MULGA RESEARCH CENTRE JOURNAL

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EDITORIAL POLICY

Contributions are welcomed for consideration by the Editorial Panel. Preference is given to contributions related to the objectives of the Mulga Research Centre. Intending contributors should submit material for consideration following a style format available from the editors. A page charge will be made depending on the level of sponsorship for a given volume.

EDITORIAL PANEL

All papers in this Journal have been critically reviewed by appropriate members of the scientific community.

AVAILABILITY

Back issues may be purchased from Mulga Research Centre, School of Environmental Biology, Curtin University of Technology, Kent Street, Bentley or GPO Box U1987, Perth, WA 6001, Australia.

CONSTITUTION OF THE MULGA RESEARCH CENTRE

(as amended at the Annual General Meeting of 6 February 1987)

1. NAME

The name of the Association shall be "Mulga Research Centre".

2. OBJECTIVES

2.1 To promote field and laboratory studies in the biology, ecology, pharmaceutical and agricultural potential, and other appropriate uses of Western Australian trees and shrubs, with emphasis where appropriate on those of the Mulga Zone.

2.2 To sponsor field studies for educational purposes, with priority to the Mulga Zone; meetings to inform the public of the results of work undertaken; reports to cover the results of investigations to be published in the manner of a journal, with an editorial review panel, on an approximately annual basis.

2.3 To assist scholars engaged in appropriate related studies.

2.4 To raise funds from appropriate sponsors to enable 2.1, 2.2 and 2.3 above to be undertaken.

2.5 To report work undertaken in journal format.

3. MEMBERSHIP

Membership shall be by invitation to scientists active in pursuit of studies compatible with the objectives of the Mulga Research Centre. Associate membership may be granted to students who participate in appropriate studies, field work or related investigations.

Representatives from Companies and other organisations sponsoring activities will be invited to attend the Annual General Meeting, to be classed as financial members, and to vote on changes or additions to this Constitution (see below).

4. SUBSCRIPTIONS

Subscriptions shall be minimal. At each Annual General Meeting the Treasurer shall recommend a subscription which shall be approved or otherwise.

5. OFFICE BEARERS

Office Bearers shall consist of a Director, a Secretary, and a Treasurer and two or more Committee Members.

6. MANAGEMENT COMMITTEE

The appointed Office Bearers and two or more Committee Members shall constitute the Management Committee. The Management Committee is empowered to co-opt additional persons to assist with organising any function held in pursuance of Objective 2.1, and to appoint suitably qualified persons to advise on any matters which may arise should funds generated by Objective 2.4 need to be divided.

7. FUNCTIONS OF THE COMMITTEE

A suitably responsible person, not a member of the Mulga Research Centre, shall be appointed Honorary Auditor. The auditor's report shall be read at the Annual General Meeting. An annual report will be prepared to cover each calendar year, this is to be available as soon as practicable in the following year.

8. MEETINGS

Members shall meet together in a formal manner on a day to be appointed by the Secretary each year. This meeting will constitute the Annual General Meeting. Items for discussion should be lodged with the Secretary prior to the meeting. Other meetings of members may be held in conjunction with organised public meetings should any pressing business require that a formal meeting be held. At any meeting where there will be voting on proposed amendments to the Constitution, financial members unable to attend may approve proxies (in writing) to the Secretary.

9. QUORUM

A quorum shall be 20 percent of the financial members, excluding associate members, provided that the Director and Secretary (or a nominee) are present.

10. PATRONS

A Patron of the Mulga Research Centre is appointed in recognition of considerable financial assistance. Patrons are entitled to one copy of all publications sponsored or produced by the Mulga Research Centre.

11. GENERAL ADMINISTRATION

11.1 Location: The location of the Mulga Research Centre is c/- School of Environmental Biology, Curtin University of Technology, Kent Street, Bentley, WA 6102.

11.2 Bank Account: An account will be opened at the South Bentley branch of the R & I Bank. Signatories shall be the Director or Secretary and the Treasurer or a nominee of the Committee should the appropriate office bearer be overseas or otherwise unable to fulfil his duties.

11.3 Changes or Additions to Constitution: Any changes in the Constitution of the Mulga Research Centre must be carried by a majority of two-thirds voting, after notice of motion has been circulated to all financial members one month prior to the meeting when the matter is to be discussed.

This manuscript was prepared at Curtin University of Technology by Miss Louise Dutton for the Mulga Research Centre.

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PRELIMINARY OBSERVATIONS ON ECOTYPIC VARIATION IN *SANTALUM SPICATUM* 1. PHENOTYPIC VARIATION

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SUMMARY

Environmental factors such as rainfall, temperature or soil type may contribute to variation in morphological attributes between ecotypes in widespread species. *Santalum spicatum* is distributed over a broad geographical range in Western Australia and is thus subject to a range of environmental conditions. Populations were examined from several soil types, a latitudinal range of 22-32° and mean annual rainfall from 206-526 mm. Attributes considered included potential phenotypic variation in nut and kernel size, viability, germination time, seedling growth rate, and adult leaf characteristics.

There were significant differences in mean values for both nut and kernel sizes between different *Santalum spicatum* ecotypes. Nut dimensions were larger from near coastal locations but smaller and lighter from inland locations. Both viability and germination times were highly variable between ecotypes. Batches of higher viability commenced germination sooner and of these some 50% of nuts germinated in about 3 weeks. The ecotype with lightest nut weight germinated most rapidly. There was a tendency for ecotypes with heavier fruit to require a longer time to germinate. For the progeny sets examined from germinated nuts there were significant variations in 60 day growth expressed as seedling height, leaf number and hypocotyl diameter between *Santalum spicatum* ecotypes.

Leaf samples taken from adult trees of *Santalum spicatum* revealed that the leaf size characteristics of length, width, length/width ratio, area and dry weight were all significantly different between *Santalum spicatum* ecotypes. The sandalwood ecotypes from the drier inland regions had higher leaf length/width ratios and smaller leaf areas than those of the milder, more coastal regions. This is believed to be an adaptive feature related to dry environments. Leaves taken from ecotypes of higher latitudes had significantly higher chlorophyll contents than those from lower latitudes.

INTRODUCTION

Santalum spicatum (R. Br.) A. DC., the Western Australian sandalwood, is an obligate root hemi-parasitic species valued for its aromatic wood. The early settlers of Western Australia realised the value of the wood and by the 1840s it was the primary export earner for the fledgling State (Richmond, 1983). The sandalwood industry today, though relatively less important, still produces a gross export income in excess of $11 million per annum. The wood is exported to Taiwan, Hong Kong, Singapore, Thailand and Malaysia, where it is mainly used in powdered form for the manufacture of joss sticks, burnt as offerings in temples. Due to the significant reduction in sandalwood there is much interest in replenishing stocks and concern for the genetic resources of the species (Kealley, 1991). The tree may be grown to provide a nut crop, providing useful intermediate yields (Barrett, 1987).

Santalum spicatum, a member of the Santalaceae family, is a shrub or small tree growing to 4 m in height, rarely taller. It has rough, grey bark and a trunk diameter of 100 to 300 mm. Leaves are lanceolate to narrowly elliptic, flat and obtuse, dull grey-green in colour, with a lamina 20-70 mm long, 3-15 mm wide and a petiole 3-5 mm long. The flowers are bisexual, numerous in panicles and mildly carrion scented, attracting flies and other insects (Barrett, 1989). The fruit is a drupe, with a green or brown epicarp. The mesocarp is firm and usually adheres to the smooth endocarp, when ripe (Hewson and George, 1984). The kernel is eaten (Barrett, 1987). It is distributed through most of southern Western Australia (Figure 1), to Shark Bay in the west and the southern Pilbara in the north. It is absent from the Kimberleys and the extreme south-west (Kealley, 1991). It occurs in some drier areas of South Australia. *S. spicatum* occurs over a wide range of climatic conditions and its distribution has been linked to the presence of endemic host plants, such as species of *Acacia* (Loneragan, 1990), although it is catholic in its parasitism (Herbert, 1925; Gardner, 1928).

Sandalwood has extensive lateral roots, which may run for 25 to 30 m from the tree. Along the whole length of the lateral roots, fine feeder roots develop, which have the potential to parasitise suitable host roots. The fine feeder roots make cup like connections, called haustoria, to the host plant (Herbert, 1925). Sandalwood is capable of photosynthesis but requires some nutrients from its hosts to survive. Struthers *et al.* (1986) observed that the nutrients K, Ca, N, and Cu were transferred from the host roots of *Acacia acuminata* Benth. to the parasite.

Santalum spicatum is generally considered to be a slow growing species, requiring around 60-90 years to reach commercial size in the more arid areas in which present day harvesting occurs. Flowers are produced at 3-4 years

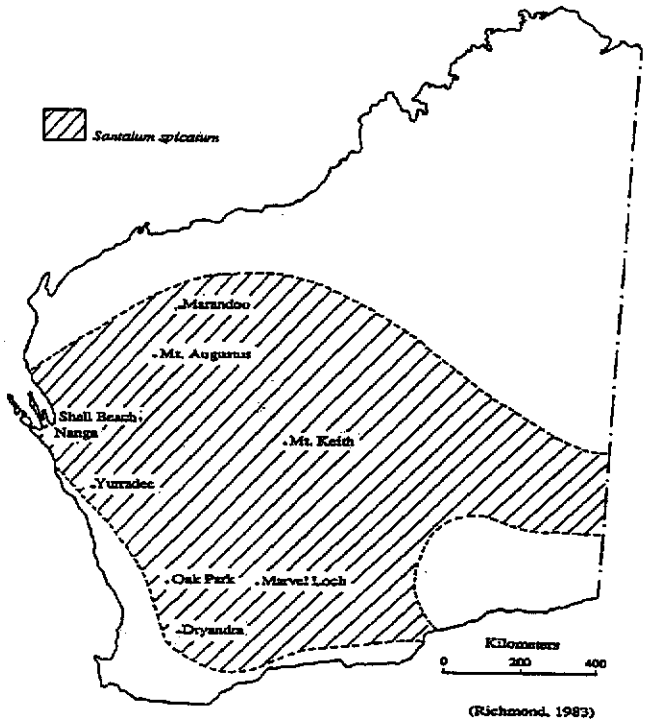

FIGURE 1. Distribution of *Santalum spicatum* in Western Australia and the location of ecotypes examined.

and the first seed crop at 6-7 years. The commercial size for harvesting is a trunk diameter of 127 mm, at 150 mm above the ground (Kealley, 1991). Uplike other commercial tree species, *S. spicatum* is not cut or sawn at the base, but the whole tree is pulled out of the ground. This is because of limited coppicing ability and the roots, butt, stem and branches are all valuable commercial products (Kealley, 1989 and 1991). It is susceptible to fire and the foliage is preferentially browsed by all herbivores.

Cultivation and biology of *Santalum spicatum* has been investigated at Curtin University of Technology since 1981 (Barrett, Wijesuriya & Fox, 1985). Fruit production on trees cultivated on the Bentley campus of Curtin University indicates that nut and kernel size is variable between trees. Total yields are also variable and trees producing larger quantities tend to have smaller kernels. Seed size, as a variable characteristic, has been associated with emergence time. Larger seeds and/or earlier emergence can increase plant size, survival, and reproduction in some species (Harper, Lovell & Moore, 1970; Schaal, 1980; Weis, 1982). In other plant species, smaller seed size or later germination may be more successful (Baker, 1972).

If the fruit from sandalwood trees were to be used as a commercial crop it would be desirable to have high yielding trees with large kernels. It is possible that either individuals could be selected or that particular populations could provide superior yielders. The present study sought to investigate some aspects of variation in nut size and other characteristics from populations of *Santalum spicatum* growing in different environments. It was hypothesized that nut weights would be heavier and sizes larger in higher rainfall areas than in the more arid areas of distribution, where germination opportunities are uncertain. This paper reports the extent of phenotypic variation encountered on a selection of populations from the range of the natural distribution of *Santalum spicatum*. Genotypic variation is covered elsewhere (Brand, 1993).

METHODS

Material from nine different populations (Figure 1) of *Santalum spicatum* was obtained. Seven to ten trees were sampled from each population between October 1990 and July 1991. These are referred to as ecotypes in the results. Mature sandalwood fruits were gathered from the ground beneath each sampled tree. After fruit exocarps were removed, the weights and diameters of 100 nuts from each population were obtained. Random sub-samples of 50 nuts were cracked to obtain weight and diameter of kernels. Nut and kernel weights were measured to 0.01 g, and diameters measured to 0.1 mm.

Fifty leaves were collected from each of the 7-10 trees sampled in 5 populations (Table 1). Leaves were collected no less than 10 leaf pairs back from a new shoot. After collection each of: leaf length, width, thickness,

TABLE 1. Locations of sandalwood populations examined, and whether leaves were obtained and nuts sown.

Population location	"n"	Latitude and longitude	Soil type	Rainfall (mm) *	Leaves taken	Nuts sown
Marandoo	7	22° 44', 118° 09'	red earth	325	-	-
Mt.Augustus	7	24° 19', 116° 55'	red earth	213	-	+
Shell Beach	10	26° 12', 113° 46'	shells, sand	225	-	+
Nanga	8	26° 15', 113° 49'	red sand	225	+	+
Yurradee	7	28° 08', 114° 42'	sandy loam	489	-	+
Mt. Keith	10	27° 11', 120° 33'	clay loam	206	+	+
Oak Park	10	31° 18', 116° 51'	sandy loam	340	+	+
Marvel Loch	10	31° 30', 119° 32'	sandy loam	265	+	+
Dryandra	10	32° 50', 117° 06'	loam	526	+	+

"n" refers to number of trees sampled in a population.
** Mean annual rainfall from Gibbs (1966), and Hall, Wainwright & Wolf (1981).*

area, chlorophyll content and dry weight were obtained in the laboratory. Leaf length and width were measured to 1 mm and leaf thickness was measured to 0.1 mm. Leaf area was measured to 0.01 cm^2, using an area meter, model LI-3000 (Lambda Instrument Company). Leaves were then oven-dried for 24 hours and the dry weights taken to 0.01 g.

Leaf samples for chlorophyll analysis were divided into 10 replicates of 1 g each, and cut into small pieces, using scissors. Each replicate was placed into a clean mortar, 80% v/v acetone added and leaf material ground to a fine pulp, using a mortar and pestle. The extract was filtered, using a conical flask, funnel and number 3 Whatman filter paper. Filtrate volume was made up to 100 ml with acetone. Aliquots of 1-2 ml were put into a glass cell. The cell was set into a spectrophotometer and optical density of the chlorophyll was measured at 645 nm, 652 nm, and 663 nm. These measurements were read against an acetone solvent blank. The amounts of chlorophyll 'a', 'b' and 'total' (mg chlorophyll g^{-1} leaf tissue) for each plant were then determined using equations taken from Witham, Blaydes & Devlin (1986).

