B and C (heartwood) show black globules in the ray and vertical parenchyma and to a lesser extent in the vessels and tracheids. Scale is same for A – I; bar = 0.1 mm.
There were also present in the heartwood, globules of a substance, which did not stain with any of the three stains used routinely, or with the stains for phenolics. There were occasional fungal hyphae in natural heartwood. Fungus was more frequent in the induced heartwood, and in some cases spread from the areas of induced heartwood into the sapwood in white finger-like projections. These infected areas were free of starch and had little polysaccharide but the lipid droplets remained.

8.5. Discussion

8.5.1. Heartwood Formation

It is possible to induce heartwood formation in five-year old *S. album* trees. Eight months after treatment heartwood formed around the injection site, and extended 15cm below, and up to 60cm above the injection site. The extent of heartwood formation following stem injections of paraquat and ethrel was greater than that of distilled water and the concentrations of total volatile oils were greater in induced heartwood than in sapwood. Histochemical analysis showed that injections of paraquat and ethrel into the sapwood of *Santalum* lead to the depletion of starch and most polysaccharides and the synthesis of lipids. Thus on this basis the hypothesis that paraquat and ethrel induces heartwood formation is accepted. Findings of histochemical analysis in *Santalum* are consistent with those reported for induced heartwood in *Az. indica* (Shah *et al.* 1981; Nair and Shah 1983) and *Ac. auriculiformis* Cann. (Bacqui *et al.* 1984).

The depletion of starch grains and polysaccharides and the increase in concentration of lipids in the induced heartwood suggest starch and most polysaccharide compounds were hydrolysed for the synthesis of lipids and extractives. Shah *et al.* (1981) observed in *Az. indica* that parenchyma cells at the ethrel treatment site showed stimulation of phosphatase, lipase, ATPase and succinate dehydrogenase which coincided with the disappearance of starch and the presence of high lipid and phenolic contents in the cells. Bacqui *et al.* (1979) found that succinate dehydrogenase was significantly active only in the transition zone of *Melia azedarach*. Ethrel is an ethylene releasing compound, which is involved in the stimulation of respiration and phenol synthesis during the responses to wounding and senescence of xylem tissues (Shain and Hillis 1973).

In many species a transition zone separates heartwood from sapwood (Hillis 1987). In *S. album*
tretrazoilium chloride reagent applied to freshly cut disks indicated living cells continued from the cambium up to the boundary of the induced heartwood. However, a thin zone peripheral to the induced heartwood region did not stain black with IKI reagent, and analysis of sections showed the depletion of starch in this region.

The volatile oil concentration of induced heartwood in *Santalum* was similar or greater (up to 3 times greater) than that of naturally formed heartwood in five year old trees. However, large variations in the total volatile oil concentration were observed between trees given the same treatment (Table 8.4). Similarly, high variation was recorded with oleoresin production following stem injections of paraquat in *Pinus* species (Wolter and Zinkel 1976). Higher total volatile oil concentrations in induced heartwood of *Santalum* suggest more efficient conversion of metabolites and greater accumulation of heartwood extractives compared to that of naturally formed heartwood at this age. In *Pinus* species the oleoresin content was up to 40% near the wound site where paraquat was administered compared with 2% in naturally formed heartwood (Roberts *et al.* 1973). Oleoresin production was most intense at the treatment site and diminished upward and downward and from the treatment site to the xylem centre. However if natural heartwood already existed around the pith new resin deposits did not form within this heartwood (Stubbs *et al.* 1984).

It is reported that the concentration of total oils and proportion of α- and β- santalol in naturally formed heartwood increases with tree age (Srinivasan *et al.* 1992), so tree age may have strong influences on the yield of santalol within induced *Santalum* heartwood. Heartwood extractives are formed at the sapwood : heartwood interface from stored and translocated primary metabolites (Hillis 1987; Streit and Fengel 1994). Hillis *et al.* (1962) suggest the amount of extractives found in the heartwood depends on the amount of carbohydrates within the sapwood available for translocation to the sapwood : heartwood boundary. Thus treatment of trees at the time of year when sapwood starch content is highest should induce greater quantities of heartwood and/or higher oil content in the induced wood.

In our study the proportion of α- and β- santalol within induced heartwood was poorly correlated to the total yield of volatile oils. In contrast Li YingLan *et al.* (1994) found a good correlation between total oil concentration in induced heartwood and santalol concentration. They found higher concentrations of total oil and santalol within the stem near the treatment site than in the root system of the same tree, but no data were presented on how far the induced heartwood extended along the stem.

The extent of induced heartwood, concentration of volatile oils and proportion of α- and β-
santalol were not substantially enhanced by the addition of ethrel to the paraquat treatment. This suggests that the two chemicals were not synergistically linked in their mode of action, which contrasts with findings by Peters and Roberts (1977) and Peters et al. (1978) in *P. elliottii*.

The extent of induced heartwood formation vertically in *Santalum* was 60cm above ground level and was greatest in the paraquat treatments. Nair and Shah (1983) observed heartwood formed up to a height of 3.5 m from the treatment site in *Az. indica* following paraquat treatment. Paraquat diffuses into the xylem tissue and enters into the tracheids and vessels before being transported upward in the transpiration stream and downward translocation in phloem is indicated by the formation of induced heartwood below the treatment site (Stubbs et al. 1984). In *Pinus* species, induced heartwood extended upward 10-15 times farther than it extended downward. If paraquat moves too rapidly to the crown very little heartwood would form before the tree was killed. In one such instance stem injections of 45ml of 8% paraquat killed all trees of *Pinus* within 30 days due to the movement of paraquat to the crown (Stubbs et al. 1984).

Our study shows that santalol compounds formed following sapwood wounding around a drill hole filled with water. However the induced heartwood zone that surrounded such wound sites was relatively small (Figure 8.1). Wounding *Santalum* sapwood is an inappropriate method of inducing heartwood, as multiple drill holes would be required to obtain a small amount of heartwood, drill holes degrade the appearance of the wood and provide infection sites for fungi (Li YingLan et al. 1994). Similarly, Kadambi (1954) concluded that injury type treatments such as including severe branch pruning, stem coring, partial bark girdling, complete ring barking, mechanical wounding and root pruning were largely unsuccessful in inducing heartwood formation in *Santalum* in India. In *Pinus* multiple wounding and paraquat treatments along the stem did not enhance oleoresin production (Nix 1977; Enos et al. 1978). It has yet to be determined whether multiple injection sites for paraquat into *S. album* would give improved yields of induced heartwood.

Once heartwood begins to form naturally, there is a steady increase in its volume as the tree grows (Hillis 1987). Paraquat and ethrel may cause the formation of induced heartwood in young trees, though whether heartwood formation continues once the effect of the chemicals is finished, is still under investigation. In any event, treatment of trees may have practical use. Natural heartwood formation in *S. album* in natural stands does not occur until 15-20 and 23-29 years of age in India and Indonesia, respectively (Srinivasan et al. 1992; Haffner 1993). Injections of paraquat and/or ethrel may be given to either induce heartwood in trees too young
to initiate the formation of heartwood naturally, or possibly to convert extra sapwood to heartwood prior to harvest in trees which already contain natural heartwood.

8.5.2. Inorganic Compounds

The concentration of potassium and magnesium in induced heartwood were about half of those in the sapwood. This suggests that there was considerable resorption of K and Mg when sapwood was treated with paraquat and ethrel. Redistribution of nutrients is known to occur prior to natural heartwood formation (Ellis 1965; Higuchi et al. 1967; Ziegler 1968; Bamber 1976; Lambert 1981; Lambert et al. 1983). Interestingly nitrogen concentration in Santalum sapwood and induced heartwood was not noticeably different. In Populus it has been observed that the nitrogen concentration in sapwood tissue was about 2 times higher than that of the naturally formed heartwood (Merrill and Cowling 1966). Nitrogen is a major constituent of amino acids, enzymes and nucleic acids and for Populus it was suggested that death of cells during aging of sapwood is accompanied by retrieval of nitrogen from those cells. Heartwood formation following stem injection of paraquat and ethrel is likely to be more rapid than that of naturally formed heartwood and the high nitrogen concentration in Santalum heartwood suggests that negligible nitrogen was retrieved from the sapwood tissue before the cells died.

8.5.3. Moisture Content

The moisture content of paraquat and ethrel induced heartwood was consistently lower than that of the sapwood. This is consistent with findings in paraquat treated Az. indica (Nair and Shah 1983). It is widely accepted that sapwood moisture content is greater than that of natural heartwood (Bauch 1980; Nobuchi et al. 1984; 1985). In softwood species paraquat administered into the sapwood has an almost immediate effect of drying, reducing the moisture content to less than one-fifth of the surrounding sapwood (Hillis 1987). Generally, low heartwood moisture content is inversely related to a high gas volume in heartwood cells. Hillis (1987) suggests that that when the water/gas ratio in the cell lumens fall below a specific threshold the changes from sapwood heartwood or wound-wood may be initiated. However, Sachsse (1967) found no relationship between moisture content in the wood and the initiation of heartwood formation in Fagus and Populus. There is an indirect relationship between this ratio and the vitality of the parenchyma cells in these species (Hillis 1987). Gaseous ethylene, which is physiologically active at low concentrations, appears responsible for initiating the reactions leading to polyphenol synthesis (Shain and Hillis 1973; Hillis 1987). Oxidation of sapwood with air may play an important role in the formation of heartwood (Frey-
Wyssling and Bosshard 1959; Yazawa et al. 1967) and this may explain why the distilled water treatment induced wound-wood immediately adjacent to the drill hole.

8.6. Conclusion

In S. album heartwood diameter is positively correlated to total stem diameter (Widiarti 1991) and therefore high early growth of S. album is important to maximise heartwood production. We have shown that administering low concentrations of paraquat or ethrel into the sapwood of young plantation-grown Santalum induced heartwood and increased santalol oil content. Paraquat was the most effective treatment but the practical use and feasibility of inducing heartwood with stem administered paraquat on an operational scale is still unclear. Information is required on heartwood induction following stem injections of paraquat in older trees, the effect of the duration of treatment and the effect of season on administering paraquat.
~ CHAPTER 9 ~

GENERAL DISCUSSION AND SYNTHESIS

This study presents new information concerning the biology, physiology and silviculture of *Santalum* and its hosts, as a contribution towards developing a reliable system for the effective culture of *S. album* in plantations. In addition, the study demonstrates the experimental potential of a fast growing tropical *Santalum* species for investigating root hemi-parasite : host interactions. Despite the species' commercial importance the development of a reliable system for culture in plantations has been severely hampered by the large deficiencies in the understanding of *Santalum* : host silvicultural and physiological relations under plantation conditions (Havel and McKinnell 1993).

In addition to satisfying the species hemi-parasitic requirements to maximise growth for high plantation productivity, the commercial feasibility of *Santalum* plantation culture is dependent on significant heartwood formation and accumulation of santalol compounds. In effect, successful development of *Santalum* plantations is dependent on understanding two complicated, but distinctly different physiological phenomena, growth of a hemi-parasite and heartwood formation, making *Santalum* plantations very different to mono-cultural plantations of *Eucalyptus* or *Pinus* species.

The thesis has demonstrated that the presence of a pot host and the period of *Santalum* : pot host association prior to field establishment significantly influenced the survival, growth and allometry of *Santalum* following outplanting in the field. Survival and growth of *Santalum* in the field was enhanced when plants were grown in nursery pots with the herbaceous *Alternanthera nana* (Chapter 2). In general, the longer the period of association of host and parasite in the pots the better the growth and survival of the parasite in the field. The timing of introduction of the *Alternanthera* however is conditional upon the seedlings being large enough not to be over-topped by the *Alternanthera*. Under our conditions planting the pot host into pots with 54 day-old *S. album* seedlings and growing the plants for a further 134 days before field planting gave greatest growth. Many previous studies have assessed the effect on *Santalum* growth of different pot host species during the period within the nursery. It was found
here that the growth of *Santalum* was the same regardless of the period of time it was associated with *Alternanthera* in the nursery (or even without a pot host) but that subsequent field survival was very different. This study highlights the importance of evaluation of pot hosts and other nursery cultural treatments both in the nursery and after field establishment.

Biomass partitioning in *Santalum* was directed to the shoot following attachment to a host. A strong positive relationship between *Alternanthera* DW and *Santalum* root : shoot ratio suggests *Santalum* uses the root system of *Alternanthera* as an extension of its own to support the large shoot biomass. Thus water and nutrient deficits would result for *Santalum* should it lose its pot host on outplanting. This is an important practical consideration as in the field an intermediate host is planted up to 2 metres from the *Santalum* (so that both trees can develop a well-shaped crown) and thus *Santalum* will not be in immediate contact with this host.

Once the *Santalum* : pot host association is transferred to the field an ‘intermediate host’, a perennial woody species, is provided to maintain high growth rates of *Santalum*. In other species it has been shown that heartwood diameter is positively correlated to total stem diameter (Widiarti 1991) and therefore high early *Santalum* plantation growth is important to maximise heartwood production. This early growth is driven by the ‘quality’ of the intermediate host species. As outlined in chapter 3, the interactions between various potential intermediate host species and *Santalum* was examined by growing them in pairs in large (25 litre) pots in a nursery. Three N$_2$-fixing species (*Sesbania formosa*, *Acacia trachycarpa* and *A. ampliceps*) were better hosts than the non N$_2$-fixing *E. camaldulensis* in promoting *Santalum* growth. These findings are consistent with the general conclusion that xylem-tapping root hemi-parasites grow best when associated with N$_2$-fixing legume hosts, apparently as a result of greater N concentrations in xylem of legumes compared to non legumes (Subbarao *et al.* 1990; Cechin and Press 1993; Seel and Press 1993; Tennakoon and Pate 1996a; Tennakoon *et al.* 1997a, b, c).

Of the legume hosts *S. formosa* was the superior host in terms of promoting *Santalum* growth, but by the end of the 33 week study period, growth was beginning to decline, whereas plants grown in association with *A. trachycarpa* continued to grow, suggesting *A. trachycarpa* may be a more sustainable host. These findings indicate the importance of screening host species over long periods to determine the performance of hosts under parasitism. A longer term field trial of *Santalum* with the same intermediate host species has been established and is being monitored but results are not available within the time frame of this thesis.

An interesting finding from this study was that the growth of *Santalum* when grown without a
host was greater than that of plants grown with *E. camaldulensis*. This suggests *Santalum* competed poorly against *E. camaldulensis* for the available nutrients in the pot medium. In many nutrient poor field situations in which *Santalum* is likely to be cultivated it will be commercially beneficial to use well nodulated legume hosts rather than fertiliser N to meet the N requirement of the parasite.

The root : shoot ratio of *Santalum* without a host increased exponentially over time whilst for the *Santalum* in association with hosts the ratio remained relatively constant. Thus whilst unattached *Santalum* directs biomass partitioning into its root system at the expense of its shoot suggesting that unattached *Santalum* explores the rooting medium for potential host roots.

The root : shoot ratio of *Santalum* grown in association with *E. camaldulensis* was consistently higher than that of seedlings grown with legume hosts. It is known that N deficiency results in abnormally greater partitioning of assimilates to the root as opposed to the shoot (Linder and Rook 1984; Nambiar 1990; Stoneman and Dell 1993). In chapter 5, it was shown that a low N : P ratio existed in the foliage of *Santalum* when grown on *E. camaldulensis* compared with that when grown on legume hosts. N-deficiency of *Santalum* may be the stimulus for a high root : shoot ratio, and connection to a N rich host root is the trigger for a reduction in the root : shoot ratio. The relatively poor performance of *Santalum* grown in association with *E. camaldulensis* in comparison to that of N$_2$-fixing hosts suggests there may have been minimal uptake of N from the transpiration stream of the host in question. This aspect was followed up by studies on organic solute transfer from host to *S. album* in chapter 4.

The effectiveness of hosts was evaluated by examining various aspects of dry weight accumulation in host and parasite as well as leaf area of *Santalum*. It was suggested that leaf area indices for *Santalum* might be a used as an index for plantation productivity. In chapter 7, which details a study of the influence of host quality on plantation productivity leaf area was used as an estimate of *Santalum* vigour.

Despite extensive field and pot culture studies on the water relations, leaf gas exchange and mineral nutrition of root hemi-parasites and their hosts (Lamont and Southall 1982; Schulze and Ehleringer 1984; Struthers et al. 1986; Pate et al. 1991a; review by Pate 1995a), limited information exists on how root hemi-parasites benefit from their hosts in terms of photosynthetic rates, water use efficiencies, heterotrophic gains of carbon and uptake of nutrients.

In chapter 4 an assessment was made of composition of the sugars, organic acids and amino
acids passed from hosts to *Santalum*. There were large compositional variations in amino acids in haustoria and xylem sap of *Santalum* compared with the host xylem sap. High N content and low C:N ratios of the xylem saps of *Santalum* when grown with N\textsubscript{2}-fixing provided evidence of the N benefit passed to *Santalum* when grown in association with N\textsubscript{2}-fixing hosts. *S. formosa* carried a xylem stream richest in N, whereas the eucalypt host, was ranked lowest in terms of N content of its xylem sap and did not promote growth of *Santalum*. Asparagine, glutamate and aspartate dominated the composition of amino acids of xylem streams of *Santalum* and N\textsubscript{2}-fixing hosts. These compounds are considered to be readily absorbed by haustorial tissues and to then pass unmetabolised into the parasite xylem (Tennakoon and Pate 1997).

An interesting finding in chapter 4 was the negligible amount of proline within the xylem sap of *S. album*, which contrasts the findings of Tennakoon *et al.* (1997b) for *S. acuminatum*. High proline levels are considered indicative of the high levels of water stress, and summer drought is experienced in the coastal south-west Western Australia heath where the study on *S. acuminatum* was conducted. Proline is suggested to carry an osmoprotective function, which may contribute to a lowering of water potential of the parasite, and thereby facilitate acquisition and retention of host-derived water under stress conditions. Future investigations of *S. album* in the field will include studies on proline levels in the xylem sap during extended periods of droughting.

In chapter 5, it was shown that increased N derived from the xylem stream of N\textsubscript{2}-fixing hosts resulted in *Santalum* foliage high in N, high photosynthetic performance, large increases in leaf area, and ultimately improved dry matter production. Improved rates of photosynthesis in *Santalum* when grown in association with legume hosts provided circumstantial evidence that these parasites may have a lower dependence on their hosts for carbon than those grown in association with *E. camaldulensis*.

Following on from improved leaf photosynthesis when grown with N\textsubscript{2}-fixing hosts increased instantaneous water use efficiency (WUE) of *Santalum* was found. In chapter 5 it was shown that *Santalum* exhibited lower values for WUE than its host. For example, when attached to *A. trachycarpa* and *A. ampluscops* WUE values of 0.74 and 0.73 mmol mol\(^{-1}\) were recorded for *Santalum*, whereas those of the corresponding hosts were 0.86 and 1.23 mmol mol\(^{-1}\), respectively. This is consistent with a number of studies on other parasitic angiosperms (Schulze *et al.* 1984; Press *et al.* 1987; Shah *et al.* 1987; Press *et al.* 1988; Davidson *et al.* 1989).

Transpiration rates of *Santalum* were significantly lower when attached to *E. camaldulensis*
than when attached to N$_2$-fixing hosts. These findings are consistent with studies on other root hemi-parasites by Press et al. (1993) and Seel et al. (1993), but contrast markedly to mistletoe where transpiration rates are generally much greater than those of their hosts (Glatzel 1983; Hollinger 1983; Schulze et al. 1984; Ehleringer et al. 1985; Davidson et al. 1989; Stewart and Press 1990). In respect to diurnal variations in transpiration, Santalum proved to be closely similar to the host it was parasitising, consistent with findings for S. acuminatum by Tennakoon et al. (1997a), but different from a number of other root hemi-parasites, including Striga (Press et al. 1987; Shah et al. 1987; Press et al. 1988).

Consistent with data from a number of other root parasites and mistletoes, Santalum showed midday leaf water potential some 2 MPa more negative than in the corresponding host (Glatzel 1983; Schulze et al. 1984; Ullman et al. 1985; Ehleringer et al. 1986; Davidson et al. 1989; Davidson and Pate 1992; Veenendaal et al. 1996). The large change in leaf water potential occurs irrespective of the rate of transpiration on the different hosts. It is unclear if this is indicative of differences in resistance to water flow from host to parasite and warrants further investigation.

Santalum exhibited little capacity for osmotic adjustment when associating with different hosts which suggest that the bulk tissue water relations of S. album are not appreciably compromised when grown in association with either N$_2$ or non-N$_2$ fixing hosts.