Eighty nuts from 8 of the populations, i.e. all except Marandoo (Table 1), were counted into batches of 2 replicates of 40. Each batch was sown in punnet trays of 340 by 280 by 60 mm containing coarse sand 50 mm deep, on 5 April 1991. Nuts were positioned 1 cm below the sand surface. The trays were placed on rail benches in a glasshouse and watered every 2-3 days with scheme water. Germinants, described as nuts with 1-2 mm of protruding radicle, were counted on a daily basis for 100 days from the time of sowing. For each replicate the following measures were obtained:

$$\textbf{Germination rate} = \frac{(n_1 t_1 + n_2 t_2 + ... + n_x t_x)}{\Sigma n}$$

where

n_1 = number of germinants on the first day of germination
t_1 = time (days) to first germination
n_2 = number of germinants on the second day of germination
t_2 = time (days) to second germination
x = number of days to final germination
Σn = total number of seed germination over experiment

Germination Value (G.V.) = P.V. x M.D.G.

where

$$\text{P.V.} = \frac{\text{Max. Cumulative \% germination}}{\text{N°. days from start}}$$

$$\text{M.D.G.} = \frac{\text{Final \% germination}}{\text{N°. days to final germ. \%}}$$

Germination rate is calculated by summing the products of germinants and days from initial treatment and dividing by the total number of germinants (Hartmann & Kester, 1975). Peak value (P.V.) is the highest level of cumulative percentage germination divided by the number of days from the start. It is thus a measure of the steepness of the germination gradient. Mean daily germination (M.D.G.) is calculated by dividing percentage germination achieved by the number of days taken for the last recorded germinant to germinate. Germination value (G.V.) combines rate of

germination and viability. It is the product of peak value and mean daily germination (Czabator, 1982).

At 2-5 days after germination, seedlings were planted one to a pot, in 70 mm x 70 mm x 100 mm pots containing a 1:1:1 mixture of coarse sand, fine sand and peat. Pots were carefully labelled to preserve identification of the parent source. Seedlings were placed on rail benches in the glasshouse and watered every 2-3 days with scheme water. Height, hypocotyl diameter and leaf number were recorded each week until a final measurement at 60 days. Seedling height was taken in mm, using a ruler from the soil surface to the base of the highest leaf bud. Hypocotyl diameter was measured using vernier callipers to 0.1 mm, at 5 mm above soil surface. The total number of leaves greater than 5 mm in length on each seedling was also recorded. These seedlings were then used in isozyme analysis (Brand, 1993).

One-way analysis of variance was used to determine if there were differences in phenotypic characteristics between *Santalum spicatum* populations. Scheffe tests were used to separate means. Regression analysis was used to seek relationships between measured characteristics and several general environmental parameters.

RESULTS

One-way analysis of variance revealed highly significant differences ($p<0.001$) for each of nut and kernel weights and diameters between ecotypes. Ecotypes from Nanga and Shell Beach were the heaviest (Table 2) and those of the inland locations Mt. Augustus, Mt. Keith, Marvel Loch and Marandoo were the lightest. Mean nut weight was negatively correlated with longitude ($r=0.798$). The pattern of difference in nut diameter between ecotypes was similar to that of nut weight and these mean dimensions had a strong linear relationship (r of 0.991; $p<0.001$; Figure 2a).

Correlation of mean kernel weight and diameter for ecotypes was less than for nuts, but still highly significant (r of 0.911; $p<0.001$; Figure 2d). The inland sources were again smaller but the rank order between ecotypes differed. Generally those ecotypes with larger nuts had larger kernels and there was a significant linear correlation between nut and kernel weights (Figure 2c; r of 0.841; $p<0.001$). The Yurradee ecotype straddled this range. Those of Marvel Loch and Mt. Keith, with kernels of least weight, had similar proportions of kernel to nut weight as Nanga and Shell Beach (0.32-0.33). Marandoo and Mt. Augustus had the highest proportions of kernel to nut weight (0.45-0.46). Nut diameter predicted kernel weight (r of 0.868; $p<0.001$; Figure 2b) more efficiently than did nut weight (r of 0.841). There were no significant relationships between mean annual rainfall and any of nut weight ($F=0.188$, $p=0.68$), nut diameter ($F=0.30$, $p=0.59$), kernel weight ($F=1.22$, $p=0.31$) or kernel diameter ($F=0.95$, $p=0.36$).

Most of the *Santalum spicatum* nuts germinated between 3 and 6 weeks after sowing (Figure 3). Three batches (Nanga, Oak Park and Yurradee) gave less than 20% germination. There were also differences in time taken for germination between the ecotypes.

No seed germinated until 2 weeks from setting out (Table 3). The Mt. Keith and Marvel Loch sets gave highest final percentage germination of 96 and 92% respectively. Mt. Keith had highest germination value and germinated fastest (24 days). Ecotypes with lowest germination were Nanga (9%) and Yurradee (14%). Nanga and Yurradee had very low germination values of 0.01 and 0.02 respectively.

TABLE 2. Mean values for nut and kernel dimensions (weight and diameter) of *Santalum spicatum* ecotypes. Same letters indicate ecotypes which are not significantly different ($p<0.05$) using the Scheffe test.

Ecotype	Nut dimensions		Kernel dimensions		Kernel / Nut
	Weight (g)	Diameter (mm)	Weight (g)	Diameter (mm)	Ratio
Nanga	4.11 a	20.8 a	1.30 a	14.6 a	0.316
Shell Beach	3.72 ab	20.5 ab	1.18 ab	13.3 ab	0.317
Dryandra	3.29 bc	19.7 bc	1.22 ab	13.6 ab	0.371
Yurradee	3.07 cd	18.7 cd	1.12 abc	12.7 ab	0.365
Oak Park	2.89 cd	18.6 cd	1.19 ab	12.6 ab	0.412
Marvel Loch	2.60 de	17.7 de	0.85 cd	12.4 bc	0.327
Mt. Augustus	2.16 ef	17.0 ef	0.98 bc	12.7 ab	0.454
Marandoo	2.04 f	16.5 f	0.94 bc	11.9 bc	0.461
Mt. Keith	1.90 f	16.1 f	0.60 d	10.4 c	0.316

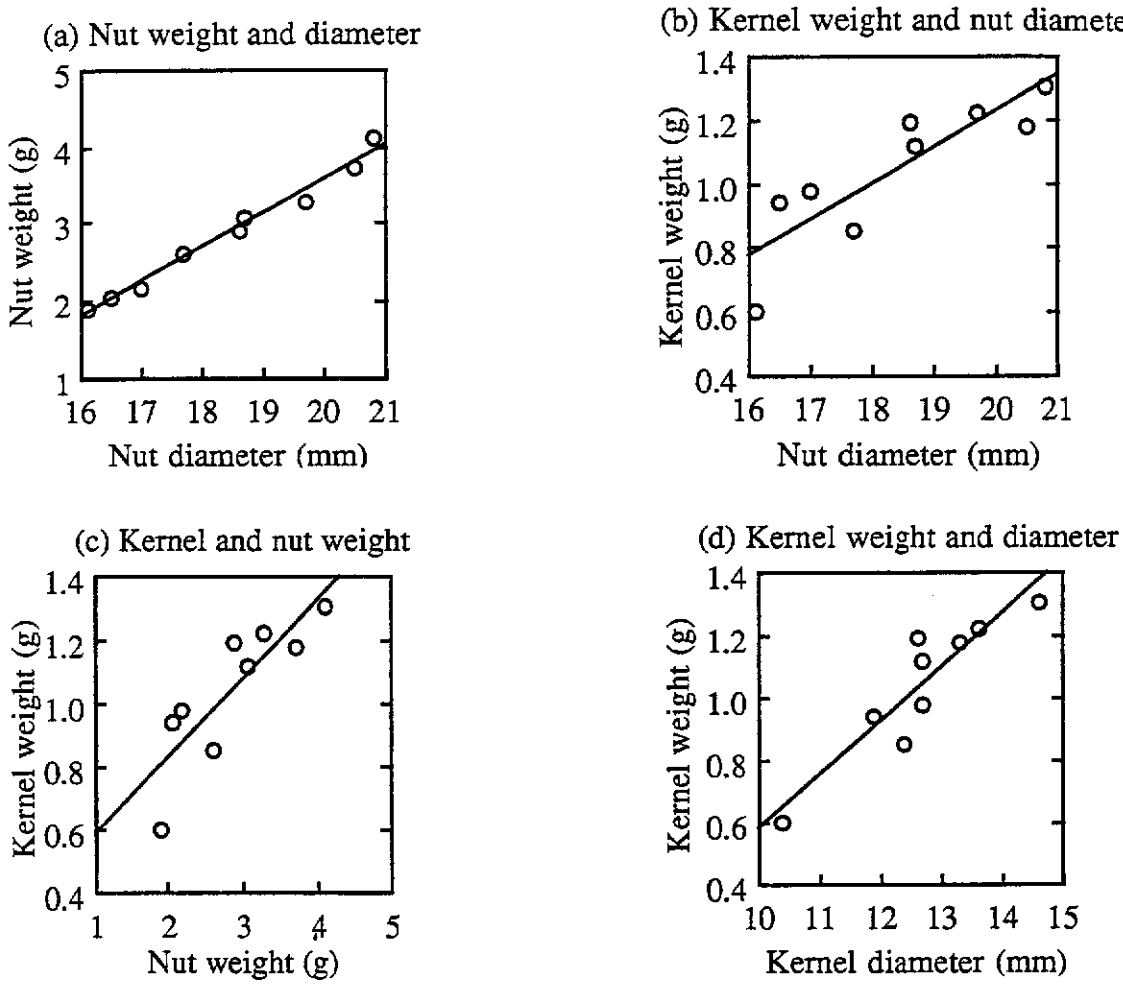

FIGURE 2. Nut and kernel correlations for ecotype mean dimensions

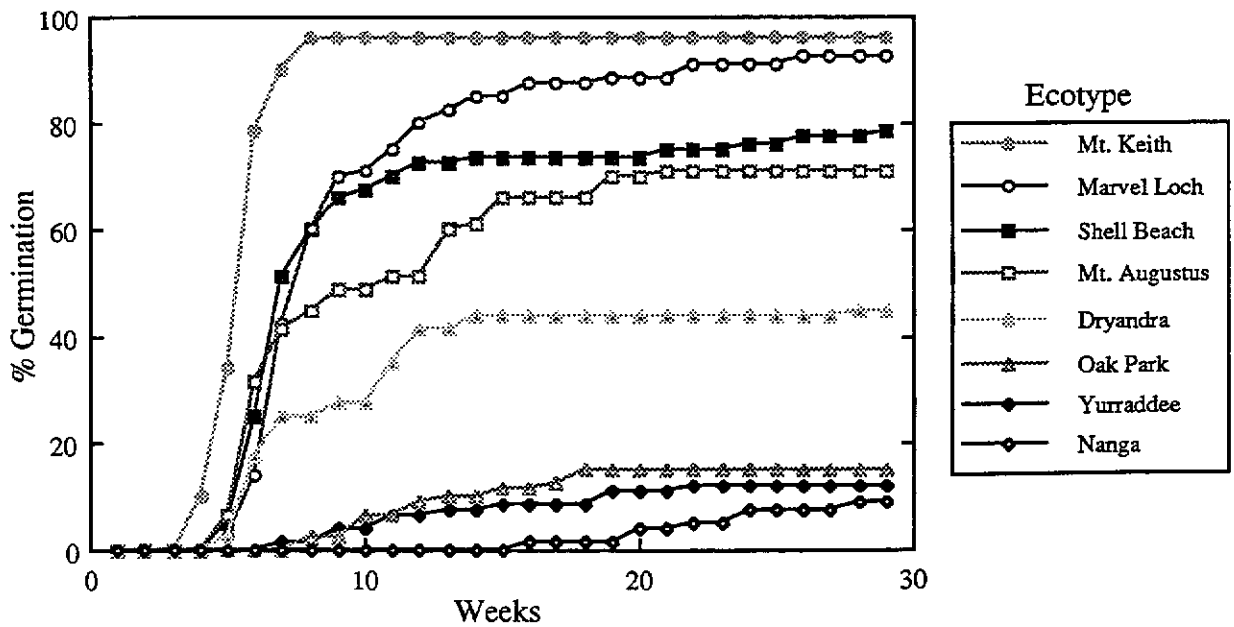

FIGURE 3. Percentage germination of sandalwood ecotypes over 14 weeks.

TABLE 3. Mean germination parameters for *Santalum spicatum* ecotypes.

Ecotype	Germination values assessed					
	Final	Days to germination of:			Germination	
	%	First	50%	Final	rate	value
Shell Beach	78	17	21	110	28.8	1.28
Nanga	9	58	-	114	81.0	0.01
Mt. Augustus	71	18	37	73	33.6	0.85
Mt. Keith	96	16	18	24	21.4	9.17
Yurradee	14	24	-	78	44.0	0.02
Oak Park	15	27	-	58	39.5	0.05
Marvel Loch	92	21	25	86	29.0	0.77
Dryandra	45	21	-	109	33.4	0.26

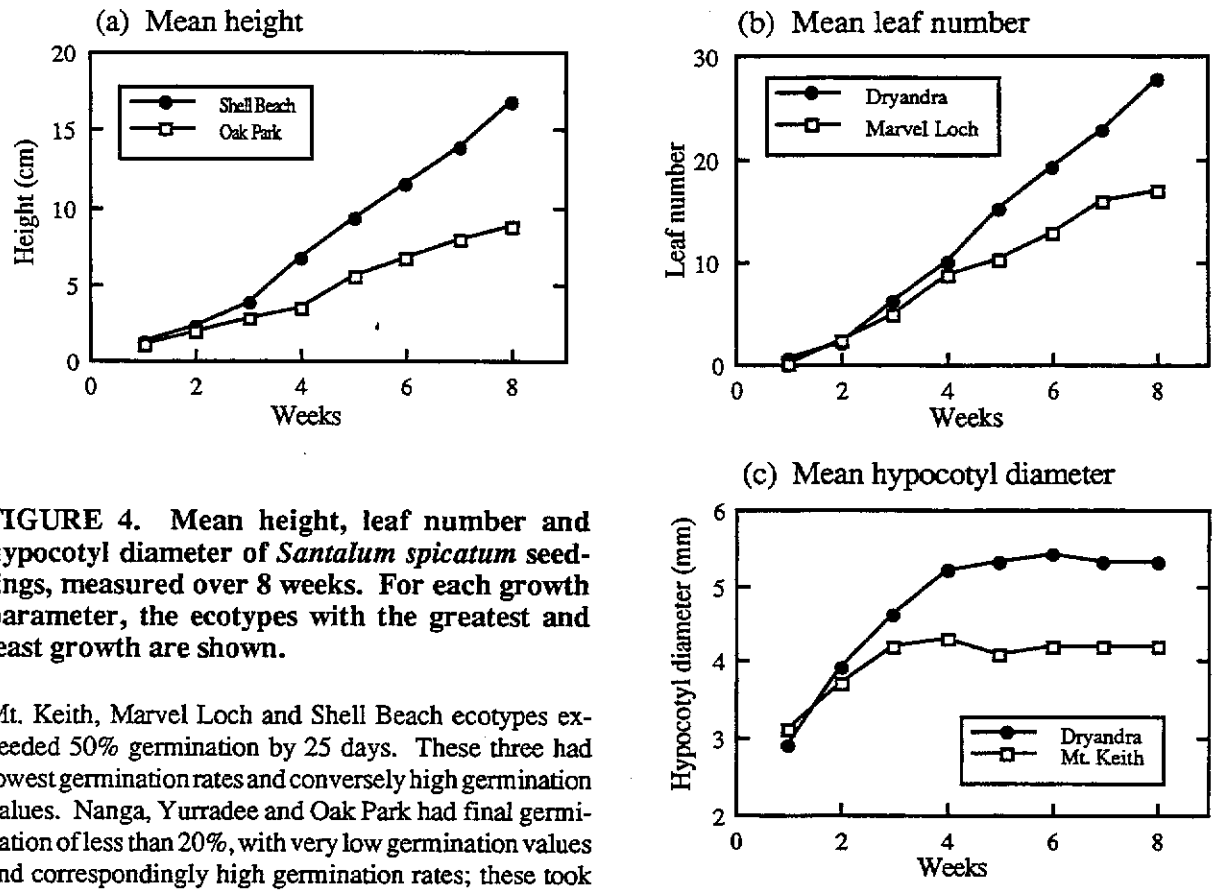

FIGURE 4. Mean height, leaf number and hypocotyl diameter of *Santalum spicatum* seedlings, measured over 8 weeks. For each growth parameter, the ecotypes with the greatest and least growth are shown.