Attempts were made to quantify benefits gained from different hosts and thus rank them in terms of suitability as donors of organic and inorganic solutes to Santalum. Benefit to Santalum from legume hosts occurs from the abstraction of fixed N, whereas, conversely, Santalum partnered with E. camaldulensis grew extremely poorly compared to those plants on legume hosts and amassed only half the dry weight of plants grown without hosts. Comparisons were made of relationships between transpiration, photosynthesis, C and N gain and xylem concentrations of C and N in terms of Santalum growth in Table 6.4. The relationship between Santalum's physiological characteristics and its mode of nutrition, the extent of Santalum's host dependence and what impact Santalum has on the host and how this information may be used in plantation management warrants further investigation.

In chapter 6, a poor relationship was found to exist between the total number of haustorial attachments to the root system of a host and the growth of the attached Santalum, whereas there was a very strong relationship between the number of haustoria attached to host roots and root
dry weight of that host. These findings contrast those by Tennakoon et al. (1997c) for *Olax phyllanthi* (Labill.) R. Br. A strong positive relationship existed between the number of haustoria connected to nodules and *Santalum* shoot and root DW. Self-parasitism (haustoria of *Santalum* attachment to its own root system) was evident in all *Santalum* : host associations including plants of *Santalum* grown without a host. Studies on haustorial numbers and sitings of attachments in chapter 6 provide further evidence of the indiscriminate nature of haustorial attachment in root hemi-parasites (Hocking and Fineran 1983; Pate et al. 1990a; Tennakoon et al. 1997a; Tennakoon and Pate 1997). In the case of all three *Santalum* : legume host associations haustoria were found penetrating bacterial tissue of nodules, which appeared to degenerate rapidly following attachment. Haustoria attached to the root xylem of hosts provide longer term benefit than those attached to nodules.

One of the primary aims of chapter 6 was to quantify the heterotrophic inputs of carbon to *Santalum* through uptake of xylem solutes from different legume hosts over a 9-week interval. A novel method for estimating heterotrophic gains (H) of C by *Santalum* is proposed. Estimates are based on matching the C : N ratio of the total organic solutes of xylem sap of *Santalum* grown with N\textsubscript{2}-fixing host (described in chapter 4) with the incremental gain of N of *Santalum* when grown on the same host and the incremental gain of N of *Santalum* cultured without a host. Estimates of H indicated that *A. ampliceps* was the best provider of C, then *S. formosa* and lastly *A. trachycarpa*. Expressed in terms of the percentage of the net C gain in dry matter of the parasite *A. ampliceps* was again the best provider of C (57.9% of net C gain of parasite), then *A. trachycarpa* of (45.5%) and finally *S. formosa* (34.6%).

*Santalum* grown in association with N\textsubscript{2}-fixing hosts showed substantially higher K concentrations than their hosts, but conversely on the non-beneficial *E. camaldulensis* host *Santalum* had lower K concentrations than that of its accompanying host. This is consistent with a number of studies on both mistletoes and root hemi-parasites (Lamont and Southall 1982; Glatzel 1983; Schulze and Ehleringer 1984; Struthers et al. 1986; Seel and Press 1993; Pate 1995a). As accumulation of K is a passive process high transpiration rates for *Santalum* grown with legume hosts (described in chapter 5) provide evidence for the mechanism for the accumulation of K in high concentrations in *Santalum* when grown with the hosts in question. The high concentrations of Na in foliage of *Santalum* compared to the associated hosts are consistent with findings from other root hemi-parasites (Struthers et al. 1986; Fer et al. 1994). High Na is thought to contribute to osmotic gradients, which, coupled with high transpiration rates, ensures the efficient capture of water from the host.

The main objective of this thesis was to develop an understanding of the silvicultural
requirements and physiological relationships between Santalum and its host with the view to
determining a system for the effective culture of Santalum in plantations. As described in
chapter 7, a young plantation, within the Ord River Irrigation Area, consisting of Santalum and
three native legume tree hosts, exhibited signs of declining vigour. This assessment was based
on high growth rates and continued good health of both parasite and hosts when sampled at age
3 years, and the poor health evident when the plantation was remeasured at age 5 years. This
phenomenon, known as ‘plantation decline’, is expressed as a marked reduction of the growth
rates of Santalum and, flowing from this of plantation productivity. A modeling procedure was
used to quantify the relationships between Santalum and its host based on host quality and the
distance of hosts from the parasite. A ‘plantation host index’ was developed to predict
Santalum crown health and future plantation productivity. The ‘plantation host index’, which
was a composite index of host quality and distance of the host from the parasite, was a more
reliable predictor of Santalum crown health than host quality or host distance alone. It is clear
that Santalum productivity in plantation culture will be sub-optimal unless highly beneficial
woody host species are selected, and can be shown to withstand long term parasitism.

These findings highlight a major deficiency in our understanding for the long term effective
culture of Santalum in plantations. At the time of planting Santalum and host seedlings in the
field a decision has to be made on the parasite : host ratio. Thus, one must have an
understanding of the ‘carrying capacity’ of each host species and the effect parasitism may
have on longevity of hosts. Research directed at determining appropriate Santalum : host stand
densities is urgently needed. Calculations of oil yield and eventual profit from commercial
plantations will be equivocal until such information is available. A plantation has been
established with parasite : host ratios of 5:1, 2:1, 1:1, 1:2 and 5:1, but analysis of the results are
clearly beyond the time frame of this thesis.

The primary objective for the plantation culture of Santalum is to maximise heartwood yield on
relatively short rotations. Under natural stands in Timor and southern India the age of
heartwood development in S. album varies from 10-46 years of age (Rai 1990; Haffner 1993).
Thus clear economic benefits exist if heartwood formation can be induced in young plantation
grown Santalum. As described in chapter 8, stem injections of paraquat and ethrel in 5-year old
trees were shown to induce limited heartwood formation. The induced heartwood was similar
anatomically to natural heartwood and had a similar or higher concentration of total volatile
oils. The induced heartwood also had depleted starch and polysaccharides, increased lipids,
lower moisture content and lower K and Mg concentration than sapwood, consistent with these
properties in natural heartwood.
One might expect the quality of induced heartwood in young plantation grown *Santalum* to be inferior to that of naturally formed heartwood in older trees, as the amount of extractives formed depends on the amount of carbohydrates within the sapwood (Hillis *et al.* 1962). The level of carbohydrates available as substrate may increase as trees age. An experiment has been established to investigate the effect of stem injections of paraquat on inducing heartwood in ten year-old *Santalum*, but the analysis of results are beyond the time frame of this thesis.

The presence of a transition zone (i.e. living tissue with depleted starch reserves) around the periphery of the induced heartwood region may lead to the formation of natural heartwood and further research on this possibility is needed. Despite the promising findings from chapter 8, the feasibility of paraquat and ethrel induced heartwood on an operational scale within *Santalum* plantations is still unclear, but its commercial potential is clearly reason for further investigation.

In conclusion, the studies on the silviculture and physiology of the root hemi-parasite *S. album* in this thesis collectively provide a clearer understanding of the complex interactions of the parasite and its host during the early stages of *Santalum* plantation growth. Undoubtedly a whole range of questions will continue to emerge relating to ‘mid-rotation’ aspects of *Santalum* plantation culture and heartwood development, and research on these questions will allow prescription of a complete methodology for the silviculture of one of the world’s most valuable timber species.
APPENDIX I

Potential for irrigated tropical forestry in northern Western Australia.

Andrew M. Radomijic1, 2, Syd R. Shea1, F. H. McKinnell1 and Jcn A. McComb2

1Dept. of Conservation and Land Management
Locked Bag 104, Bentley Delivery Centre 6983 Western Australia
2Division of Science, Biological Sciences, Murdoch University, Perth 6150 WA

Revised manuscript received 5 February 1998

Quote

"... the devastated remains of the (ancient) synagogue. All that was left were four walls, and the ribs of a roof. And where once the magnificent sandalwood ark had stood, there was little more than a niche in the wall pointing towards Jerusalem ...

Allan Gold, The Lost Testament.

Summary

Several high value exotic tropical timber trees have been screened in irrigated species selection experiments, on both predominant soil types (the Cununurra clay and Cockatoo sand), as potential plantation species for the Ord River Irrigation Area (ORIA), northern Western Australia, over the past ten years.

Santalum album (Indian sandalwood) has demonstrated potential as a plantation species on the flood irrigated Cununurra clay sites. S. album, a root hemi-parasite, requires a range of host species over its entire rotation length. Consequently, a relatively complex multi-species plantation system has been developed to maintain high plantation productivity. Wood quality issues, plantation policy and environmental implications of irrigated ORIA S. album plantations are also discussed.

Damage from Mastotermes darwinianus prevents long term survival of S. album and many other hardwoods on light textured soils in the region. The re-investigation into the reforestation of these soils has commenced with a range of previously untested species. The early performance of a species selection experiment on a trickle irrigated Cockatoo sand site is presented. At seven months of age Tectona grandis (teak), Pterocarpus indicus Willd. (narra, kayu merah), grow successfully on these sites.

The highly valued Santalum album L. (Indian sandalwood) has shown potential as a plantation species on the Cununurra clay (McKinell 1990; Havel and McKinell 1993; Radomijic and Borouh 1995). Due to its high heartwood santalol oil content, diverse utilisation and inherent religious significance, Indian sandalwood is the most important species from the widely distributed and economically important Santalum genus. It has been the ‘corner-stone’ of the global sandalwood industry for 20 - 300 centuries (Srinivasan et al. 1992). An innovative silvicultural research program has developed an intensive silvicultural system for S. album. It is perhaps one of the world’s most complex plantation systems, as it involves a flood irrigated plantation system for a xylem-tapping obligate root hemi-parasite in a semi-arid sub-tropical environment.

Western Australia has maintained an entirely export orientated sandalwood industry for the past 150 years. Based on the native S. spicatum (R. Br.) DC. A S. album plantation resource may ultimately supplement the S. spicatum greenwood harvest from natural stands (Kealley 1991).

World sources of sandalwood are declining rapidly (Srinivasan et al. 1992; Harisetyono and Suriamiharja 1991; Radomijic and Borouh 1995) and most sources will be exhausted over the next decade (Figure 1) (Havel and McKinell 1993). Current world annual sandalwood heartwood production estimates are around 5100 tonnes (Table 1). Assuming a heartwood yield of 20 tonnes hectare1 with a rotation length of 25 years, a plantation resource of around 6400 hectares is suggested to meet current global sandalwood consumption. That is, an annual planting program of 255 hectares.

Introduction

The potential for irrigated tropical forestry in northern Western Australia has been made possible by an immense (980 - 1050 km2) man-made body of water, Lake Argyle situated 50 km south of Kununurra (lat. 15°46’ S. long. 128°44’ E.). Water, mostly gravity fed, flood irrigates 13,000 hectares of agricultural land known as the Ord River Irrigation Area (ORIA). Recent feasibility studies for the expansion of the irrigated area to 70,000 hectares, with one third situated within the neighbouring Northern Territory, indicate potential for significant forestry development in the region.

The soils of the ORIA have been described in detail by Riley et al. (1993). The most suitable for flood irrigated plantations are the cracks clay soils of the Cununurra series, the area of which is fringed by the deep, freely drained light textured Cockatoo sands. There is an extensive area of these lighter soils which remain relatively under-utilised by the horticultural industry due to their low water holding capacity, low fertility and the potential for damage from the giant termite (Mastotermes darwinianus Frogg). Arboretum trials on the Cockatoo sands in the 1970s have shown that some commer-

The sandalwood plantation system

A bio-diverse farm forestry system appears possible due to S. album’s parasitic silvicultural requirement for a series of host species. That is, a range of species are required to perform as hosts at various stages in the plantation rotation.

Initially, S. album is propagated from seed in nursery containers. Although it can grow photo-autotrophically for several months, vigour and growth decline without attachment to a host (Figures 2 and 3). Two to three months following S. album germination, cuttings of a herbaceous pot host, Alternanthera spp. Forssk., are planted into each S. album
seedling container. The parasite: host combination continues for up to 18 months following plantation establishment. The simultaneous establishment of other host species, termed intermediate and long term hosts, is essential. These additional hosts are strategically placed within the plantation. The long term host, which must persist as final host for the entire rotation length (25-30 years), is planted several metres from the S. album and Alternanthera spp. combination. This is to avoid obstruction of the growth of the S. album as both S. album and long term host have large crowns. An intermediate host is therefore required to bridge the pot host and the long term hosts - a period of 2-5 years. We have found that the ideal intermediate hosts are fast-growing, short-lived leguminous trees. Once parasitism is initiated S. album remains highly heterotrophic and this sequence of hosts is required to maintain high plantation productivity.

Table 1. Origin, santolol oil content and heartwood production of the world’s commercial Santalum species.

<table>
<thead>
<tr>
<th>Country / State</th>
<th>Species</th>
<th>Heartwood santolol oil content (%)</th>
<th>Heartwood volume production (tonnes annum⁻¹)</th>
<th>Royalty ($/tonne)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Western Australia (Australia)</td>
<td>S. spicatum (R. Br.) A. DC.</td>
<td>2</td>
<td>2200⁴</td>
<td>SAUD7200 (5400)⁵</td>
</tr>
<tr>
<td>Queensland (Australia)</td>
<td>S. lanceolatum R. Br.</td>
<td>1</td>
<td>1400⁴</td>
<td>SAUD1200 (900)⁵</td>
</tr>
<tr>
<td>Karnataka (India)</td>
<td>S. album L.</td>
<td>6-7</td>
<td>500⁴</td>
<td>Rp340000 (20000)⁵</td>
</tr>
<tr>
<td>Tamil Nadu (India)</td>
<td>S. album L.</td>
<td>6-7</td>
<td>500⁴</td>
<td>Rp340000 (20000)⁵</td>
</tr>
<tr>
<td>Indonesia</td>
<td>S. album L.</td>
<td>6-7</td>
<td>300⁴</td>
<td>InrRp6000000 (3750)⁵</td>
</tr>
<tr>
<td>Vanuatu</td>
<td>S. austrocaledonicum Vieillard var. austrocaledonicum</td>
<td>3.5</td>
<td>80⁴</td>
<td>Vt300000 (2590)⁵</td>
</tr>
<tr>
<td>Papua New Guinea</td>
<td>S. macgregorii F. v. Mueller</td>
<td>(4-6) ?</td>
<td>60⁴</td>
<td>PNGK2000 (1500)⁵</td>
</tr>
<tr>
<td>New Caledonia</td>
<td>S. austrocaledonicum Vieillard</td>
<td>3-5</td>
<td>40⁴</td>
<td>CPF170000 (1800)⁵</td>
</tr>
<tr>
<td>Fiji</td>
<td>S. vari Seeman</td>
<td>5</td>
<td>14¹</td>
<td>SF6270 (4580)⁵</td>
</tr>
</tbody>
</table>

Estimated total volume 5094


Figure 1. Declining trend in sandalwood heartwood production in (i) India, (ii) Indonesia, (iii) Fiji and (iv) Vanuatu: from 1970 - 1996. Sources: ¹. Verma et al. (1984): Srinivasa et al. (1992); Surata et al. (1994); Bulai (1994); Vira and Smith (1994). Note different scales on Y axis.

Rather than considering S. album’s parasitic requirements an impediment to plantation development, a potentially highly valuable and biodiverse farm forestry system may arise as a result of S. album’s enigmatic heterotrophic existence. Thus, CALM has conducted research on the suitability of long term host species based on several principles (i) host: parasite compatibility, (ii) bio-climatic matching, (iii) suitability for flood irrigation and the soil conditions, (iv) market familiarity and (v) declining global supply and strong market demand. Many tropical forests are over exploited and are exposed to adverse land uses which have resulted in the declining supply of high valued tropical timbers whilst market demand remains strong (Evans 1986).

Long term host species that produce a valuable timber may offer the potential for two or more timber products from one plantation system. Successful long term host species introductions to date include the CITES-listed Swietenia macrophylla King (Brazilian mahogany), S. mahogoni Jacq., (Cuban mahogany) and Dalbergia melanoxylon Guill. and Perr. (East African Ebony).

Wood quality issues

The commercial value of sandalwood depends entirely on the santolol oil content of the heartwood and the quantity of heartwood per tree. In relatively short rotations it is important to maximise heartwood production and ensure that oil content is high. It appears there is considerable variation between tree age at which heartwood formation is initiated. From 30 trees sampled in West Timor, Indonesia, the apparent age of heartwood initiation varied from 14 to 46 years, and two of the trees contained no heartwood (Haffner 1993). In contrast, Rai (1990) states that in India the same species usually commences heartwood formation at 10 - 13 years of age. At present, ORIA plantings are using seed originally sourced from India. From limited sampling carried out in trial plantations most trees have initiated heartwood formation by the age of 10 years.

The rotation length of ORIA S. album plantations remains unclear, perhaps 20 - 30 years. However, there needs to be a compromise between avoiding high compound interest costs and producing a log with good wood quality suitable for oil distillation.
However, it is not necessary to fell all trees in a plantation at a young age. Retaining some trees would be attractive for the production of large logs for the wood carving and furniture industry, for which premium prices are paid.

**Plantation policy and environmental implications**

All indications, from early growth studies, are that sandalwood plantations grown with appropriate host combinations and following a specific silvicultural regime may be a profitable crop. The Department of Conservation and Land Management (CALM)

![Image](image_url)

**Figure 2. Santalum album** seedling growth 4 months after field establishment on a flood irrigated Cununurra clay site, Ord River Irrigation Area, north Western Australia. Left seedling parasitised to *Alternanthera* spp. as a host. Right seedling with no host. Rule is 1 metre.

<table>
<thead>
<tr>
<th>Diameter (mm)</th>
<th>Survival (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>80</td>
</tr>
<tr>
<td>60</td>
<td>70</td>
</tr>
<tr>
<td>70</td>
<td>60</td>
</tr>
<tr>
<td>80</td>
<td>50</td>
</tr>
<tr>
<td>90</td>
<td>40</td>
</tr>
<tr>
<td>100</td>
<td>30</td>
</tr>
<tr>
<td>110</td>
<td>20</td>
</tr>
<tr>
<td>120</td>
<td>10</td>
</tr>
<tr>
<td>130</td>
<td>0</td>
</tr>
</tbody>
</table>

**Figure 3.** Performance of *Santalum album* (i) mean survival, (ii) mean shoot dry weight and (iii) mean diameter at 10 cm with the (*) presence or (o) absence of *Alternanthera* spp. pot host; after establishment on a flood irrigated Cununurra clay site, Ord River Irrigation Area, north Western Australia. All treatments are significantly different using Tukey’s pairwise t-test (*p* < 0.05), except for survival data at 69 and 115 days after field establishment. Survival and diameter data are from 5 replicates and shoot dry weight data are from 3 replicates. Bars show standard errors.

does not intend to establish a large state-owned *S. album* plantation resource. Rather, it looks to the private sector: both established and new farmers within the ORIA, and investment companies, to carry out the bulk of the planting to establish a viable industry in the region. There is already strong interest from existing farmers and from investors in plantation development.

The sandalwood plantation enterprise will complement the existing successful horticultural industry in the ORIA, taking advantage of the infrastructure development already there, and contributing both economic and environmental benefits.

Coupled with commercial benefits, a *S. album* farm forestry system also may offer a cost effective method to control the early onset of rising water tables within the ORIA. Most irrigated agricultural systems inevitably lead to rising ground water table levels. Even though at present the ORIA sub-soil and ground water salt levels are comparatively low, waterlogging poses a significant long term risk to agricultural productivity. The Cununurra clay constitutes a large proportion of the intensively cropped land, and when saturated it becomes anaerobic and vehicle accessibility is greatly reduced. The extensive use of commercial tree plantations, planned on a landscape scale and integrated with agricultural activities, holds out considerable potential for a sustainable land management system for the region.

**Species selection trial on Cockatoo Sand**

Initial reforestation experiments using *S. album* and its potential hosts were performed on both the Cununurra clays and the light textured soils. Due to high seedling mortality caused by *M. darwinianus* damage, *S. album* failed on the Cockatoo sand sites (McComb unpublished data). This resulted in the emphasis on *S. album* silvicultural research shifting to the clays, on which sophisticated silviculture has been developed for *S. album* and its hosts. The development of sugar cane crops has raised the value of flood irrigated agricultural land at the time when agricultural crops (e.g. peanuts) on the Cockatoo sands have declined, hence attempts to identify suitable high value tropical timber species for these soils has recommenced. In this paper we report preliminary results, up to age seven months, from a trickle irrigated species selection experiment with exotic high value timbers on a Cockatoo sand site. Early seedling survival in the presence of *M. darwinianus* is being tested concurrently with growth performance. Eight species: *T. grandis*, *P. angolensis* DC. (musinga), *P. macrocarpus* Kurz. (Bunya padauk), *P. marapuu* Roxb., *P. indicus*, *S. mahogany*, *Khaya anthotheca* Welw. C. DC., *W.* and *K. senegalensis* (Dess) A. Juss., (both
African mahogany), propagated in 1.4 litre pots, were planted in a fully randomised complete block design in July 1996. Each plot consisted of 25 trees (5 x 5) planted at 3m x 3m, replicated four times.