Mt. Keith, Marvel Loch and Shell Beach ecotypes exceeded 50% germination by 25 days. These three had lowest germination rates and conversely high germination values. Nanga, Yurradee and Oak Park had final germination of less than 20%, with very low germination values and correspondingly high germination rates; these took longer to start. Correlation of germination parameters suggested those ecotypes with larger nuts took a longer time to germinate.

Correlation coefficients were as follows:

	Nut weight	Kernel weight
Days to first germination	+0.652	+0.539
Germination rate	+0.684	+0.632

when 0.707 required for $p<0.05$, and 0.622 for $p<0.10$.

Shell Beach seedlings grew most rapidly and were approximately twice the size of Oak Park seedlings at 4-8 weeks from germination (Figure 4). Dryandra and Marvel Loch ecotypes had similar leaf counts of around 10 at 4 weeks , but after 8 weeks Dryandra had increased to 27, while Marvel Loch had less than 20. Mean hypocotyl diameters of Dryandra and Mt. Keith at 4 weeks were 4 and 5 mm, respectively. Between 4-8 weeks hypocotyl diameters changed little.

At 60 days seedlings of ecotypes showed significant differences in each of mean height, leaf number, and hypocotyl diameter (Table 4). Shell Beach seedlings maintained the early lead; these and seedlings of Mt. Keith and Yurradee were significantly taller than Oak Park at 60 days. Dryandra and Mt. Augustus had significantly more leaves than other ecotypes. There was less difference in hypocotyl diameter range between ecotypes, however Dryandra, Shell Beach and Mt. Augustus seedlings had significantly thicker hypocotyls than those of Marvel Loch and Mt. Keith.

Seedling sets which germinated first tended to be taller at 60 days, evidenced by negative correlation between germination and height (Table 5). There was a tendency for seedlings of ecotypes with heavier kernels to be leafier and have thicker hypocotyls.

Foliage from adult trees of 5 different *Santalum spicatum* ecotypes differed significantly in means for each of leaf length, width, length/width ratio, area and dry weight (Table 6). There were no significant differences in leaf thickness between the ecotypes (F=1.86, p=0.172), which varied between 0.8 mm and 1.0 mm. Mt. Keith and Dryandra ecotypes had longer leaves than Marvel Loch, Oak Park and Nanga trees. The mean leaf widths of Dryandra, Nanga and Oak Park were broader than those of Mt. Keith and Marvel Loch. Mt. Keith and Marvel Loch had mean leaf length/width ratios significantly greater than the Nanga ecotype. The mean leaf areas from Dryandra and Oak Park trees were greater than Marvel Loch. Mean leaf dry weights of Dryandra and Oak Park were also heavier than Mt. Keith, Nanga and Marvel Loch.

Leaves from adult trees of the 5 different *Santalum spicatum* ecotypes sampled differed significantly in each of mean chlorophyll 'a', chlorophyll 'b', and total chlorophyll contents (Table 7). The ecotypes with highest chlorophyll 'a' contents were Dryandra, Oak Park and

TABLE 4. Mean values for seedling dimensions of *Santalum spicatum* ecotypes at 60 days. Same letters indicate ecotypes which are not significantly different (p<0.05) using the Scheffe test.

Ecotype / Analysis of variance	Height (mm)	Leaf number	Hypocotyl diameter(mm)
Nanga	12.0 bc	18.7 b	4.8 ab
Shell Beach	17.0 a	20.9 b	5.1 a
Dryandra	12.1 bc	29.5 a	5.3 a
Yurradee	14.2 ab	18.5 b	4.9 ab
Oak Park	8.8 c	23.5 ab	4.6 ab
Marvel Loch	12.7 bc	17.5 b	4.3 b
Mt. Augustus	11.9 bc	29.4 a	5.1 a
Mt. Keith	15.5 ab	17.8 b	4.2 b
F value =	3.31	6.04	2.77
p level =	0.001	0.001	0.014

TABLE 5. Correlation coefficients for 60 day seedling dimensions with nut and germination values (* = p<0.05; # = p<0.10).

Nut characteristic / germination	Height (mm)	Leaf number	Hypocotyl diameter(mm)
Nut weight	+ 0.032	- 0.095	+ 0.482
Kernel weight	- 0.335	+ 0.311	+ 0.704#
Kernel wt / nut wt	- 0.611	+ 0.757*	+ 0.379
1st germination	- 0.349	- 0.249	0.000
Germination rate	- 0.336	- 0.167	+ 0.167

TABLE 6. Mean values for leaf length, width, length/width ratio and area for mature plants of *Santalum spicatum*. Same letters indicate ecotypes which are not significantly different (p<0.05) using the Scheffe test.

Ecotype / Analysis of variance	Length (mm)	Width (mm)	Length / width	Area (cm^2)	Dry weight (g)
Dryandra	58.8 a	13.3 a	4.4 bc	5.2 a	0.15 a
Marvel Loch	51.9 b	9.9 b	5.6 ab	3.0 b	0.07 b
Mt. Keith	60.2 a	9.9 b	5.9 a	4.1 ab	0.08 b
Nanga	46.4 b	12.1 a	3.8 c	4.2 ab	0.08 b
Oak Park	49.9 b	11.9 a	4.4 bc	4.9 a	0.13 a
F value =	32.8	46.3	41.0	29.9	32.3
p level =	0.001	0.001	0.001	0.001	0.001

TABLE 7. Mean chlorophyll content (mg chlorophyll g^{-1}) for leaves from mature *Santalum spicatum*. Same letters indicate ecotypes which are not significantly different (p<0.05) using the Scheffe test.

Ecotype / Analysis of variance	Chlorophyll 'a'	Chlorophyll 'b'	Total chlorophyll
Dryandra	0.89 a	0.37 a	1.34 a
Marvel Loch	0.75 ab	0.28 bc	1.08 bc
Mt. Keith	0.63 b	0.23 c	0.90 c
Nanga	0.65 b	0.23 c	0.92 c
Oak Park	0.84 a	0.32 ab	1.21 ab
F value =	10.63	32.45	29.72
p level =	0.012	0.001	0.001

TABLE 8. Correlation coefficients for adult foliage values with latitude and longitudes of ecotype origin (= p<0.01; * = p<0.05).**

Adult leaf characters	Latitude	Longitude
Length	+ 0.228	+ 0.704
Width	+ 0.313	- 0.723
Length / width	0.000	+ 0.972**
Area	+ 0.232	- 0.405
Dry weight	+ 0.649	- 0.237
Chlorophyll 'a'	+ 0.926*	- 0.095
Chlorophyll 'b'	+ 0.915*	- 0.055
Total chlorophyll	+ 0.923*	- 0.077

Marvel Loch. Ecotypes with lowest chlorophyll 'a' contents were Nanga and Mt. Keith. Dryandra and Oak Park had the greatest mean chlorophyll 'b' contents and total chlorophyll. Mt. Keith and Nanga had the lowest chlorophyll 'b' contents and also total chlorophyll. In summary, the mean chlorophyll measurements for Dryandra and Oak Park ecotypes were significantly more than for Mt. Keith and Nanga ecotypes.

Overall, some of the differences observed between ecotypes may be associated with geographical location of the sources (Table 8). The most striking correlation, albeit on these few samples, was the tendency for ecotypes from higher latitudes (more southern locations) to have more chlorophyll in the leaves. There was also an indication that selections from the more inland areas had narrower leaves, exemplified by a highly significant correlation of mean leaf length/width ratio and longitude.

TABLE 9. Correlation coefficients for seedling dimensions at 60 days with adult foliage values (= p<0.01; * = p<0.05; # = p<0.10).**

Adult leaf characteristics	Seedling dimensions		
	Height (mm)	Leaf number	Hypocotyl diameter(mm)
Length	+ 0.613	+ 0.303	0.000
Width	- 0.545	+ 0.844#	+ 0.963**
Length / width	+ 0.663	- 0.463	- 0.744
Area	- 0.421	+ 0.834#	+ 0.723
Dry weight	- 0.542	+ 0.971**	+ 0.760
Total chlorophyll	- 0.574	+ 0.894*	+ 0.665

Examination of correlation coefficients between adult foliage characters and 60 day seedling dimensions for the 5 ecotypes for which both data sets were available (Table 9) suggests several associations between characteristics. These would appear to have no direct adaptive or ecological significance and may be most related to the growing conditions at Perth *vis a vis* the parent location. More data would be needed to confirm or refute this possibility.

DISCUSSION

Studies on plant species growing in their natural environments indicate that seed size is genetically determined and may be related to environmental factors, such as temperature, latitude or moisture (Baker, 1972; McWilliams, Landers & Mahlstede, 1968; Schimpf, 1977). The present study revealed significant variation in mean values for nut and kernel size (weight and diameter) between different *Santalum spicatum* ecotypes. Nut dimensions were larger from near coastal locations but smaller and lighter from inland locations. Baker (1972) related dry conditions at establishment being associated with larger seeds enabling seedlings to emerge from depth and to tap into available water prior to the onset of drought. Schimpf (1977) also found that seed weight was higher in populations of *Amaranthus retroflexus* growing in drier environments. These observations differ from the present findings. No significant correlation was found between average annual rainfall and nut and kernel size in *Santalum spicatum*. If, however, the Shark Bay ecotypes (Shell Beach and Nanga), with large nuts from a low rainfall coastal area, and the Marandoo set, with small nuts from the northern limit of the species distribution, are excluded then there was a consistent pattern of increased nut dimensions with increased rainfall. This suggests that moisture resources affect nut and kernel filling to limit the size of nuts over much of the range of *Santalum spicatum*. Effective rainfall in the period prior to collection of nuts examined was not available and it is possible that ecotypes from Shark Bay had experienced above average, and Marandoo below average, rainfall resulting in atypical nut and kernel dimensions. There are certainly indications of a positive relationship between higher rainfall in a particular year and nut yield in *Santalum spicatum* (Davies, 1976) and between higher mean annual rainfall and heavier mean seed weight in *Acacia harpophylla* (Coaldrake, 1971), and by extension, probably many tree species. The implication is that, under the particular rainfall regimes of the distribution of *Santalum spicatum* in Western Australia, nut dimensions in the higher, and more predictable rainfall zones, may be generally larger than in the inland and northern areas where rainfall may be less predictable and less effective.

McWilliams *et al.* (1968) studied populations of *Amaranthus retroflexus* with regard to seed weight and latitude. Seed weight decreased significantly from higher to lower latitudes in North America. This was interpreted as an adaptation for reaching mature size more rapidly where the growing season is shorter. In the case of *Santalum spicatum* in Western Australia, the effective growing season over the latitudinal range is moisture dependant. At higher latitudes winter rainfall is consistent but at lower latitudes summer rainfall may be less effective, and in the arid interior rainfall is less predictable. Nut dimensions in *Santalum spicatum* were more closely correlated with longitude than latitude, this may be temperature related but further analysis would be required to substantiate such a hypothesis. The occurrence of a higher kernel to nut weight ratio in the ecotypes from low latitudes suggests that this characteristic may be worthy of further attention. The edaphic conditions pertaining to sites of nut collection threw no light on variation in nut dimensions. However at Nanga the species occurs on deep red sands and it is possible that larger nuts there have evolved partly in response to soil moisture availability.

Significant correlation observed between nut and kernel dimensions is important for future breeding programmes

to develop *Santalum spicatum* as a nut crop species. Size grading would be more useful than weighing nuts as nut diameter appears to be the better predictor of kernel weight than is nut weight.

Santalum spicatum nut viability and germination time were highly variable between ecotypes. There was an earlier start to germination in batches of higher viability. The best batches attained 50% germination in a little over 3 weeks and of those tested the lightest (Mt. Keith) germinated most rapidly. Nuts were of different ages from fall when tested and were all incubated at the same temperature and moisture. Consequential questions of possible dormancy and varied development with temperature were not considered. Most of the variation must be attributable to seed quality and several general hypotheses remain to be considered in relation to germination. Is there for example a difference in temperature response with latitudinal origin? Seed of *Betula papyrifera* from higher latitudes have thinner pericarps which allow greater light transmittance to the embryo, present less mechanical resistance to radicle growth, and were considered adapted to germinate at lower temperatures (Bevington, 1986).

Differences in germination characteristics between plant populations have been related to initial starting capital, in particular higher viability and/or faster germination have been associated with larger seed size (Waller, 1985; Stickler & Wassom, 1963). In contrast the present study indicated a tendency for ecotypes with heavier fruit to require a longer time to germinate, a characteristic also, for example, of *Rumex*, in which smaller seeds germinate first (Maun & Cavers, 1971). Nuts of *Santalum spicatum* which were lighter in weight appeared more viable whereas in many species there is a higher percentage germination from larger seeds than smaller seeds (*e.g. Rumex crispus*, in Cideciyan & Malloch, 1982). There is scope for weighing out individual nuts of a batch to confirm whether heavier *Santalum spicatum* nuts require a longer time to emerge and if faster seedling development within a batch is associated with initial capital.

Growth differed significantly between ecotypes of *Santalum spicatum* in each characteristic assessed. At 60 days the tallest batch was twice as big as the shortest, earlier germinating ecotypes were taller. There was also considerable variation in leaf development among the ecotypes. Differences observed in initial seedling growth measurements of *Santalum spicatum* ecotypes are comparable with other studies (*e.g.* Turnbull, 1979). Larger seedlings with more leaves have strong advantages in terms of survival and later fruit production (Solbrig, 1981). A study of *Atriplex triangularis*, found that larger seeds and/or seeds which germinated earlier, produced larger plants (Ellison, 1987). In a comparative study Gross (1984) observed that plants from smaller seeds have the potential to grow faster than those from larger seeds, but this characteristic is dependent upon the microhabitat in which the seedlings grow. In competitive habitats with more plants present, seedlings from large seeds had a competitive advantage. In non-competitive cover types, such as on bare or recently disturbed soil, seedlings from smaller seeds with high growth rates had the advantage if they were able to outgrow seedlings with lower growth rates. In *Santalum spicatum*, despite the observed differences, correlations between nut characters and early growth were not clear, other than that larger hypocotyl diameter and greater leafiness were associated with heavier kernels. Nevertheless the differences in nut size and growth rate may be considered appropriate adaptations to particular environments. In arid inland Western Australia where vegetation is sparse, low cover might favour trees producing small nuts and seedlings with high growth rates. In the south-west of Western Australia with higher rainfall and more vegetation cover, large nuts may have an advantage when competition is important.

Foliage samples from adult trees of *Santalum spicatum* confirmed leaf size parameters of length, width, length/width ratio, area and dry weight all contributed to significant differences between ecotypes. It is of particular interest that ecotypes from the drier inland regions had higher leaf length/width ratios and smaller leaf areas than those from the milder, more coastal regions. Similar differences in leaf length/width ratio between regions of different average annual rainfall have been observed in *Eucalyptus cloeziana* (Turnbull, 1979). These may represent adaptations to reduction of water loss in dry environments, in that decreased exposed leaf surfaces reduce transpiration (Goble-Garratt, Bell & Loneragan, 1981). Sandalwood leaf size characteristics may be affected to a lesser extent by environmental pressures than other species as it is able to draw nourishment from the root systems of host plants during unfavourable conditions, such as drought (Loneragan, 1990).

All measures of leaf chlorophyll taken for parent trees of *Santalum spicatum* indicated significant differences between ecotypes. Sampled ecotypes from higher latitudes (Dryandra and Oak Park) had significantly higher values for each chlorophyll parameter than those from lower latitudes (Nanga and Mt. Keith). Winstead & Toman (1972) demonstrated that populations of *Acer negundo* from higher latitudes in North America contained higher chlorophyll levels than more southern populations. *Xanthium strumarium*, also shows a similar pattern (Abdulrahman & Winstead, 1977). The growing season for the higher, northern latitudes of North America is much shorter than the lower, southern latitudes. The increased chlorophyll content at higher latitudes may be an adaptation related to productivity, to compensate for the shorter growing season (Tieszen, 1970). *Santalum spicatum* ecotypes growing in the south of Western Australia receive most rainfall in winter where the shorter daylengths reduce photosynthesis and higher chlorophyll contents may compensate.

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PRELIMINARY OBSERVATIONS ON ECOTYPIC VARIATION IN *SANTALUM SPICATUM* 2. GENOTYPIC VARIATION

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SUMMARY

An account is given of a preliminary investigation into genetic diversity within *Santalum spicatum*. Sets of seedlings grown from nuts collected from 8 distinct populations from the species range were used in allozyme analysis. The aim was to examine genotypic variation to determine whether *Santalum spicatum* ecotypes are composed of different genotypes. Six enzyme systems were examined and the following three were used : Glucose-phosphate isomerase (Gpi), Glutamate-oxaloacetate transaminase (Got), and Menadione reductase (Mdr). There were genetic differences between *Santalum spicatum* ecotypes, and a geographical trend was observed. Genetic distance between sandalwood ecotypes increased linearly with increases in geographic distance. The most variable populations were those from Dryandra, Oak Park, Mt. Keith and Marvel Loch. Two populations from Shark Bay, although different in tree form and height, differed less than selections from the south-west of Western Australia. Here environmental factors may be more important.

An appreciation of the total gene pool for *Santalum spicatum* will assist in the possibility of selection from the available range of genotypes. An adequate set of populations would need to be preserved. This is considered of importance both for selection of trees with high quality wood (*i.e.* high oil content) and for production of high quality fruit (*i.e.* large kernels).

INTRODUCTION

Present day stocks of sandalwood (*Santalum spicatum*) are much reduced and there is considerable current interest in managing and replenishing stocks. There is also concern for the genetic resources of the species (Kealley, 1991). It is possible that some populations of sandalwood contain better sources of genes for oil production, early heartwood development, or prolific fruit. It is desirable that sufficient reserves be available for adequate conservation of the range of genetic material in this economically important species. Distinct stands appear to differ in phenotypic characters sufficiently that it seems appropriate to refer to them as ecotypes. Studies of genetic diversity require examination of both phenotypic and genotypic variation within and between populations (Schwaegerle, Garbutt & Bazzaz, 1986). Differences between individuals in survival and reproduction as the basis of genetically determined evolutionary change link phenotypic characters and fitness. Seed size and emergence may affect plant size, often the strongest determinant of fitness (Solbrig, 1981). Limited observations of phenotypic characters had suggested that whereas there may not be a great deal of variation within plants of sandalwood from the same general area, there did appear to be a considerable range in a variety of characteristics across the latitudinal, and through the climatic zones of the species distribution. It was hypothesized that such clinal variation could be associated with broad climatic factors coupled with discontinuous variation due to disjunct factors such as soil type (Burley, Hughes & Styles, 1986).

Gel electrophoresis can be used to investigate genotypic variation within a species. Active proteins, such as enzymes, are commonly examined for variation. Enzymes have multimolecular forms, called allozymes, which can be separated by relative migration rates. Variation in allozymes has a genetic basis and allozymes represent different alleles at the same enzyme locus (Turnbull, 1979). When only one allozyme occurs at a given enzyme locus it is termed homozygous; when two or more occur the locus is termed heterozygous. The amount of heterozygosity within a population can be used to measure the extent of genetic variation within a population. Populations with greater heterozygosity will be genetically more diverse (Richards, 1990). Allozyme analysis can also be used to determine genetic variation between populations (Nei, 1975). It was hypothesized that allozyme analysis would be useful in indicating the extent of genetic variation in *Santalum spicatum*. The work reported here sought to determine whether *Santalum spicatum* ecotypes were distinguishable on the basis of different genotypes and whether the geographical distance between populations affected their genetic distance.

METHODS

Seedlings were grown from nuts collected from 8 populations (Table 1) of *Santalum spicatum*, referred to as ecotypes (Fox & Brand, 1993). Germinated seedlings were raised in a potting mixture of coarse sand, fine sand and peat, with a seedling of *Medicago sativa*, as a host, planted with each *Santalum spicatum*. Pots were carefully labelled and seedlings were grown for 8 weeks when two juvenile leaves, of 5 - 10 mm in length, were collected

TABLE 1. Sources of ecotypes sampled.

Ecotype location	Latitude and longitude	Soil type	Rainfall (mm)
Mt. Augustus	24° 19', 116° 55'	red earth	213
Shell Beach	26° 12', 113° 46'	shells, sand	225
Nanga	26° 15', 113° 49'	red sand	225
Yurradee	28° 08', 114° 42'	sandy loam	489
Mt. Keith	27° 11', 120° 33'	clay loam	206
Oak Park	31° 18', 116° 51'	sandy loam	340
Marvel Loch	31° 30', 119° 32'	sandy loam	265
Dryandra	32° 50', 117° 06'	loam	526

from 7-12 seedlings from the 8 different populations. The leaf tissue was then ground in 0.4 ml of a grinding buffer composed of 50 mg ml^{-1} polyvinyl-pyrrolidone (PVP), 0.8 mM NAD, 0.4 mM NADP, 1 mM Na_2EDTA, 1 mM ascorbic acid, 0.1 % w/v bovine serum albumin (BSA), with 10 % w/v sucrose in deionised water and equilibrated to pH 7.0 with 0.5 M Tris. After equilibration 1.5 mg ml^{-1} dithiothreitol was added. The ground leaves were then transferred to sample cells and directly loaded onto plates using the applicator. Before loading, the plates were soaked in running buffer for 10 minutes. The buffer consisted of 80 mM Tris, 1 mM Na_2EDTA, 5.7 mM maleic acid, 1 mM $MgCl_2$ and 8 mM citric acid with a pH of 8.6. After loading the plates were placed face down in the tank with ends resting on blotting paper. The plates were electrophoresed for 40 minutes at 20 mA and 5°C (Egerton-Warburton, 1990).

The plates were stained with 6 different enzyme systems: Alcohol dehydrogenase (Adh), Glutamate dehydrogenase (Gdh), Glucose-phosphate isomerase (Gpi), Glutamate-oxaloacetate transaminase (Got), Menadione reductase (Mdr) and Phosphoglucomutase (Pgm). Gpi, Got and Mdr were found to be more active with *Santalum spicatum* than the other three and were therefore used to examine allozyme variation. Chemical compositions of the active enzyme systems were as follows :

(a). Glutamate-oxalacetate transaminase (Got, EC 2.6.1.1)
4 ml 0.1 M Tris pH 8.0, 10 mg pyridoxial-5-phosphate, 0.4 ml α-ketoglutarate (50 mg ml^{-1}) pH 8.0, 0.4 ml L-aspartate (50 mg ml^{-1}) pH 8.0, 12 mg Fast Garnet (GBC).

(b). Glucose-phosphate isomerase (Gpi, 5.3.1.9)
4 ml 0.1 M Tris pH 8.0, 10 mg fructose-6-phosphate, 0.4 ml NADP, 0.4 ml $MgCl_2$, 0.2 ml MTT, 0.2 ml PMS, 4 i.u. glucose-6-phosphate dehydrogenase.

(c). Menadione reductase (Mdr, EC 1.6.99.22)
4 ml 0.1 M Tris pH 7.0, 8 mg menadione, 5 mg NADH, 0.4 ml MTT.

Genotypic variation was determined using allele frequency data obtained from allozyme analysis. Three different measures of genetic variation within a population were employed :

(a) Average expected heterozygosity. This measure (H_e) is an estimation of the mean heterozygosity of individual loci (h). It is equal to the average probability of two genes chosen at random being identical,
where $H_e = \Sigma h_I / r$ for r loci,
$h_i = 1 - \Sigma x_i^2$ for the I-th locus and x_i is the frequency of the i-th allele.

(b) Effective number of alleles. This function (n_e) is the inverse of homozygosity, averaged over individual loci, where $n_e = 1 / \Sigma x_i^2$, x_i is the frequency of the i-th allele.

(c) Square-root sum. This measure (Sq Σ) is averaged over loci by the arithmetic mean value and is useful for indicating populations with a greater number of alleles, where $Sq\,\Sigma = \Sigma\sqrt{x_i} - 1$, x_i is the frequency of the i-th allele. (After Turnbull, 1979).

To estimate variation between populations, gene frequencies were used to determine Nei's (1972) genetic distance (D) between populations. This measure is an estimate of the accumulative number of gene substitutions per locus between two populations.

$D = - \log_e I$ where,
$I = J_{xy} / \sqrt{J_x J_y}$, between populations X and Y
J_x = mean over all loci of $j_x = \Sigma x_i^2$ in population X
J_y = mean over all loci of $j_y = \Sigma y_i^2$ in population Y
J_{xy} = mean over all loci of $j_{xy} = \Sigma x_i y_i$ in populations X and Y
x_i = the frequency of the i-th allele in population X
y_i = the frequency of the i-th allele in population Y

The genetic distances (D) between populations were subjected to hierarchical cluster analysis. The populations with the highest similarity (least genetic distance) were merged, forming nested classes (or clusters). This process

was repeated until all populations belonged to a single cluster. A dendrogram of the hierarchical classes was then used to represent the genetic relationship among populations.

The proportion of genetic diversity (G_{ST}) due to the among-population component (Nei, 1975) was calculated as follows:

$$G_{ST} = D_{ST}/H_T$$

where, $D_{ST} = H_T - H_S$

$H_T = 1 - \Sigma pi^2$, pi^2 is the mean frequency of the i-th allele

$H_S = 1 - \Sigma Pi^2$, Pi^2 is the frequency of the i-th allele

RESULTS

Variation within populations

The allele frequencies at 3 polymorphic enzyme loci are presented in Table 2. Variability was found in all populations, at all 3 loci except for Mdr-1 which was monomorphic in the Shell Beach and Nanga ecotypes. These allele or allozyme frequencies were used to determine H_e, n_e and Sq Σ (Table 3). The average expected heterozygosity of individual populations (H_e) is equal to the probability of two genes chosen at random being identical (Nei, 1975). This function ranged from 0.333 to 0.543, with the most variable populations being Dryandra (0.543), Oak Park (0.534), Mt. Keith (0.526) and Marvel Loch (0.524). The average expected heterozygosity of the south-west ecotypes were also much higher than Shell Beach (0.333) and Nanga (0.357). This indicates that the south-west ecotypes were more variable than those from Shark Bay.

The effective number of alleles function (n_e), increases proportionally with an increase in the number of alleles, at a given locus (Turnbull, 1979). Since the number of alleles at each locus did not vary much between populations (Table 2), this diversity measure was similar to average expected heterozygosity. The effective number of alleles at Dryandra, Oak Park, Mt. Keith, Mt. Augustus and Marvel Loch, ranged from 2.153 to 2.278. While the n_e values from Shell Beach, Nanga and Yurradee ranged from 1.667 to 1.931.

The square root sum (Sq Σ) is a measure similar to the effective number of alleles, but it gives more weight to alleles present at low frequencies (Turnbull, 1979). The highest values came from Dryandra (0.515), Oak Park (0.511), and Mt. Keith (0.509), while Shell Beach (0.276) and Nanga (0.363) had the lowest (Table 3). These results provide more evidence that the south-west ecotypes were more diverse than the Shark Bay ecotypes.