The site was irrigated for 24 hours prior to planting by a single line of drip irrigation T-Tape, TSX 515 buried to a depth of 5 cm and located 30 cm from the rip line. Following establishment the experiment was irrigated at the rate of 10 - 15 ml per day. On 2nd and 3rd December 1996 a line of drip irrigation T-Tape, TSX 525 was installed, 60 cm from the planted row, on the opposite side of the planted row to the initial line of T-Tape. From 10th December 1996 to 11th January 1997 the experiment was irrigated very infrequently and from 12th January to 5th March 1997 was not irrigated due to wet season rains (Figure 4). Thereafter the experiment was irrigated at the rate of 7.2 - 9 ml per day. Natural vegetation was low open woodland with the climate dominated by a very long dry period, from March to November (Figure 4). Hence a non-irrigation treatment was not tested as field establishment occurs during the dry season (July), when soil moisture levels are very low.

The experiment received an application of soluble Lorsban™ (active ingredient chlorpyrifos 500 g/kg) at 6L/ha for M. darwiniensis control.

Seven months following field establishment all seedlings were measured for survival, height and diameter growth at 10 cm above the ground.

Survival in all species was high (93 - 100%) except for S. mahogani (70%) which was significantly lower (p < 0.05) than all other species (Figure 5ii). P. macrocarpus height was significantly greater than all other species followed by T. grandis, K. senegalensis and P. angolensis (Figure 5ii). K. senegalensis, P. macrocarpus and T. grandis diameter was significantly greater than the other species (Figure 5i).

This study suggests several commercial tropical timbers can be successfully established on a Cockatoo sand site within the ORIA, in particular T. grandis and a number of *Pterocarpus* species. However, suitable sawlog growth and the quality of timber production remains untested, except for a recent study by Brennan and Radomiljac (1998) on *T. grandis* and *K. senegalensis*.

A monospecific silvicultural system is far less complicated than the S. album multispecies plantation system developed for the flood-irrigated Cununurra clay soils, described earlier. However significant risk to plantations on the lighter textured soils is posed by the potential for infestation by *M. darwiniensis* and therefore screening for species with low susceptibility to *M. darwiniensis* is important on these soils.
In this study S. mahogani mortality was caused entirely by *M. darwiniensis* damage despite chloprypfos applications. Mature *T. grandis* has been reported as resistant to *M. darwiniensis* attack due to the presence of the phenolic heartwood compounds anthraquinone and tectoquinone (Tewari 1992). However, *T. grandis* and other plantation species seedlings were reported to be susceptible to damage in the Northern Territory (Cameron 1985).

Although the data are only preliminary they are sufficiently encouraging to continue species introduction and silvicultural studies on the promising plantation species. Further species selection research is planned to identify a wider group of species with high value timber that may be able to contribute to an extensive development of tropical timber plantations. *Tectona, Khaya* and *Pterocarpus* displayed encouraging growth in experiments in the Northern Territory and it was considered possible that some of the more valuable tropical cabinet timbers could be managed on selected sites (Cameron 1985).

Conclusion
While a relatively small production level of sandalwood would make a viable industry, much larger quantities of other species would be required to establish a commercially viable tropical hardwood timber industry. For this reason, the potential for afforestation of the ORIA Cockatoo sands, which are currently under-utilised, is particularly encouraging. Apart from making an economic contribution to the ORIA farm forestry would be an essential component of a sustainable land management system that uses intensive tree plantations to control ground water levels and also provide windbreaks, shelter and other amenity benefits.

Acknowledgments
We are grateful to the Australian Centre for International Agricultural Research for funding some of the early *S. album* silvicultural research within the ORIA. Staff at Agriculture Western Australia’s Frank Wise Institute for Tropical Agricultural Research are acknowledged for their collaboration in this project. We thank W. and G. Bloeker for providing the site and irrigation system for the experiment and their considerable efforts in its maintenance. Thanks are also due to Craig Palmer for his continued diligence in raising the seedlings for this study.

References


Radomiljac, A. M. 1995b. Australian Centre for International Agricultural Research - India trip report. ACIAR project 9043 - Sandalwood research. 9th - 16th October 1995. Department of CALM., Western Australia.


Srinivasan, V. V., Sivaramakrishnan, V. R., Rangaswamy, C.


APPENDIX 2

Radomiljac AM. 1998. The influence of pot host species, seedling age and supplementary nursery nutrition on *Santalum album* Linn. (Indian sandalwood) plantation establishment within the Ord River Irrigation Area, Western Australia. Forest Ecology and Management, 102; 193-201.
The influence of pot host species, seedling age and supplementary nursery nutrition on *Santalum album* Linn. (Indian sandalwood) plantation establishment within the Ord River Irrigation Area, Western Australia

Andrew M. Radomiljac *

*Department of Conservation and Land Management, P. O. Box 942, Kununurra, Australia, 6743*

Received 12 February 1996; accepted 5 May 1997

Abstract

A factorial experiment investigated the effect of six pot host species treatments (*Alternanthera nana*, *Sesbania formosa*, *Atalaya hemiglauca*, *Acacia hemignosta*, *Crotalaria retusa* and no pot host), two *Santalum album* seedling age treatments (24 and 17 weeks at field establishment) and a supplementary nursery nutrition treatment (2 × 100 ml 5% Ca Waxal®) on *Sa. album* survival and growth 287 days after field establishment. Significant variation exists between pot host species in increasing *Sa. album* survival and growth. *Al. nana* and *Se. formosa* pot host species significantly increased *Sa. album* survival, height and diameter. *Sa. album* survival, height and diameter was significantly better with supplementary nutrition. *Sa. album* survival and height was significantly greater and pot host species survival was significantly poorer with older *Sa. album* seedlings. Older seedlings and supplementary nursery nutrition gave higher levels of *Sa. album* field survival and growth when parasitised to poor pot host species but not when parasitised to satisfactory pot host species.

© 1998 Elsevier Science B.V.

*Keywords: Sandalwood; Parasitism; Host; Propagation*

1. Introduction

The *Santalum* L. genus, consisting of 16 known species (Hamilton and Conrad. 1990), is characterised by obligate hemi-parasitism and highly valued aromatic heartwood. The prized heartwood resource, in both western and eastern cultures, has endured centuries of exploitation (Brennan and Merlin, 1993). Considerable global concern has arisen over the survival of many *Santalum* species due to acute unsustainable exploitation and other adverse influences, such as habitat loss, uncontrolled fire and grazing, illegal cutting and government resource ownership and control (Husain, 1983; Murthy, 1985; Rai, 1990; Havel and McKinnell, 1993; Suriamhardja and Susila, 1994). Earlier attempts to regenerate *Santalum* species artificially have been largely unsuccessful, due to an inappropriate silvicultural understanding of the genera’s obligate hemi-parasitic nature (Iyengar, 1965; Scheffel, 1990; Harisetijono and Suriamhardja, 1991).
Table 1

Sa. album pot host species utilised in a field experiment in the Ord River Irrigation Area, Western Australia

<table>
<thead>
<tr>
<th>Treatment code</th>
<th>Pot host species treatment</th>
<th>Family</th>
<th>Succinct description of pot host species</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>No pot host</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>A</td>
<td>C. retusa L.</td>
<td>Papilionaceae</td>
<td>Nitrogen fixing herbaceous annual</td>
</tr>
<tr>
<td>B</td>
<td>Ac. hemigraecus F. Muell.</td>
<td>Mimoseaceae</td>
<td>Nitrogen fixing woody perennial</td>
</tr>
<tr>
<td>C</td>
<td>Se formosa (F. Muell.) N. Burb.</td>
<td>Papilionaceae</td>
<td>Nitrogen fixing woody perennial</td>
</tr>
<tr>
<td>D</td>
<td>At. hemigraecus (F. Muell.) F. Muell. ex Benth.</td>
<td>Sapindaceae</td>
<td>Woody perennial</td>
</tr>
<tr>
<td>E</td>
<td>Al. nana R. Br.</td>
<td>Amaranthaceae</td>
<td>Herbaceous annual</td>
</tr>
</tbody>
</table>

As a result of global decline in Santalum resources and the inherently strong demand for sandalwood products interest exists in establishing Sa. album Linn. plantations within the Ord River Irrigation Area (ORIA), Western Australia (Applegate and McKinnell, 1993). Preliminary plantings established in the mid 1980’s indicated that acceptable growth rates were achievable. Subsequent research was required to refine silvicultural techniques to increase nursery and field survival (McKinnell, 1990).

Silvicultural research has been conducted on Sa. album in Indonesia (Suramihardja et al., 1991; Surata, 1992) and India (Srinivasan et al., 1992). Similar research requires replication within the ORIA due to climatic, site and technological differences. Host selection, in particular, needs investigation as it is the single most important silvicultural parameter influencing Sa. album plantation establishment (Havel and McKinnell, 1993). Research elsewhere has identified a range of suitable pot host species for Santalum species plantation establishment, such as Desmanthus virgatus (L.) Willd., Alternanthera spp. Forsskal and Crotalaria juncea L. for Sa. album in Timor (Surata, 1992), Calotropis procera (Aiton) W. T. Aiton, Cassia siamea Lam. and Calliandra calothyrsus Meisn. (Shinde et al., 1993) and Cajanus cajan Huth. (Rai, 1990) for Sa. album in India, Al. sessilis (L.) D.C. for Sa. australacadianicum Vieillard in Vanuatu (Bule and Daruhi, 1990) and New Caledonia (Chauvin, 1988).

Due to the hemi-parasitic characteristic, Sa. album plantation silviculture is vastly more complex than mono-culture plantation species. Hosts are generally categorised into three groups: pot, intermediate and long term host. All are critical for adequate Sa. album survival and growth at various stages during the plantation rotation (Radomiljac and Borough, 1995).