Variation between populations

Allele frequencies (Table 2), were also used to determine the genetic distance (D), a measure of variation between populations (Table 4). The 8 ecotypes were arranged into 3 sets, according to their geographic location. Ecotypes from Shell Beach and Nanga formed the Shark Bay set. Mt. Augustus, Yurradee and Mt. Keith were grouped into the central-west set. Oak Park, Marvel Loch and Dryandra ecotypes, formed the south-west set. The genetic distance between ecotypes from Shark Bay, central-west and the south-west were very high. For example the genetic distance between Shark Bay and the south-west ecotypes ranged from 0.112 to 0.355. Genetic distances within each set were relatively low. Within the Shark Bay set, the genetic distance between Nanga and Shell Beach was only 0.029. In the central-west set, genetic distances ranged from 0.006 to 0.077 and in the south-west set they ranged from 0.010 to 0.062. To illustrate the genetic relationships between each of the ecotypes a single linkage cluster analysis was performed (Figure 1). The cluster analysis showed that the south-west ecotypes were more closely related to the central-west, compared to Shark Bay. These results provide evidence that the genetic distance (D) was related to the geographic distance.

TABLE 2. Allele frequencies for 3 polymorphic loci in 8 populations of *Santalum spicatum* and number of seedlings sampled (N) from each population.

		Got - 1			Mdr - 1		Gpi - 1	
Ecotype	N	1	2	3	1	2	1	2
1. Shell Beach	12	0.00	0.50	0.50	1.00	0.00	0.50	0.50
2. Nanga	7	0.17	0.58	0.25	1.00	0.00	0.50	0.50
3. Mt. Augustus	12	0.36	0.36	0.28	0.79	0.21	0.50	0.50
4. Yurradee	12	0.00	0.50	0.50	0.67	0.33	0.50	0.50
5. Mt. Keith	12	0.34	0.32	0.34	0.71	0.29	0.50	0.50
6. Oak Park	12	0.25	0.33	0.42	0.62	0.38	0.40	0.60
7. Marvel Loch	12	0.23	0.31	0.46	0.33	0.67	0.43	0.57
8. Dryandra	12	0.28	0.36	0.36	0.37	0.63	0.50	0.50

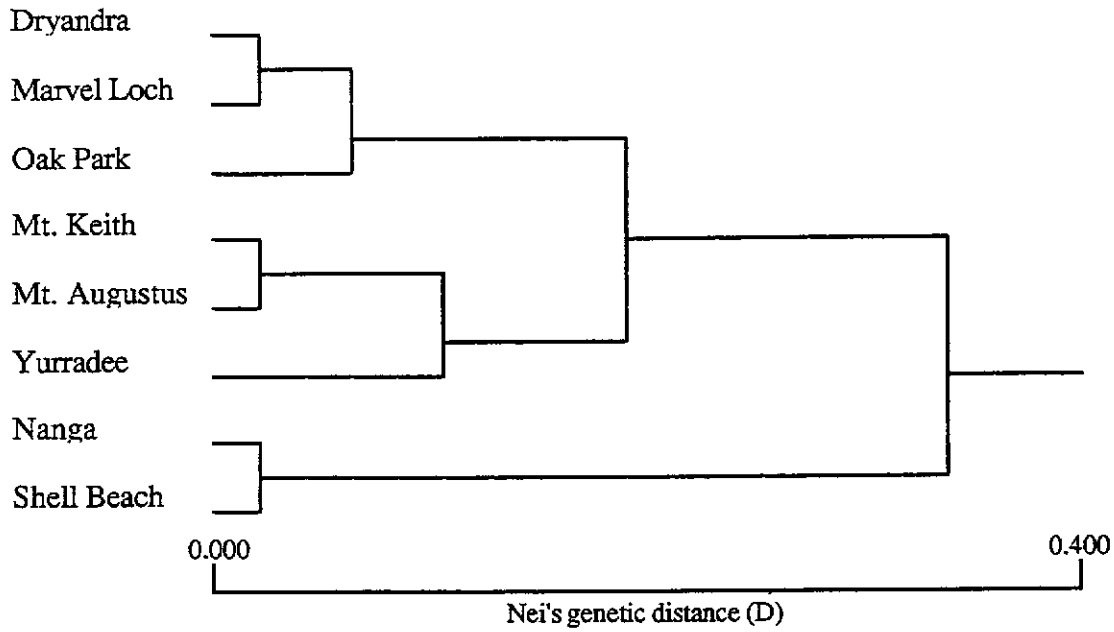

FIGURE 1. Clustering of the *Santalum spicatum* ecotypes, based on Nei's (1972) genetic distance.

TABLE 3. Gene diversity functions within *Santalum spicatum* ecotypes.

Ecotype	Average exp. heterozygosity (H_e)	Effective no. of alleles (n_e)	Square root sum (Sq Σ)
1. Shell Beach	0.333	1.667	0.276
2. Nanga	0.357	1.779	0.363
3. Mt. Augustus	0.498	2.153	0.497
4. Yurradee	0.481	1.931	0.407
5. Mt. Keith	0.526	2.233	0.509
6. Oak Park	0.534	2.230	0.511
7. Marvel Loch	0.524	2.176	0.506
8. Dryandra	0.543	2.278	0.515
Mean	0.474	2.056	0.448
Std. Dev.	0.083	0.232	0.090

TABLE 4. Matrix of genetic distance (Nei, 1972) for the 8 *Santalum spicatum* ecotypes.

Ecotype		Shark Bay		Central-west			South-west		
		1	2	3	4	5	6	7	8
Shark	1	-							
Bay	2	.029	-						
Central-	3	.075	.060	-					
west	4	.055	.093	.077	-				
	5	.094	.095	.006	.064	-			
South-	6	.112	.138	.039	.042	.019	-		
west	7	.330	.355	.180	.117	.122	.062	-	
	8	.297	.302	.135	.105	.089	.057	.010	-

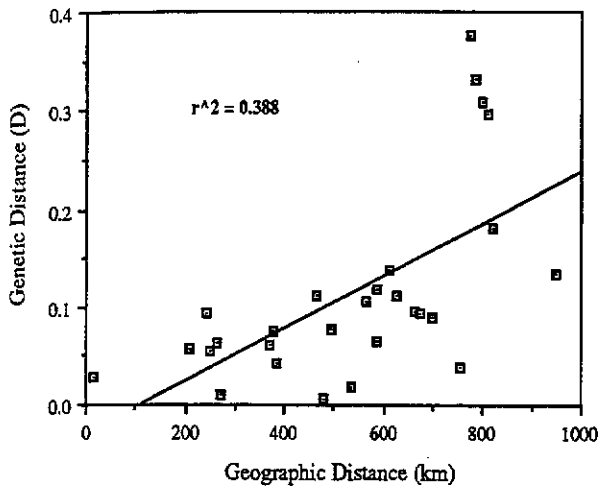

FIGURE 2. Correlation between genetic distance (D) and geographic distance between the *Santalum spicatum* ecotypes.

TABLE 5. Genetic diversity among *Santalum spicatum* ecotypes.

Locus	H_T	H_S	G_{ST}
Got-1	0.643	0.609	0.056
Mdr-1	0.443	0.338	0.311
Gpi-1	0.499	0.496	0.006
Mean	0.528	0.481	0.124

Where H_T = total genetic diversity
H_S = mean genetic diversity within populations
G_{ST} = proportion of gene diversity among populations

To determine whether genetic distance (D) variation between *Santalum spicatum* ecotypes had a geographic pattern, a regression analysis was used (Figure 2). A significant correlation ($F=16.49$; $p=0.001$) was found and the relationship had a correlation coefficient of 0.388. This indicates that an increase in geographic distance between *Santalum spicatum* ecotypes would result in a linear increase in genetic distance (D). However, in this relationship there were four major outliers, plotted at the top right hand corner of Figure 2. The outlying points were genetic and geographic distances between ecotypes from Shark Bay and the south-west. The genetic distances between these points ranged from 0.297 to 0.355, and the geographical distances were all about 800 km. If these points were to be excluded, the regression line would be lowered and the correlation coefficient would increase.

Genetic variation within and between *Santalum spicatum* ecotypes was compared using genetic diversity measures (Table 5). The genetic variation between ecotypes was 0.124, however the genetic variation within ecotypes was 0.481. Therefore more genetic variation occurred within rather than between ecotypes.

DISCUSSION

Within Populations

The measures of genetic diversity within populations (average expected heterozygosity, effective number of alleles and the square-root sum) all revealed a high variation within *Santalum spicatum* ecotypes. The most variable ecotypes came from Dryandra and Oak Park, both in the south-west set. Ecotypes with lowest variability were Shell Beach and Nanga, comprising the Shark Bay set. Turnbull (1979) observed differences in the amount of diversity within populations and suggested that climatic factors may have been responsible for differences in genetic variability within populations of *Eucalyptus cloeziana* in Queensland. For example a minor decrease or increase in moisture availability to *E. cloeziana* populations growing at the extremity of the species range could make its environment either intolerable or provide an opportunity for colonisation. Variable selection pressures may therefore have resulted in these populations being more diverse.

Genetic diversity within *Santalum spicatum* ecotypes was higher than between ecotypes. Moran & Hopper (1987) also observed higher genetic variations within compared to between populations of *Eucalyptus* species. It was concluded that a high percentage of a species total gene pool can be represented within a single population. It is possible that genetic diversity within *Santalum spicatum* ecotypes could be just as important as between ecotypes in determining the species total gene pool. However only three loci were examined which may have biased the assessment of genetic diversity measures. Nei (1975) indicated that where the number of loci used is small, the bias of the estimate of average heterozygosity becomes large. A more accurate estimate of variation within *Santalum spicatum* ecotypes would have been obtained if data from more loci had been available.

Between Populations

There were significant differences in the genetic distance (D) between populations indicating genetic differences

between *Santalum spicatum* ecotypes. Genotypic variability between populations of a species has been found to be associated with 5 principal factors: selection, mutation, migration, drift and non-random mating (Stanley, 1979). Selection is the non-random reproduction of genotypes. The individuals which possess characteristics which enable them to survive and produce more offspring in their environment will take over successive populations. These successful characteristics will appear in more and more individuals and the nature of the population will gradually change (Moore, 1983; Endler, 1986). Mutation of alleles can change the proportions of particular alleles in a population, however mutation rates are generally low, therefore population change is very slow. Migration is defined as the movement of individuals from one population to another. The movement of alleles that accompany such migration is called gene flow. Experimental studies have shown that limited gene flow will cause genetic differentiation between populations and also within a population (Hamrick & Allard, 1972; Schaal, 1975; Levin & Kerster, 1974). Genetic drift is known as the random loss of alleles from a population. The effects of genetic drift on population differentiation may be of particular importance when populations are founded by a small number of individuals (Bryant, 1981). Non-random mating causes the frequency of a particular genotype to differ greatly from the expected. In plants, non-random mating can occur from self-fertilization. If a plant population is self-fertilized and consists mainly of homozygotes, there will be less heterozygotes than expected in the next generation (Stanley, 1979).

There were large genetic differences between *Santalum spicatum* ecotypes from the three designated groups: Shark Bay, the south-west and the central-west. The genetic distance between ecotypes increased linearly with increased geographic distance. The relationship between genetic distance and geographic distance indicates that genetic drift may have been a major cause for the high genetic diversity between populations (Sytsma & Schaal, 1985). Selection would also have been important as populations occur on a range of soil types and in regions of differing climatic conditions. Turnbull (1979) also observed a similar relationship between genetic distance and geographic distance in *Eucalyptus cloeziana*. There were marked differences between populations of this species growing in southern regions compared to northern regions of Queensland. Coates & Sokolowski (1989) found that the genetic distance between populations of karri (*Eucalyptus diversicolor*), growing in the south-west of Western Australia was not related to geographic distance. Karron (1987) examined such relationships between geographic and genetic distance in a number of widespread and restricted distribution plant species. Species of restricted distribution displayed lower geographic patterns than widespread species. Since *Santalum spicatum* is distributed over a broad range in Western Australia, it would be expected to show geographic trends, similar to that found in this investigation.

Interestingly the genetic distance between Shell Beach and Nanga was very low and variable selection pressures may not yet have affected genetic make-up in these two Shark Bay populations. These two populations appear dramatically different in terms of tree height and form. Trees at Shell Beach are much shorter than those at Nanga. Low genetic distance suggests the difference in tree height is due more to environmental than genetic factors. Possible environmental influences include exposure to salt wind, soil differences or tree age. Firstly, trees at Shell Beach are more exposed to strong salt winds which may have had a pruning effect on the branches. Secondly, the soil at Nanga is composed of red sand, while at Shell Beach it consists mainly of ancient shell deposits which may reflect nutrient deficient soil, with increased salt levels. These environmental factors have been shown to limit plant growth (Meyer *et al.*, 1973). Another explanation for differences in tree size could be that the Shell Beach trees were much younger than those at Nanga. Older trees at Shell Beach may have been previously harvested or cleared. At present there is no suitable method of estimating sandalwood age (Loneragan, 1990). To determine whether environmental factors are responsible for differences in tree size and shape it would be necessary to compare these two ecotypes under the same environmental conditions. Further allozyme analysis may also help to confirm that the two ecotypes are genetically similar and are basically one population.

The observed phenotypic (Fox & Brand, 1993) and genotypic variation between *Santalum spicatum* ecotypes provides evidence that ecotypes examined were composed of different genotypes. If the total gene pool of *Santalum spicatum* is to be determined, a broad range of ecotypes would need to be examined and catalogued. Through cataloguing the different genotypes within a species, an appreciation of its genetic pool can be derived, allowing the possibility for it to be conserved for the future (Brown, 1978). This genetic pool can also be used as a reservoir from which to artificially select superior genotypes, for particular traits (Adams, 1983). This information could be used to identify different sandalwood ecotypes which need to be conserved, to prevent loss of genetic diversity. A knowledge of the total gene pool may assist in the possibility of selecting trees with superior genotypes, such as high quality wood or larger kernels. If sandalwood is to be cultivated for its nut crop, it would be advantageous to obtain trees which produce large kernels.

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SANDALWOOD NUTS AS FOOD

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SUMMARY

Kernels of the fruit of *Santalum spicatum* can be used in cooking as a nutritious and acceptable substitute for more traditional nut ingredients such as peanuts. Kernels can also be dry roasted and made acceptable as a snack food. Further studies on the preparation of the nut should be aimed at improving their subtle flavour. The effects of consuming sandalwood nuts on human metabolism are not known but it is important that this issue is resolved before the nuts are marketed.

INTRODUCTION

Sandalwood (*Santalum spicatum*) has been a tree of commerce in Western Australia for nearly 150 years. The wood contains aromatic oils used in perfumery and also in religious observances. The tree is slow growing, taking perhaps 50-90 years to reach a harvestable size. Regeneration in nature is slow and erratic, with much of the new growth that does occur being eaten by herbivores. The range of sandalwood has diminished and the number of trees has been greatly reduced. This is causing concern amongst those who regard sandalwood as a potentially renewable resource yielding a valuable source of timber, and amongst those who wish to see a natural resource preserved.

To redress the decline of sandalwood active regeneration programmes must be supported. It is necessarily a long term venture to grow sandalwood as a crop. Agriculturalists who initiate and finance planting programmes may see no direct benefits from the sale of wood in their lifetimes. However, the presence of trees on a property will increase the value of the land asset and regeneration programmes will be seen as desirable if other benefits ensue.

The sandalwood nut has long been a minor source of food to people living and working in the bush. The hard-shelled nut is usually cracked between rocks, with a hammer or pliers. The kernel is eaten plain or more rarely, toasted on an open fire.

Sandalwood trees under cultivation on the Curtin University campus have commenced flowering and fruiting at four years of age. If the nut could be developed as a viable food crop, comparable perhaps with macadamias, this would provide continuing cash benefits for growers down the years until the trees become ready for harvesting.

This study on the palatability and nutritional value of sandalwood nuts is part of a larger investigation which includes quantifying fruit production and examining the biological basis of fruit set. Two aspects of the use of the nut have been considered. The nut was used on its own as a snack food, and also as an ingredient in prepared food. A partial analysis of nutrient content is also reported.

MATERIALS AND METHODS

Sandalwood fruits were obtained from trees growing near Narrogin, Western Australia. They had an outer dry, fibrous covering (exocarp) which was removed by hand, and a hard inner shell (endocarp) which had to be removed by cracking to reveal the kernel (endosperm and embryo). Kernels were halved or chopped for all tests.

Sandalwood kernels were analysed for moisture (drying in a laboratory drying oven at 105°C until constant weight); protein (semi-micro Keldahl analysis using conversion factor 6.25); fat (Soxhlet extraction by diethyl ether); calcium, sodium and potassium (EEL flame photometric determination from ash). Ash was obtained by heating overnight at 500°C in a muffle furnace; kilojoule content (Baird and Tatlock adiabatic bomb calorimeter); and carbohydrate, including fibre (calculated by difference). Lees (1971) provides descriptions of the methods of analysis used.

Three hundred and seventy five grams of unshelled nuts gave 85 g of kernels.

In snack food studies kernels were prepared in one of three ways:

Untreated - eaten raw.
Deep pan-fried in Copha.
Baking by spreading on an oven tray (greased with Copha), and heating at 120°C for 15, 25 or 35 minutes.

Copha is a solidified coconut oil used as a shortening in cooking.

When used as an ingredient in a prepared food, sandalwood kernels were substituted for peanuts in a well-tried recipe with proven acceptability known as Deane's Muesli and Apricot Bar (Appendix 1). A number of modifications to the original recipe were tested: the quantity of apricots was reduced, the amount of liquid was increased,

and different quantities of sandalwood nuts were added. The products eventually evaluated contained either half a cup (125 ml) of peanuts (original recipe, used as a control), or chopped, lightly roasted sandalwood nuts (125 ml or 250 ml).

Product evaluation was conducted on the snacks and the muesli bar to assess community acceptability and potential markets for the sandalwood products. Accidental quota sampling (that is, non-random sampling) was the basis of data collection.

Snack evaluation was conducted at Perth Domestic Airport. Forty men and forty women comprised the sample interviewed. Small unlabelled packages of two samples (macadamia, prepared and obtained commercially, and dry-roasted sandalwood nuts), were given to respondents to allow them to consider flavour. Each person was asked to answer 14 questions relating to texture, flavour and acceptability contained in a structured questionnaire, (Edmiston, 1988). Respondents were also asked to compare sandalwood nuts with a number of other commercial nuts (not supplied) on a five point labelled category scale.

Muesli bar evaluation was conducted at Curtin University. There were 104 respondents with a mean age of 23 years. Seven questions were asked concerning the sensory characteristics of the three muesli bars using an undifferentiated bipolar scaling system. Samples of muesli slice containing either half a cup peanuts, half a cup sandalwood kernels or one cup of sandalwood kernels, were coded and randomly presented to lessen the likelihood of response bias. The results were analysed by analysis of variance.

RESULTS

Composition of Nuts

The nutritive value of sandalwood nuts compared with other nuts is given in Table 1. Sandalwood kernels are similar in composition to other nuts in common domestic use. They have high levels of fat (61%) and are rich in protein (18%).

Taste and Flavour of Nut Preparations

Snack Nuts

Initial preparation trials were evaluated by a small group of interested respondents.

Raw Nut

The taste was bland and left a chewy aftermass in the mouth.

Deep Oil Fried

The nut retained a chewy aftermass and a bland flavour and became slightly browned.

TABLE 1. Sandalwood composition (per 100 g) in comparison to other nuts*.

Type of nut	Moisture (g)	Protein (g)	Fat (g)	Ash (g)	Carbo-hydrate (g)	Calcium (mg)	Potassium (mg)	Sodium (mg)	Kilojoules
Sandalwood	3.5	17.7	60.7	1.7	16.4	36	309	10	2945
Walnut	4.1	14.8	63.7	-	14.9	84	491	3	2713
Pinenut	4.3	22.0	53.9	-	16.0	12	-	-	2491
Peanut	5.4	26.5	47.9	-	17.5	57	700	4	2374
Brazil	6.6	14.0	64.5	-	11.1	181	697	1	2663
Almond	4.8	19.5	53.8	-	18.9	245	773	4	2504
Macadamia**	1.9	9.0	70.5	1.5	17.2	-	-	-	-

* Data for other nuts taken from Thomas and Corden (1977) and departmental research analyses, Home and Consumer Studies, Curtin University.

** Macadamia results are not strictly comparable as the nuts were commercially prepared by frying in fat.

Roasted

At 120°C for 15 minutes: The nut became crisper but still had a chewy aftermass and bland flavour.

At 120°C for 25 minutes: The kernel was even crisper with very little chewy aftermass, a more distinct flavour and a slightly browned appearance.

At 120°C for 35 minutes: The nuts became crisp with no chewiness. They were a little dry with a slightly burnt flavour and a definite browned surface.

On the basis of this evaluation it was decided that chopped or halved kernels, roasted at 120°C for 25 minutes gave the best overall result. This preparation was used in acceptability trials.

Macadamia nuts were classed similarly to peanuts and almonds. Cashew nuts were considered to have the best flavour. The flavour pleasantness of sandalwood kernels as a snack food was rated lower than macadamia nuts (Figure 1). Nonetheless 23% of the respondents thought that sandalwood kernels had a good to very good flavour and 42% rated the flavour average or better (Table 2).

Respondents suggested that the rather subtle flavour of the sandalwood nuts would be improved by the addition of spices or by smoking. It was considered that a price slightly higher than that for peanuts would be appropriate and that the price would play a large part in how the nut was consumed. Most people thought that sandalwood kernels should be used for cooking and for snacks (Table 3), and that their value was in their nutritive content rather than their flavour.

Muesli Bars

The muesli bars, whether containing peanuts or different quantities of sandalwood nuts, were all found to be equally acceptable (95% confidence limit) to respondents in terms of flavour pleasantness, nuttiness, eating texture and overall acceptability (Figure 2). Respondents to the questionnaire suggested the muesli bar would be suitable for morning or afternoon tea, as a snack health bar, or for school lunches.

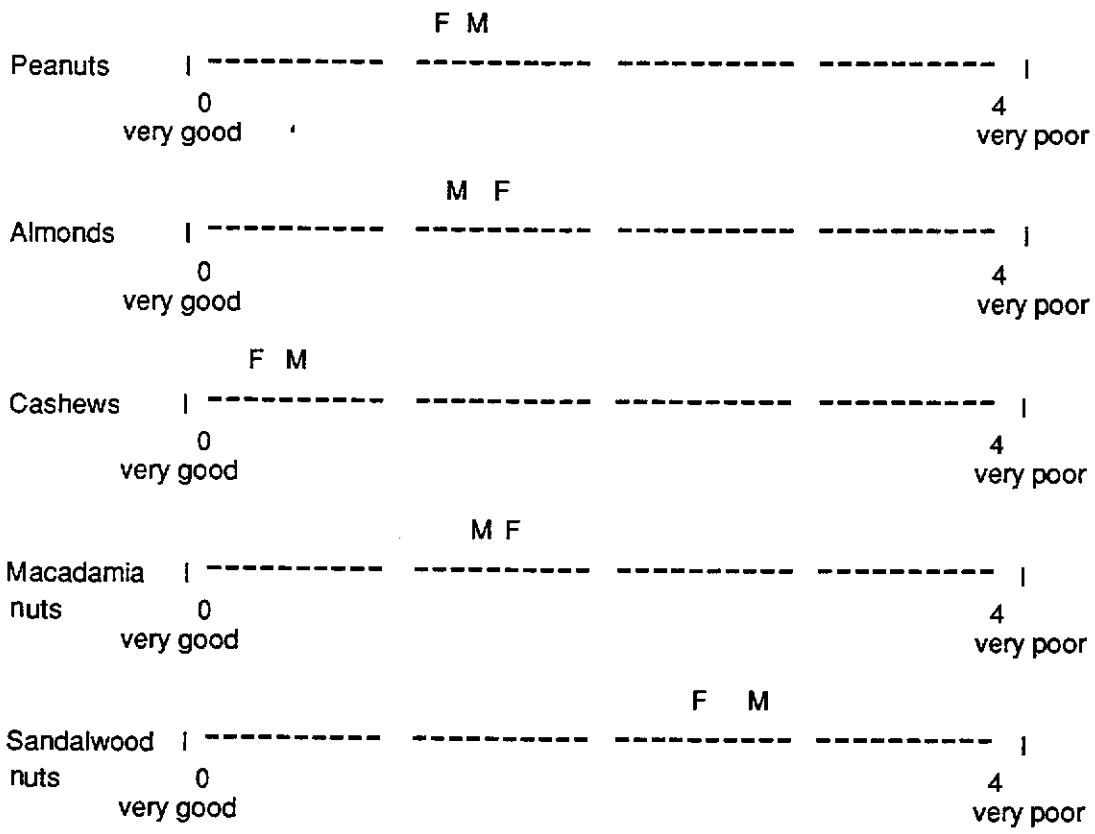

FIGURE 1. Profiles showing the mean flavour pleasantness of sandalwood nuts compared with other commonly used nuts, as rated by male (M) and female (F) respondents.

TABLE 2. Comparisons of flavour pleasantness given as percentages of male (M) and female (F) respondents.

Type of nut	Respondent	Rating					
		Very Good	Good	Average	Poor	Very Poor	Weighted (mean)
Peanut	M	15	55	27	0	3	1.2
	F	20	53	27	0	0	1.1
Almond	M	27	48	18	7	0	1.1
	F	33	37	20	8	2	1.1
Cashew	M	62	15	18	5	0	0.7
	F	75	18	5	0	2	0.4
Macadamia	M	33	25	30	5	7	1.3
	F	22	45	15	10	8	1.4
Sandalwood	M	5	15	17.5	40	22.5	2.6
	F	3	22	20	30	25	2.5

TABLE 3. Uses of sandalwood nuts as suggested by male (M) and female (F) respondents.

	main meal	snack	cooking	special occasions
% (M)	8	52	50	20
% (F)	10	58	60	28

Seventy seven percent of the people surveyed had heard of sandalwood, although many (41 percent of the females and 12 percent of the males) were not sure of its uses. All respondents were unaware that the tree produced edible nuts. Some respondents noted that it was good to see Western Australian products being considered for the market.

DISCUSSION

Sandalwood nuts used were collected from the ground under trees. They were all at least a year old which probably accounts for the low percentage of usable kernels. Fresher nuts provide a greater number of full and edible kernels. Sandalwood kernels are very similar in protein, fat, carbohydrate, calcium, potassium and sodium content to other commonly used nuts. Nutritionally, they are a good substitute for other nuts. It is known that oil in the kernels of sandalwood nuts contain about 33% santalbic acid (Jones 1988). The effects of consuming santalbic acid on human metabolism are not known but are currently under investigation (Dr Jones - pers. comm.). It is important that this issue is resolved before the nuts are marketed. From anecdotal evidence it is known that many harvesters and others living in the bush regularly eat sandalwood nuts and have done so for years with no apparent ill effects.

The roasted sandalwood kernel was considered to have a good to very good flavour when consumed as a snack by 23% of the respondents. Other respondents considered the nut could be improved by further preparation such as roasting then salting, smoking, devilling, treating with spices or coating in honey.

Many people showed an interest in consuming a local nut. This initial positive response and interest is encouraging. Further development of the sandalwood nut as a snack is warranted. Particular emphasis should be given to promoting the local origin of the nut.

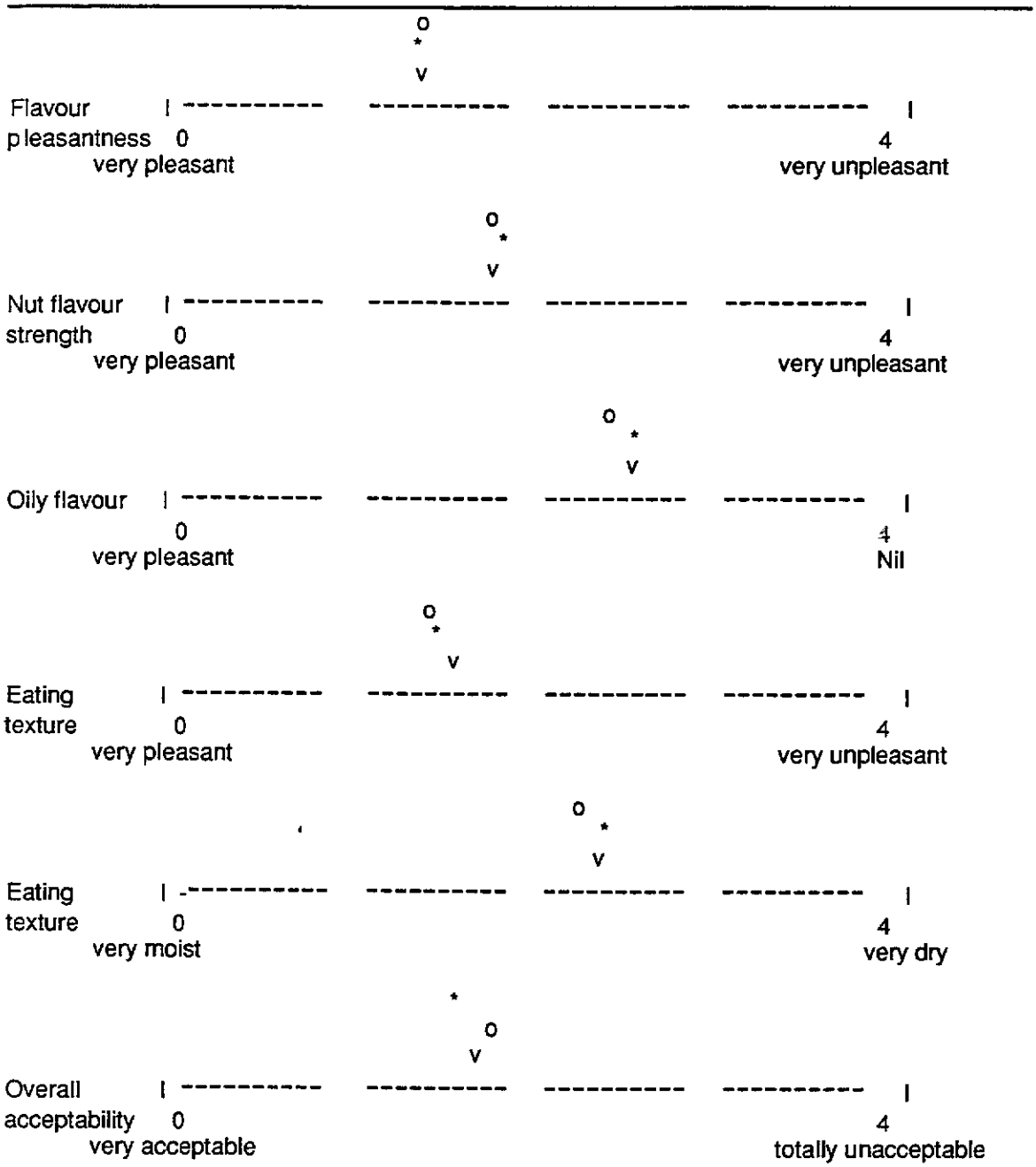

FIGURE 2. Sensory evaluation of muesli bars containing peanuts (o), sandalwood nuts (*) and double quantity of sandalwood nuts (v) by 104 respondents at Curtin University.