The pot host is planted into a Sa. album seedling container during nursery propagation. The pot host: parasite relationship continues after plantation establishment and should persist until the second host plant is parasitised, the intermediate host. An intermediate host seedling, usually a fast growing perennial legume, planted up to 2 m from the Sa. album seedling is parasitised 9–12 months after field establishment.

Characteristics for suitable pot host selection include fine root growth, an even distribution of root growth within the pot, ability to withstand top pruning, low level of competition, low allelopathic influences, low growth structure, hemi-parasite compatibility and persistence in the field after out planting (Fox and Doronila, 1993).

This paper reports a factorial experiment designed to test the effect of three factors on the survival and growth of Sa. album seedlings after field establishment: the nutrition of seedlings during nursery propagation, the age of Sa. album seedlings at the time of field establishment and the presence of perennial and leguminous pot host species (Table 1).

2. Materials and methods

2.1. Experiment design and treatments

The experiment consisted of 24 treatments (6 pot host species × 2 seedling age × 2 supplementary nutrition treatments). Each treatment plot consisted of a single line of 10 Sa. album seedlings planted at 3 m spacings. A 1.8 m wide buffer row separated the experiment rows. The experiment was replicated in 3 fully randomised blocks.
2.2. Pot host species and seedling age treatments

On 15 April 1993 when *Sa. album* seedlings were 14 weeks old (24 week old treatment, seed sown 7 January 1993) or 7 weeks old (17 week old treatment, seed sown 22 February 1993) the pot host species treatments were propagated into pots containing *Sa. album* seedlings (Table 1). The *Sa. album* seedlings and pot host species treatments remained in the nursery for a further 12 weeks, with or without supplementary liquid fertiliser applications (described below), before transfer to the field on 7 July 1993.

2.3. Supplementary nutrition treatments

Liquid fertiliser Wuxal suspension Calcium (16% N, 17.2% Ca, 1.8% Mg, 0.08% B, 0.0016% Mo, 0.0006% Co, 0.064% Cu, 0.08% Fe, 0.16% Mn and 0.032% Zn) at 0.5% (w/v) in 100 ml of distilled water was applied twice to the supplementary nutrition treatment *Sa. album* seedlings, on 28 April 1993 and 25 May 1993. Non-supplementary nutrition treatment seedlings received 100 ml of distilled water on the same days.

2.4. Seedling propagation

Fresh *Sa. album* seed was collected from the Department of Conservation and Land Management’s Kununurra arboretum. The fleshy exocarp was removed and the seed was dried for 24 h in ambient conditions. Seed was soaked in 0.05% gibberellic acid solution for 15 h, washed and then sown. Germinants were transplanted into pots with a 1.5 l volume, 180 mm height and 100 mm diameter, approximately 6 weeks after sowing. The pot medium consisted of sand: peat: perlite at 3:2:2, respectively. Osmocote plus, a slow release fertiliser, (16% N, 3.5% P, 10% K, 1.2% Mg, 4.1% S, 1.5% Ca, 0.15% Fe, 0.06% Mn, 0.05% Cu, 0.02% B, 0.02% Mo and 0.015% Zn) was incorporated into the mixture at 300 g m⁻³.

All five pot host species are of local ORIA origin. *Se. formosa*, *At. hemiglaucua*, *Al. hemignosta* and *C. retusa* seed was sown on 18 March 1993. *Se. formosa*, *Ac. hemignosta* and *C. retusa* seed was soaked in hot water for 2 minutes. *Al. nana* was propagated vegetatively by cuttings. *Al. hemiglaucua* seed received no pre-treatment.

2.5. Site description

The field experiment was established on the Kununurra clay soil. Northcote Key classification is Ugl 5.29 (Northcote, 1984). The soil is dark brown (2.5Y 4/2) heavy clay, very plastic when wet and very hard when dry. Analysis of a composite sample of

Table 2
Analysis of variance table for final *Sa. album* survival, height, diameter, and pot host survival 287 days after establishment in a field experiment in the Ord River Irrigation Area, Western Australia

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>Sa. album survival</th>
<th>Sa. album height</th>
<th>Sa. album diameter</th>
<th>Pot host species survival</th>
</tr>
</thead>
<tbody>
<tr>
<td>Block</td>
<td>2</td>
<td>0.25</td>
<td>5.92 *</td>
<td>88.3</td>
<td>1.26</td>
</tr>
<tr>
<td>Species (S)</td>
<td>5</td>
<td>0.89</td>
<td>21.40 * * * *</td>
<td>4479.1</td>
<td>64.02 * * *</td>
</tr>
<tr>
<td>Age (A)</td>
<td>1</td>
<td>0.325</td>
<td>7.82 *</td>
<td>310.3</td>
<td>4.43 *</td>
</tr>
<tr>
<td>Nutrient (N)</td>
<td>1</td>
<td>0.768</td>
<td>18.48 * * * *</td>
<td>1134.9</td>
<td>16.22 * * *</td>
</tr>
<tr>
<td>S x A</td>
<td>5</td>
<td>0.089</td>
<td>2.14</td>
<td>454.3</td>
<td>6.49 * * *</td>
</tr>
<tr>
<td>S x N</td>
<td>5</td>
<td>0.082</td>
<td>1.98</td>
<td>136.7</td>
<td>1.95</td>
</tr>
<tr>
<td>A x N</td>
<td>1</td>
<td>0.001</td>
<td>0.04</td>
<td>39.9</td>
<td>0.57</td>
</tr>
<tr>
<td>S x A x N</td>
<td>5</td>
<td>0.135</td>
<td>3.26 *</td>
<td>124.9</td>
<td>1.78</td>
</tr>
</tbody>
</table>

Species, is 6 pot host species treatments: *Al. nana*, *Se. formosa*, *At. hemiglaucua*, *Ac. hemignosta*, *C. retusa* and no pot host control. Age, is two *Sa. album* seedling age treatments at the time of field establishment: 17 and 24 weeks. Nutrition, is two nursery nutrition treatments: two applications of liquid fertiliser Wuxal suspension Calcium (16% N, 17.2% Ca, 1.8% Mg, 0.08% B, 0.0016% Mo, 0.0006% Co, 0.064% Cu, 0.08% Fe, 0.16% Mn and 0.032% Zn) at 0.5% in 100 ml of distilled water and two applications of 100 ml of distilled water on 28 April 1993 and 25 May 1993.

Significant values of *F* are denoted by *P < 0.05*, **P < 0.01 or *P < 0.001.*
Table 3
Pot host species treatment means of final *S. album* survival, height, diameter and pot host survival 287 days after field establishment

<table>
<thead>
<tr>
<th>Pot host species treatment</th>
<th>Pot host survival (%)</th>
<th><em>S. album</em> survival (%)</th>
<th><em>S. album</em> height (cm)</th>
<th><em>S. album</em> diameter (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>A. nana</em></td>
<td>86.7a</td>
<td>97.1a</td>
<td>96.2a</td>
<td>2.04a</td>
</tr>
<tr>
<td><em>S. formosa</em></td>
<td>71.7b</td>
<td>84.7b</td>
<td>93.7a</td>
<td>1.99a</td>
</tr>
<tr>
<td><em>C. retusa</em></td>
<td>0.0d</td>
<td>67.1c</td>
<td>69.8b</td>
<td>1.12b</td>
</tr>
<tr>
<td><em>A. hemignosta</em></td>
<td>30.0c</td>
<td>50.4cd</td>
<td>61.0c</td>
<td>0.89bc</td>
</tr>
<tr>
<td><em>A. hemiglauca</em></td>
<td>86.3a</td>
<td>57.4c</td>
<td>52.4d</td>
<td>0.69c</td>
</tr>
<tr>
<td>Control</td>
<td>–</td>
<td>36.7d</td>
<td>50.1d</td>
<td>0.74c</td>
</tr>
</tbody>
</table>

Means followed by the same letter are not significantly different from each other (*p* = 0.05 by pairwise *t*-test).

Soil (Lambert, 1976) indicates the topsoil (0–15 cm) consists of sand, silt and clay fractions at 44%, 18% and 38%, respectively, 2.5% organic matter, a pH (1:5 soil: water) of 7.4 and a bulk density of 1.26 g cm$^{-3}$.

2.6. Site preparation and irrigation

Site preparation involved several applications of ripping, disc cultivation and rotary hoeing to gain a fine tilth. Planting beds were formed at 1.8 m widths and a height of 20 cm above the normal soil surface. Following field establishment the experiment was flood irrigated every 7 days over a 42 day period and subsequently every 14 days over a 70 day period. The experiment was not flood irrigated during the monsoonal wet season (December 1993 to March 1994). Following the wet season, the experiment was flood irrigated every 28 days.

2.7. Assessment and data analysis

Each treatment was assessed for final *S. album* survival, height, diameter (at 5 cm above ground level) and pot host species survival 287 days after field establishment. Analysis of variance (ANOVA) was conducted on all measured parameters using a general linear model (GLM) procedure. An angular (arcsine) transformation was applied to survival data. Significant differences between treatment means were determined using pairwise *t*-tests (Snedecor and Cochran, 1968).

3. Results

Results of the ANOVA are presented in Table 2. The pot host species treatments (*S*) significantly influenced *S. album* survival, height, diameter and pot host survival. *S. album* seedling age treatments (*A*) significantly influenced *S. album* survival, height and pot host survival. Nursery nutrient treatments (*N*) significantly influenced *S. album* survival, height and diameter. In addition, the *S* × *A* interaction was significant for *S. album* height, diameter and pot host survival. The *A* × *N* interaction was significant only for *S. album* diameter. The *S* × *A* × *N* interaction was significant only for *S. album* survival. All treatment interaction effects were negligible relative to the main treatment effects, in particular the pot host species treatments. The mean square values for the pot host species treatment effects was greater for *S. album* survival (*× 6.5*), height (*× 37*), diameter (*× 12*) and pot host survival (*× 78*) over the *S* × *A* × *N* treatment interaction.