Due to the limited availability of sandalwood nuts and their unique Western Australian origin, it was envisaged that sandalwood snacks would be a specialty, possibly for use on aeroplanes or at special functions. Perth Domestic Airport was chosen as the site for acceptability testing since it was hoped to involve many of the travelling public. It proved difficult to find sufficient respondents however. One reason was that the questionnaire employed was too long. A simpler, shorter set of questions should have been used. In future tests it may be better not to compare the more delicate and unknown flavour of sandalwood kernels directly with macadamia nuts. The latter are well known and have a distinctive and strong flavour which may have masked peoples' perceptions of sandalwood nuts.

The use of sandalwood kernels in cooking has presented few problems. Sandalwood can replace equal quantities

of peanuts with little, if any, discernible alteration of flavour or nuttiness. Since the sandalwood kernel has a bland taste, larger quantities of nuts may have to be used if the distinct, although mild, kernel flavour is to be discerned. The use of sandalwood as an ingredient, a local alternative to more traditional nuts in cooking, could be limited by the availability of sufficient quantities of nuts. The supply of nuts is unlikely to be large, certainly until plantations are established and fruiting. At present a licence is required to harvest any part of the tree, including the fruit, from crown lands.

No one surveyed knew that sandalwood trees produced edible nuts. A developed product might best be promoted by providing a brief outline of the historical background of sandalwood and a few recipes and serving suggestions with each product.

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APPENDIX 1

Control Recipe - S. Deane Muesli & Apricot Bar (Deane 1981)

Ingredients

500 ml wholemeal flour
120 g raw sugar
40 g nonfat dried milk powder
5 ml baking powder
2 eggs (size 60 g)
315 ml cold water
65 ml melted copha
125 ml bran cereal
125 ml dried apricots (chopped and soaked)
125 ml raisins
125 ml sultanas
125 ml unsalted peanuts
60 g honey
125 ml rolled oats
90 ml coconut
65 ml unprocessed bran
pinch of salt

Method

1. Place all ingredients together in a bowl and mix well.
2. Place in a greased tray 280 x 180 mm for 30-35 minutes in moderate oven (180-200°C).
3. Allow to cool in tin, then cut into 10 bars.

CANOPY INVERTEBRATE COMMUNITIES IN WOODLANDS - A COMPARISON OF MORNING AND AFTERNOON SAMPLES BY CHEMICAL KNOCKDOWN

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ABSTRACT

Chemical knockdown procedures were used to sample invertebrate communities during the morning and afternoon on four species of eucalypts (*Eucalyptus capillosa, E. loxophleba, E. erythronema* and *E. yilgarnensis*) and Jam Wattle (*Acacia acuminata*) in the wheatbelt of Western Australia. There were differences between morning and afternoon samples on the wattle and bulked *Eucalyptus* trees for individual taxa of invertebrates. Some taxa were significantly more abundant in morning samples (Collembola and Coleoptera - larvae) while others were significantly more abundant in those taken during the afternoon (Araneae, Hemiptera - others, Thysanoptera, Coleoptera - adults, Diptera - adults and Hymenoptera - others). The results show the importance of standardising sampling to set times of day. In comparing samples collected at the same time of day, temperature, wind speed and cloud cover conditions should be as similar as possible.

INTRODUCTION

The distribution and behaviour of terrestrial invertebrates differs throughout the day as temperature, light intensity, wind speed and humidity change with time of day and weather conditions (Southwood 1966). Thus, standardisation of invertebrate samples by time of day and weather conditions is important, if reliable estimates are to be made of invertebrate densities.

In our studies of *Eucalyptus* canopy invertebrates we standardise procedures by restricting sampling to early morning (0700 - 1000 h) and by not sampling when it is windy or there is rain (Majer & Recher 1988, Majer *et al.* 1990). In this paper we present the results of an experiment using chemical knockdown procedures in which we took equal numbers of samples during early morning, following our normal practice, and in late afternoon, when wind intensities were similar to those encountered during the morning, but temperatures were higher. These data are useful for the evaluation of precise stopping and starting rules for invertebrate sampling methods.

METHODS

Invertebrates were sampled between September 4 and 13, 1987 in East Yorkrakine and Durokoppin Reserves near the town of Kellerberrin in the central wheatbelt of Western Australia. Five tree species were sampled; Jam Wattle (*Acacia acuminata*), Wandoo (*Eucalyptus capillosa*), York Gum (*E. loxophleba*), and two species of mallee, *E. erythronema* and *E. yilgarnensis*. The vegetation is described by Muir (1978, 1980).

Ten patches of each species were selected. Five patches were designated for morning and five for afternoon sampling. Ten 0.5 m^2 circular nets were placed in each patch at a height of 0.5 - 2.5 m above the ground, taking care that there was no overlap between adjacent nets. Nets for the morning sample were placed in position the previous evening and those for the afternoon sample were positioned from mid to late morning. Knockdowns were performed between 0630 and 0830 h in the morning and 1600 and 1730 h in the afternoon. Knockdowns were restricted to times when wind speed was less than 8 - 10 km h^{-1} which sometimes prevented samples from being taken on consecutive days. Daily meteorological data and also temperature, wind speed and cloud cover during each sample period are presented in Table 1.

The canopy above each net was fogged with 0.5% pyrethrin insecticide synergised with piperonyl butoxide using a motorised knapsack mistblower. The insecticide reached an approximate height of 7 m. After fogging, the nets were left in position for approximately 30 minutes to allow the affected animals to drop from the vegetation. At the end of this time, the canopy was shaken to dislodge any remaining animals. All animals on the nets were collected and preserved in 70% ethanol. For this particular investigation we combined the invertebrates from the ten nets from each patch. The specimens were divided into broad taxonomic groups (Order or Family) and counted. The samples were placed in filter paper funnels for 12 hours and then weighed for an approximation of the live wet weight.

The data for the four *Eucalyptus* spp. were combined and analysed for the effect of tree species and time of day by two-way analysis of variance (ANOVA). The data for *A. acuminata*, which exhibited different trends from those for the eucalypts, were treated separately by an unpaired student's t-test. In both instances, we only performed tests on taxa which were present in the majority of samples.

TABLE 1. Weather data measured by the Bureau of Meteorology at Merredin during the period of canopy invertebrate sampling at Durokoppin and Yorkrakine Nature Reserves. Merredin is the closest weather recording site to these reserves (A). The temperature, percentage cloud cover and wind speed measured on-site at the time of sampling is also shown (B).

(A)	September 1987											
DATE	3	4	5	6	7	8	9	10	11	12	13	14
Max temp °C	16	19	20	18	19	23	24	28	23	25	26*	28
Min temp °C	3	3	6	7	4	8	10	8	11	12	12	-+
Rainfall	NIL											

(B)	September 1987											
DATE	3	4	5	6	7	8	9	10	11	12	13	14
0800												
Temp °C		10	11		10	12					14	
% Cloud cover		50	80		0	0					0	
Wind speed (kmh-1)		3-5	0		3-5	3					3-5	
1600												
Temp °C		20	20		20	26		28				
% Cloud cover		30	50		0	0		0				
Wind speed (kmh-1)		3-5	3-5		3	3		3				

* Maximum temperature not available and calculated as mean of previous and following day's maximum temperature.

\+ Minimum temperature not available.

RESULTS

Twenty-two categories of invertebrate taxa were recorded from the five species of tree (Table 2). At the ordinal level, faunal diversity was greatest on York Gum, with 21 taxa, and least on Wandoo, with 18 taxa. Numbers of invertebrates were lowest on Wandoo and greatest on Jam Wattle. The latter was in part accounted for by the high numbers of psyllids on the wattle. However, when the psyllids were excluded, the overall daily count was still highest on the wattle.

On the wattle, there was a tendency for taxa to be more numerous in the afternoon samples than in those from the morning (14 vs 3 taxa respectively) (Table 2). The tendency for taxa to be favoured by time of day statistically differs from that which could be expected by chance ($p<0.01$ using the 50% probability test [Langley 1970]).

Six taxa (Araneae, Hemiptera - others, Thysanoptera, Coleoptera - adults, Diptera - adults and Hymenoptera - others) were significantly more abundant in the afternoon samples, as was the total invertebrate biomass (Table 2).

The time-trends were less clear on the eucalypts, with more taxa being abundant in the morning samples on the mallee species, more taxa being abundant in the afternoon on the Wandoo and no difference evident on the York Gum (Table 2). Using the ANOVA, three taxa exhibited statistically different time-trends; Collembola and Coleoptera - larvae were more abundant in morning samples and Coleoptera - adults were more abundant in the afternoon samples (Table 2). These trends were consistent over all four *Eucalyptus* spp. We did not perform tests on taxa on individual tree species in view of the relatively low numbers of individuals and the high variance between samples.

TABLE 2: Morning and afternoon numbers and biomass of invertebrates collected in pyrethrin knockdown samples of Eucalyptus and Acacia species in Durokoppin and East Yorkrakine Reserves during September 1987. Numbers shown are mean and standard deviation with level of significance between morning and afternoon samples.

TREE GENUS		*Eucalyptus*								AM/PM Comparison		*Acacia*		AM/PM Comparison	
TREE SPECIES		*capillosa*		*erythronema*		*yilgarnensis*		*loxophleba*				*acuminata*			
DAY / TIME OF DAY		4th AM	5th PM	7th AM	8th PM	8th AM	7th PM	5th AM	4th PM	F Value	Sig.	13th AM	10th PM	t Value	Sig.
Arachnida	Acarina	1.2±0.8	2.6±3.6	3.8±4.7	1.2±1.8	3.0±1.2	4.2±5.0	1.8±0.8	1.6±1.8		ns*	11.2±9.4	63.6±50.9		ns
Arachnida	Araneae	4.0±1.6	6.4±3.4	7.2±2.9	7.2±4.3	6.4±0.5	8.2±4.7	9.2±5.8	5.6±2.7		ns	7.0±3.7	12.4±3.6	-2.33	<.05
Crustacea	Isopoda	-	-	-	-	-	-	-	0.2±0.4		nt*	-	-		nt
Collembola		7.6±4.5	1.2±0.8	42.2±23.8	4.6±1.1	44.8±19.6	11.6±10.3	6.8±7.5	0.2±0.4	30.91	<.05	1.2±1.3	0.4±0.9		nt
Insecta	Thysanura	-	-	-	0.8±1.8	-	0.4±0.9	0.2±0.4	-		nt	-	0.6±1.3		nt
Insecta	Blattodea	0.2±0.4	0.2±0.4	0.2±0.4	-	-	-	0.2±0.4	0.6±1.3		nt	-	0.2±0.4		nt
Insecta	Mantodea	-	-	-	0.2±0.4	-	-	-	-		nt	-	-		nt
Insecta	Orthoptera	-	-	-	-	0.2±0.4	0.2±0.4	-	0.2±0.4		nt	-	-		nt
Insecta	Psocoptera	20.4±23.3	44.8±35.3	11.2±8.2	5.6±5.2	4.2±2.7	3.4±1.1	6.8±7.0	19.2±24.0		ns	7.6±3.8	12.6±4.3		ns
Insecta	Hemiptera psyllids	3.6±1.1	23.0±22.1	14.2±9.5	10.6±5.7	21.6±13.5	10.2±8.4	46.6±27.7	21.0±15.7		ns	2679±2038	5014±3810		ns
Insecta	Hemiptera others	27.0±13.4	25.0±15.7	45.4±17.7	46.2±22.7	60.2±16.6	72.6±21.4	22.2±7.0	23.4±24.0		ns	11.0±2.8	18.0±6.1	-2.33	<.05
Insecta	Thysanoptera	5.0±4.2	12.0±9.7	29.4±19.2	14.2±6.1	35.2±27.1	15.4±9.7	47.0±76.3	28.2±31.5		ns	16.0±6.4	44.2±16.9	-3.48	<.01
Insecta	Neuroptera adults	1.0±0.7	0.6±0.9	1.4±1.7	-	0.6±0.9	0.4±0.9	0.8±0.4	0.4±0.5		ns	0.4±0.5	0.4±0.9		nt
Insecta	Neuroptera larvae	-	1.4±1.3	-	0.2±0.4	0.8±0.8	-	0.2±0.4	0.2±0.4		nt	0.4±0.5	0.4±0.9		nt
Insecta	Coleoptera adults	38.8±13.2	51.2±32.2	42.6±22.5	76.8±17.8	35.8±13.6	35.8±21.0	29.4±17.8	54.8±17.2	7.90	<.05	24.6±10.0	91.6±33.6	-4.28	<.005
Insecta	Coleoptera larvae	9.8±3.6	3.0±2.1	7.4±3.4	5.2±3.5	8.0±3.2	1.8±1.3	4.0±3.0	3.2±1.3	20.13	<.05	13.2±7.8	11.2±6.5		ns
Insecta	Diptera adults	36.2±10.7	15.0±6.2	37.6±11.6	10.0±4.6	24.0±16.6	22.8±28.8	38.8±9.5	50.0±20.4		ns	19.8±10.0	100.2±49.1	-3.59	<.01
Insecta	Diptera larvae	1.2±1.3	-	1.0±0.7	0.2±0.4	0.4±0.5	-	1.0±1.0	-		nt	7.4±4.7	5.4±4.8		ns
Insecta	Lepidoptera adults	1.2±0.4	0.2±0.4	1.8±1.5	1.0±1.0	1.4±1.5	1.6±2.1	0.6±1.3	0.2±0.4		nt	0.6±1.3	1.0±1.0		nt
Insecta	Lepidoptera larvae	2.0±2.2	3.6±2.9	5.6±3.0	4.6±2.3	6.6±3.3	3.8±4.7	2.2±1.5	5.4±4.2		ns	17.0±5.6	18.8±7.5		ns
Insecta	Hymenoptera ants	5.6±5.3	10.0±12.6	22.4±18.3	36.4±26.1	20.0±6.0	9.4±5.0	9.4±11.5	26.8±25.1		ns	59.2±32.0	126.4±127.2		ns
Insecta	Hymenoptera others	16.4±7.7	20.8±8.1	21.6±6.9	11.4±9.1	18.6±12.7	19.2±9.8	24.2±10.9	36.4±12.6		ns	18.0±9.7	88.8±51.6	-3.02	<.05
Total Invertebrates		181.2±52.2	221.0±59.3	229.2±57.5	236.4±46.2	291.8±77.2	224.6±84.8	251.4±107.9	277.4±145.2		ns	2893±1998	5611±3780		ns
Biomass (g)		0.14±0.04	0.23±0.08	0.23±0.07	0.23±0.10	0.32±0.13	0.25±0.10	0.20±0.11	0.24±0.15		ns	0.36±0.11	0.93±0.47	-2.66	<.05

* nt = test not performed on this taxon
ns = not significant

DISCUSSION

There were detectable and sometimes significant differences in the abundances of invertebrates sampled during morning and afternoons.