Table 4
Age treatment means of final *S. album* survival, height, diameter and pot host survival 287 days after field establishment

<table>
<thead>
<tr>
<th>Age treatment (week)</th>
<th><em>S. album</em> survival (%)</th>
<th><em>S. album</em> height (cm)</th>
<th><em>S. album</em> diameter (cm)</th>
<th>Pot host survival (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>24</td>
<td>74.4a</td>
<td>72.7a</td>
<td>1.19a</td>
<td>41.8b</td>
</tr>
<tr>
<td>17</td>
<td>62.1b</td>
<td>68.4b</td>
<td>1.29a</td>
<td>60.4a</td>
</tr>
</tbody>
</table>

AGE treatment, is the duration of *S. album* seedling nursery propagation prior to field experiment establishment. Means followed by the same letter are not significantly different from each other (*p* = 0.05 by pairwise *t*-test).
Table 5
Nutrient treatment means of final *Sa. album* survival, height, diameter and pot host survival 287 days after field establishment

<table>
<thead>
<tr>
<th>Nutrient treatment</th>
<th><em>Sa. album</em> survival (%)</th>
<th><em>Sa. album</em> height (cm)</th>
<th><em>Sa. album</em> diameter (cm)</th>
<th>Pot host survival (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>77.7a</td>
<td>74.7a</td>
<td>1.35a</td>
<td>53.8a</td>
</tr>
<tr>
<td>−</td>
<td>58.6b</td>
<td>66.4b</td>
<td>1.14b</td>
<td>48.6a</td>
</tr>
</tbody>
</table>

+ is an application of liquid fertiliser Waxal™ suspension calcium (16% N, 17.2% Ca, 1.8% Mg, 0.08% B, 0.0016% Mo, 0.0006% Co, 0.06% Cu, 0.08% Fe, 0.16% Mn and 0.032% Zn) at 0.5% (w/v) in 1 ml of distilled water on 28 April 1993 and 25 May 1993.

− is an application of 100 ml of distilled water on 28 April 1993 and 25 May 1993.

Means followed by the same letter are not significantly different from each other (p = 0.05 pairwise t-test).

3.1. Pot host species treatment

Pot host species treatment means of *Sa. album* survival, height, diameter and pot host species survival are summarised in Table 3. *At. nana* increased *Sa. album* survival, height and diameter and maintained a higher level of field persistence than that of all the other pot host species treatments. *At. nana* was not significantly better than *Se. formosa* in increasing *Sa. album* height and diameter or than *At. hemiglauca* in field persistence. *Se. formosa* ranked second in promoting *Sa. album* growth. *Ac. hemiglauca* and control treatments gave poor *Sa. album* growth. *C. retusa* did not persist in the field.

3.2. Age treatment

Seedling age treatment means of *Sa. album* survival, height, diameter and pot host species survival are summarised in Table 4. *Sa. album* survival and final height was significantly greater with increased nursery seedling age. Conversely, pot host species field survival was lower with increased seedling age. Seedling age did not significantly influence *Sa. album* diameter.

3.3. Supplementary nursery nutrition treatment

Supplementary nursery nutrition treatment means of *Sa. album* survival, height, diameter and pot host survival are summarised in Table 5. Supplementary nursery nutrition gave significant increases in *Sa. album* survival, height and diameter. Pot host species survival was not significantly influenced by supplementary nutrition in the nursery.

3.4. Species × nutrition × age treatment interaction

The pot host *Al. nana* treatment across all age and nutrient treatments (Fig. 1). The 24 week old *Sa. album* seedling treatment (2) and supplementary nursery nutrition (+) increased *Sa. album* survival for all pot host species treatments except for *Se.

Fig. 1. Pot host species, seedling age and supplementary nursery nutrition treatment interaction on final *Sa. album* survival 287 days after field establishment. Values are mean with standard deviation. Control is no pot host treatment. (A) *C. retusa*; (D) *Ac. hemiglauca*; (C) *Se. formosa*; (D) *At. hemiglauca*; (E) *Al. nana*. (1) 17 week old *Sa. album* seedling at field establishment. (2) 24 week old *Sa. album* seedling at field establishment. (+) An application of liquid fertiliser Waxal™ suspension Calcium (16% N, 17.2% Ca, 1.8% Mg, 0.08% B, 0.0016% Mo, 0.0006% Co, 0.06% Cu, 0.08% Fe, 0.16% Mn and 0.032% Zn) at 0.5% in 100 ml of distilled water on 28 April 1993 and 25 May 1993. (−) An application of 100 ml of distilled water on 28 April 1993 and 25 May 1993.
Fig. 2. Pot host species and seedling age interaction on final Se. album (a) height, (b) diameter and (c) pot host survival 287 days after field establishment. Values are mean with standard deviation. Control, is no pot host treatment. (A) C. retusa; (B) Ac. hemiglauca; (C) Se. formosa; (D) At. hemiglauca; (E) Al. nana. (1) 17 week old Sa. album seedling at field establishment. (2) 24 week old Sa. album seedling at field establishment.

formosa. The 17 week old seedling treatment (1) and no supplementary nursery nutrition (—) gave lower Sa. album survival for all pot host treatments except for Al. nana. The control pot host, 17 week old seedling treatment and supplementary nursery nutrition gave very poor Sa. album survival (3.3%). Conversely, Al. nana pot host species, 24 week old seedling treatment and supplementary nursery nutrition gave very good Sa. album survival (99.9%). Supplementary nursery nutrition significantly increased the level of Sa. album survival for both Ac. hemiglauca and At. hemiglauca pot host species in both seedling age treatments.

3.5. Species X age treatment interaction

The Al. nana and Se. formosa pot host species treatments gave greater Sa. album height growth (Fig. 2a) and diameter (Fig. 2b) than the other pot host treatments in both age treatments. Se. formosa, At. hemiglauca and Al. nana pot host species had higher levels of field survival than C. retusa and Ac. hemiglauca (Fig. 2c) in both age treatments. The C. retusa pot host species did not survive past 90 days in the field, in both age treatments. The 24 week old seedling treatment gave lower pot host survival for all pot host species, except At. hemiglauca.

4. Discussion

The greatest influence on Sa. album survival and growth, after field establishment, was exhibited by the pot host species treatments. The efficacy of different pot host species in promoting Sa. album growth varied significantly. Thus, the selection of appropriate pot host species is critical to ensure acceptable levels of Sa. album field survival and growth.

Sa. album survival and growth was significantly greater with Al. nana and Se. formosa pot host treatments than with At. hemiglauca, Ac. hemiglauca pot hosts and no pot host treatments. Al. nana and Se. formosa, differ markedly in habit. Se. formosa is a perennial legume attaining a height of 12 m and Al. nana is a non-leguminous, usually prostrate, herb about 0.3 m tall (Wheeler et al., 1992). The other two leguminous pot host treatments, Ac. hemiglauca and C. retusa, did not increase Sa. album survival or growth relative to the control treatment which suggests the suitability of pot host species is dependent on their individual efficacy to supply nutrients and moisture to the hemi-parasite, and that legumes are not necessarily better pot hosts than non-legumes.

Like all photosynthetic, Sa. album possesses leaf chlorophyll and is able to synthesise carbohydrates. Iyengar (1965) described Sa. album as a part root parasite and part photosynthetic. Generally, parasitic plants are smaller than their hosts, such as the herbaceous root parasite Rhinanthus serotinus Schouw, (Klaren and Jansen, 1978). However, Sa. album has a large tree habit and, therefore, the host is not the sole source for all nutrients, moisture and amino acids. Diversity of opinion exists as to which nutrients are absorbed from the host and which nutrients are absorbed directly from the soil, through Sa. album's own root system (Sreenivasa Rao, 1933; Iyengar, 1960; Srinivasan et al., 1992). Parthasarathi et al. (1974) indicates that Sa. album roots possess cation exchange capacity comparable to normal
plants, indicating that *Sa. album* has the same ability to absorb nutrients from the soil. Struthers et al. (1986) suggests a marked increase in concentration of K and Na from *Sa. spicatum* (R.Br.) D.C. roots to leaves compared with *Ac. acuminata* Benth. hosts would serve to maintain a strong water potential gradient. Ensuring the unidirectional flow of water from host to hemi-parasite and, therefore, the indiscriminate absorption of mineral nutrients from the host. The pot host species efficacy in increasing *Sa. album* growth is dependent on the osmotic gradient established between the respective root system of host and hemi-parasite.

Selection of suitable pot host species is dependent on a multitude of parameters, not just initial *Sa. album* growth under nursery conditions. (i) A high level of pot host field persistence whilst parasitised is important. *C. retusa* grew prolifically in the nursery and promoted good early *Sa. album* field growth. However, following outplanting it senesced quickly breaking the host–parasite relationship which attributed to lower *Sa. album* growth and high field mortality. (ii) The pot host species must be compatible with *Sa. album*’s nursery and field cultural regimes. Unlike *Ac. hemignosta*, which was attacked by *Fusarium* spp. Link. in the nursery caused by over watering during nursery propagation. However, the water regime was adequate for *Sa. album* propagation and no evidence of *Fusarium* spp. activity was observed on other pot host species treatments. (iii) An understanding of pot host growth vigour is required. *Se. formosa* required top pruning due to excessive apical growth and *Al. nana* smothered small *Sa. album* seedlings. Consequently, a suitable pot host ‘pricking in’ regime is required. If sown too early, the pot host will compete with *Sa. album* within the pot. Conversely, if sown too late, insufficient time for adequate haustoria connections before field establishment will result in *Sa. album* outplanting stress.

Propagation of *Sa. album* for 14 weeks rather than 7 weeks prior to 10 weeks with a pot host in the nursery increased subsequent survival of *Sa. album* in the field. Nagaveni and Srimathi (1985) observed 70% of *Sa. album* seedlings initiated haustoria development within 30 days. This suggests older *Sa. album* seedlings may develop more numerous and advanced haustoria connections on the pot host root system before field establishment and in consequence is better able to withstand outplanting stress.

The presence of a woody stem in older seedlings increases *Sa. album* seedling robustness for outplanting. In India, Srinivasan et al. (1992) indicated, suitable seedlings for outplanting are 30 cm in height with a woody stem, after 6 to 8 months nursery propagation. In Indonesia, Surata (1992) indicated, *Sa. album* seedling propagation takes 8 months or more before seedlings are ready for outplanting. Conversely, in this study good *Sa. album* field survival with 4 month old seedlings was achieved when supplied with the *Al. nana* pot host, suggesting efficient pot hosts may compensate for the reduced growth of younger *Sa. album* seedlings. The utilisation of the *Al. nana* pot host may reduce the duration of *Sa. album* nursery propagation.

Increased seedling nutrient availability through two applications of 100 ml 5% Ca Wuxal® increased *Sa. album* survival and growth in the field. Nutrient omission studies reported by Wijesuriya (1984) and Struthers et al. (1986) on *Sa. spicatum* found that seedlings treated with nutrient solution lacking Ca died prematurely. Both studies indicate seedling growth without supplementary N, Fe and micro-nutrients was significantly reduced.

That *Sa. album* survival with the *Ac. hemignosta* and *At. hemiglauca* pot host species treatments was improved by supplementary nursery nutrition indicates *Sa. album* parasitised to inefficient pot hosts may require increased supplementary nutrition to sustain growth. Conversely, supplementary nursery nutrition did not significantly influence the survival of *Sa. album* parasitising the more efficient pot hosts, *Se. formosa* and *Al. nana*.

The hemi-parasitic characteristic of *Sa. album* and its host requirements still remains a significant enigma to the understanding of plantation establishment, even though first evidence of *Sa. album*’s parasitic requirements was reported by Scott (1871).

5. Conclusion

This experiment clearly demonstrates that the use of a pot host increases the level of *Sa. album* survival and growth in the field. Considerable variation exists between pot hosts in increasing *Sa. album*
survival and growth. Consequently, the utilisation of appropriate pot hosts is critical to ensure successful *Sa. album* plantation establishment. No generalisation in the identification of suitable pot hosts can be obtained due to the varied habit of the best two pot host species. Older seedlings increased the level of *Sa. album* survival and growth when parasitised to poor pot host species, *Ac. hemignosta* and *At. hemiglaucu*. Efficient pot host species, *Al. nana* and *Se. formosa*, will compensate for the reduced growth of younger *Sa. album* seedlings after field establishment. Increased seedling nutrient availability, with a liquid multi-nutrient fertiliser, gave significantly higher *Sa. album* survival and growth across the poorest pot host species, whereas, the effect on *Sa. album* seedlings with efficient pot host species was negligible.

Acknowledgements

The Department of Conservation and Land Management and the Australian Centre for International Agricultural Research for funding this research. The author acknowledge the staff of the Western Australian Department of Agriculture’s Frank Wise Institute for Tropical Agricultural Research for their technical support. Drs. F. McKinnell, J. McComb and P. Christensen for their interest in this research.

References


**APPENDIX 3**

**SANTALUM MACGREGORII IN PAPUA NEW GUINEA.**

Report from a Sandalwood Workshop funded by Australian Centre for International Agricultural Research and Papua New Guinea Forest Authority conducted in Port Moresby, 11 – 14th November 1996.
**INTRODUCTION**

Santalum species are distributed widely, from India throughout much of Australia, Papua New Guinea and many South Pacific Islands countries. The fragrant heartwood from these species, commonly known as sandalwood, is of high commercial significance to many rural based economies. With the exception of Santalum species native to Australia, the global production of sandalwood occurs in developing countries. Due to the utilisation of sandalwood in both eastern and western cultures and the long period of exploitation most Santalum species are faced with declining population sizes. S. macgregorii, endemic to southern Papua New Guinea, is one of the lesser known Santalum species and the extent of current exploitation is unknown.

**STATUS OF SANTALUM MACGREGORII IN SOUTHERN PNG.**

S. macgregorii’s former distribution extended continuously along the PNG southern savanna region and as close as 20km from Port Moresby (Department of Forests, Lae. Herbarium collections NGF 17404, 22/2/1964). S. macgregorii is now scarce in this area, presumably as a result of over-harvesting. Harvesting pressures, coupled with indiscriminate burning, have resulted in S. macgregorii’s current distribution as isolated populations in remote regions. Recent inventories have identified S. macgregorii as far west as the Paupala range, near Lese, in the Gulf Province (PNGFA data 1997). A complete inventory of the remaining populations is urgently required which will assist identifying areas that require germ plasm conservation measures. This inventory will greatly assist the South Pacific Regional Initiative on Forest Genetic Resources (SPRIG) project. This project has a strong interest in assisting developing countries in the South Pacific in areas of seed collection, assessment, tree improvement and conservation of priority forest genetic resources (Thomson pers. com. 1997). Santalum has been identified as a priority genus for the SPRIG project.

As in most areas within the Central and Gulf Provinces inaccessibility prevents S. macgregorii harvesting and extraction. Poor road access prevents buyers liaising with villages and impedes the transportation of sandalwood to Port Moresby for export. By virtue of remoteness remnant S. macgregorii populations have avoided exploitation.
It appears that two varieties of *S. macgregorii* exist in southern PNG. A coastal (eg. Manumanu) and highland (eg. Berenia) variety. The coastal variety has a broader leaf, a lighter coloured heartwood and lower scented heartwood. However, detailed morphological examination on flowers and fruits is required to determine the actual extent of this variation.

**SANTALUM MACGREGORII EXPLOITATION AND UTILISATION.**

It is critical that an understanding of the status of *S. macgregorii* is gained as it is currently being commercially exploited in an unregulated way. The recent resurgence in *S. macgregorii* exploitation commenced near Port Moresby with a merchant trader purchasing heartwood from coastal villagers between 1985-86. Exploitation then extended into the Central Province in 1994, into an area known as the Berenia region, 200km north west of Port Moresby and now occurs in the Gulf Province. Villages in southern PNG harvest *S. macgregorii* from traditionally owned forests, from which the lucrative large piece size stem heartwood is most sought after. The Papua New Guinea Forest Authority (PNGFA) does not regulate *S. macgregorii* harvesting, marketing and royalty payments when exploitation occurs on these lands.

Sandalwood buyers usually liaise directly with the traditional land owners and as a result harvested quantities are unknown. The consequences of the unregulated *S. macgregorii* exploitation are highlighted by the brief period of exploitation in the Manumanu and Berenia regions, from 1993 to 1995. Harvesting in these areas ceased due to resource decline and the subsequent market rejection of small heartwood material, which made it impossible to recover costs of harvesting, sapwood removal and transport.

Buyers were demanding large, defect free, solid wood for the lucrative South East Asian sandalwood carving and furniture market. The under-utilisation of *S. macgregorii* occurred in significant quantities and stockpiles of seemingly utilisable *S. macgregorii* root, branch or small stem heartwood remain as rejected heartwood within coastal PNG villages. Buyers refused to purchase *S. macgregorii* heartwood if the piece size was considered too small or if the heartwood scent was low, but if marketed appropriately this rejected heartwood may be powdered and used for joss stick manufacturing.

As the resource declined villagers commenced harvesting immature trees, excavated roots of
previously cut trees, harvested *S. macgregorii* on sacred grounds and cut trees of other species mistaking them for *S. macgregorii*. Eventually harvesting in the Western Province ceased when resource depletion occurred. Simultaneously, the PNGFA intervened by revoking export licences to prevent the excavation of root systems from previously harvested trees. From this area buyers moved to the Gulf Province.

The exploitation of sandalwood is dependent on buyers contacting remote villages. Road access is the key to *S. macgregorii* exploitation. To date road access from Berenia into the remote Gulf province is difficult, however, an extension of the unsealed Hiritano highway into this area is imminent.

**SEED COLLECTION AND EX SITU CONSERVATION**

Several mature *S. macgregorii* trees exist within the Port Moresby botanical gardens and attempts have been made to poach these trees. The botanical gardens may act as a valuable *S. macgregorii* seed resource and seed collection arrangements have been put in place. It appears that mature trees from natural stands produce moderate fruit crops indicating there should be reliable seed supplies for silvicultural and species introduction research, ex situ plantings and small scale plantation establishment work.

**SANTALUM MACGREGORII FOR PLANTATIONS**

Morphologically *S. macgregorii* appears quite close to *S. album* (native to southern India and eastern Indonesia) which produces the premium Indian sandalwood. If it is determined that *S. macgregorii* and *S. album* are similar then *S. macgregorii* should be tested as potential plantation species outside PNG.

In the early 1980’s the PNGFA Forest Research Institute (PNGFRI) considered the possibility of growing *S. macgregorii* from seed for plantation establishment. It was concluded that sandalwood’s silvicultural requirements were not well understood. Consequently, attempts to develop a *S. macgregorii* silvicultural system did not commence (Ross pers. com. 1996).

As with all *Santalum* species *S. macgregorii* is an obligate root hemi-parasite. This parasitic habit complicates nursery propagation and plantation silviculture. However the protocols developed for nursery and field establishment of *S. album* in India, Indonesia and Australia
and for *S. austrocaledonicum* in New Caledonia make it feasible to re-consider establishment of *S. macgregorii* as a native plantation species in PNG.

**PRICING STRUCTURE OF SANTALUM MACGREGORII HEARTWOOD**

In the mid-1980’s traditional owners were receiving only PNG Toea 5 kg\(^{-1}\) for *S. macgregorii* heartwood. By the peak of exploitation in 1995 the price had risen dramatically to PNG Toea 200 kg\(^{-1}\). However significant quantities of heartwood were purchased for less than AUD$2 kg\(^{-1}\). (PNG Toea 100 = AUD$1). Within PNG a price differential exists based on heartwood quality. The price for coastal *S. macgregorii* heartwood was less than half of the highland heartwood; i.e. PNG Toea 90 kg\(^{-1}\) compared to PNG Toea 200 kg\(^{-1}\), respectively.

A probable explanation for the low price received for *S. macgregorii* heartwood is that traditional landowners have a limited understanding of the global sandalwood industry. This allows buyers to exploit remaining *S. macgregorii* populations below their real value. For comparison purposes, *S. album* heartwood in India is sold for between AUD$15 – 18 kg\(^{-1}\) and the lower oil containing *S. spicatum* from Western Australia is sold for between $6- 8 kg\(^{-1}\).

In addition, it appears there is limited cultural attachment to *S. macgregorii* in PNG. This in contrast to the utilisation of sandalwood for medicinal, religious and commercial purposes in Indonesia, India and several South Pacific countries. Surprisingly, there is limited traditional utilisation of sandalwood in southern PNG, which is contrary to other regions within PNG where wood carving is culturally significant.

**SANTALUM MACGREGORII HEARTWOOD OIL ANALYSIS**

The percentage content and composition of *S. macgregorii* heartwood oil is still 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*.
CONCLUSION

S. macgregorii in PNG’s southern savanna regions has been heavily exploited where human habitation exists. The species appears to be protected due to inaccessibility. This is likely to change in the near future with the extension of the Hiritano highway into the Gulf Province. Before buyers enter into the Gulf Province it is important that villagers are aware of the importance and value of S. macgregorii, and harvesting regulations need to be considered to implement sustainable harvesting levels.