Avoidance of high temperatures and desiccation (e.g. by springtails and larval beetles), increased activity with warmer temperatures (e.g. by adult flies and beetles), aggregation at food sources such as nectar or honeydew (e.g. by some wasps), and responses to diurnal changes in the physiology of the tree, are possible reasons for differences in the abundance of various taxa between morning and afternoon sampling periods. Movements between trees and/or the use of different kinds of substrates throughout the day may have also affected the kinds and numbers of invertebrates sampled at different times of the day. The different patterns of abundance observed for different taxa and the differences between tree species in the numbers, biomass and kinds of invertebrates sampled suggests that there is no simple explanation for the differences between morning and afternoon samples.

Samples of invertebrates within and between trees obtained by branch clips, chemical knockdown and visual counts are highly variable, even when taken in a standardised manner at the same time of day and during fine weather conditions (Majer & Recher 1988, Majer *et al.* 1990, Recher & Gowing unpublished data). Variability of samples arising from these changes in the distribution and behaviour of invertebrates can only be controlled by standardised sampling procedures. Samples taken at different times of the day or under different physical conditions add additional, significant variability to the data and cannot be used to compare invertebrate communities on different species of plants or on the same species in different localities or seasons. Although costs are increased, we conclude that samples of invertebrate communities need to be rigorously controlled for time of day and, in so far as is possible, taken under similar weather conditions.

The need to standardise sampling times does not just apply to invertebrates. For instance, Rollfinke and Yahner (1990) have recently demonstrated that, although winter bird counts were similar between morning and midday, they were much lower in the afternoon. Thus, for studies of birds and their invertebrate food resources, the standardising of survey times for both groups of animals is critically important if reliable data are required.

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INDUCED GERMINATION AND GERMINANT SURVIVAL IN THE MANY-FLOWERED FRINGE LILY (*THYSANOTUS MULTIFLORUS* R. BR., ANTHERICACEAE)

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INTRODUCTION

Thysanotus multiflorus (many-flowered fringe lily, Anthericaceae) is a herbaceous perennial native lily (Marchant *et al.* 1987). The species *T. multiflorus* is highly variable and includes related species such as *T. proliferus* and *T. brevipes* which were once regarded as separate taxa (Brittan 1981). Plants flower from September to January. Although the plant is much sought after as an ornamental - because of its unusual fringed purple petals - it is generally unavailable from nurseries. Difficulties with its propagation from seed appears to be the main reason. The small numbers that may be available in certain years are usually volunteer seedlings removed from the bush after a fire.

Despite a relatively high seed set, germination is sporadic in the wild. Dixon (1983) suggests that moisture stress, poor pollination and harvesting of immature seeds contribute to a high percentage of infertile seeds. Germination of untreated seeds in normal pot sowings, and even under environmentally-controlled conditions, is extremely low (Tan, pers. obs.).

The impervious seed testa prevents imbibition, which is a prerequisite for germination (Bewley and Black 1978). In this study a variety of techniques was applied to weaken the hard seed coat. Their efficacy for inducing germination, conducted at different temperatures, is reported below.

MATERIALS

Commercially available seeds (Vaughans, Gingin; Nindethana, Woogenilup) were used. Each seed lot was tested for potential viability by staining bissected seed samples with 1% (w/v) tetrazolium (Anon 1970).

The *T. multiflorus* plants raised from commercial seeds are non-tuberous and self-fertile, possessing glabrous, semi-terete leaves and growing to 400 mm. Another form, not used here but also referred to by some local nurserymen as '*T. multiflorus*', has a shorter stature (to 200 mm), distinctly terete-acicular leaves, and is completely self-sterile.

Seed Structure

The seed is roughly spherical, approximately 2.5 mm across, and is covered by a hard black testa. A yellow elaiosome, approximately one-quarter the size of the seed, is attached by a short stalk at the 'top' of the seed (Figure 1a). A slender elongated embryo is housed in a narrow cavity within the lipid-rich endosperm (Figure 1b).

METHODS

Seed Treatments

Seven seed treatments were undertaken and germination was conducted in a germination cabinet with constant temperatures of 15°C, 20°C, 25°C, or an alternating tem-

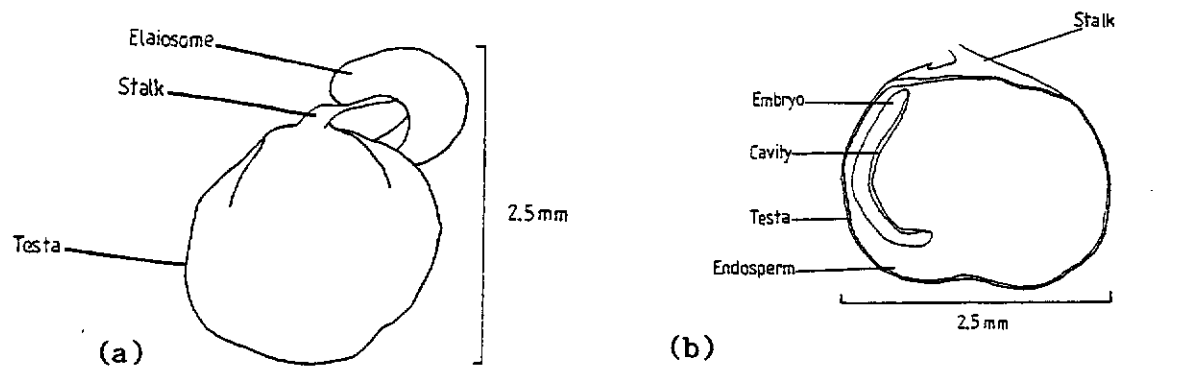

FIGURE 1. Structure and anatomy of *T. multiflorus* seed: (a) side view of intact seed, and (b) cross-section of seed.

perature regime of 25°C (13 hr)/7°C (11 hr). Lots of 100 seeds were surface-sterilized with dilute sodium hypochlorite (3% available chlorine) for 10 minutes, rinsed three times with deionised water and assigned to one of the following treatments:

1. Control - seeds were left untreated after surface sterilization.
2. Scarification - seeds were scarified by rubbing on a sheet of sandpaper with sanding cork.
3. Acid - seeds were soaked in 5 ml of concentrated H_2SO_4 for 7 minutes and then rinsed thoroughly with water.
4. Dry heat - seeds were heated in an oven at 60°C for 1 hour.
5. Hot water - seeds were scalded with boiling water and left to cool to room temperature.
6. Seed nicking near elaiosome - approximately 1 mm^2 section of testa was removed near the 'top' of the embryonic axis near the elaiosome.
7. Seed nicking opposite to elaiosome - a small piece of testa was removed from the 'bottom' of the seed.

Two seed lots, each of 100 seeds, were assigned to each treatment/temperature regime. Seeds of each lot were placed on moistened filter paper (Whatmans No. 3, 90 mm diameter) over a layer of vermiculite in a petri dish. Drops of dilute Benlate^R suspension were added to the moist filter paper to suppress fungal growth. The petri dishes were checked daily for germination and re-moistened with water and fungicide when required.

Germinant Survival

Although germination was readily induced in seeds nicked near the elaiosome, many germinants failed to develop further. Initial germination, as judged solely by radicle emergence, may have been facilitated by the partial removal of the testa's physical constraint. However, some unknown factor, possibly water-soluble compound(s) in the seed, appeared to affect further seedling development. Therefore, a second experiment was conducted to test whether germinant survival could be enhanced by additional treatment.

Four hundred seeds, nicked near the elaiosome, were agitated in 50 ml of deionised water containing 2% (w/v) of activated charcoal (North Carolina Supply Co.), contained in an Erhlenmeyer flask, at 125 rpm (rotary platform shaker, New Brunswick Scientific). A further 400 nicked seeds were similarly agitated in 50 ml of deionised water. One hundred seeds were removed after 1, 3, 7 and 14 days, and germinated at 15°C, the temperature found to be most suitable in the first part of this trial. Comparisons of germination and germinant survival were also made with 100 unnicked, non-washed seeds, and 100 nicked, non-washed seeds.

Statistical Treatment

The underlying assumption when analysing the germination or germinant survival data was that each observed value (**p**) is binomially distributed and, accordingly, a standard error can be computed for each **p** value, viz. $\sqrt{(pq/n)}$, where **q** = no. or % of non-germinated seeds, and n = number of seeds in treatment. Confidence limits (P=0.95) of each **p** value, obtained by multiplying its standard error by 1.96, are represented by a bar on the germination response curve (Fig.2) or histogram (Fig. 3). Values of **p** whose confidence bars do not overlap are inferred to be significantly different at the 5% level of probability.

RESULTS

Seed Treatments

Nicking seeds near the 'top' of the embryonic axis was the most effective means of inducing germination; zero to very low germination was recorded in all other treatments as well as in the control (Table 1). Within the seed-top nicking treatment germination was significantly lower (P<0.05) at 20°C and 25°C than at 15°C and 7°C/25°C (Figure 2). The highest germination response was obtained at 15°C.

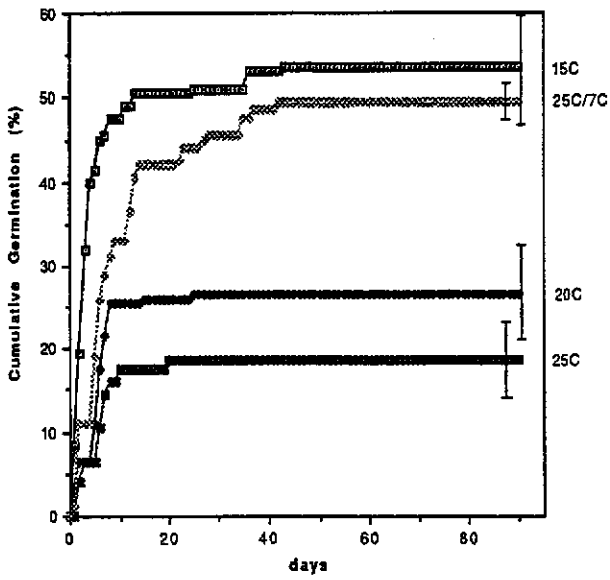

FIGURE 2. Germination response of 'top-nicked' seeds at four temperature regimes. Vertical bar indicates the 95% confidence limits.

TABLE 1. Germination (%) after 50 days for treated fringe lily seeds*

Treatment	Temperature (°C) 15	20	25	25/7
Control	0.5[a]	0.5[a]	0[a]	0[a]
Scarification	7.0[a]	3.5[a]	1.5[a]	2.0[a]
Acid	0[a]	0[a]	0[a]	0[a]
Dry heat	2.0[a]	0[a]	0[a]	0[a]
Hot water	0[a]	0[a]	0[a]	0[a]
Nicking - top	53.0[b]	26.5[b]	17.5[b]	49.0[b]
Nicking - bottom	0[a]	0[a]	0[a]	0[a]

*Within each column, values with the same superscript are not statistically significantly different at P = 0.05. * Average viability of seed samples as indicated by tetrazolium staining of dissected embryos: 62% (range: 58 - 70%)*

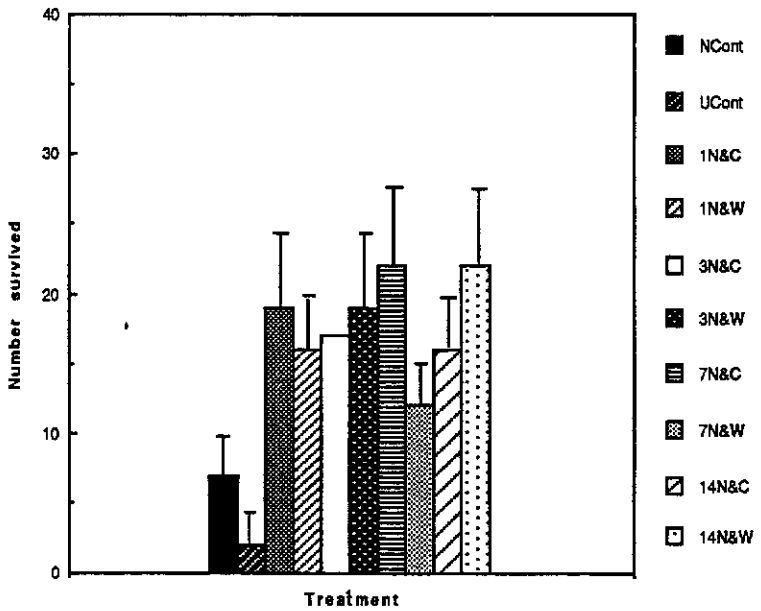

FIGURE 3. Number of surviving germinants. Vertical bars indicate the 95% confidence limits for each treatment. Abbreviations: Cont = control, N = nicked, U = unnicked, C = washing with charcoal, W = washing without charcoal.

Germinant Survival

The survival of germinants from unnicked and nicked non-washed seeds was significantly lower ($P<0.05$) than those from nicked washed seeds. The survival of germinants washed for 1, 3, 7 or 14 days (except for those washed for 7 days with charcoal) was similar whether or not charcoal was used in the washing procedure. The significantly lower survival of seedlings washed in charcoal suspension for 7 days is being regarded as a chance aberration at this stage. The presence of activated charcoal, an effective adsorbant of harmful gases and compounds, was not found to influence the results. There was also no significant improvement in germinant survival with washing for more than 1 day (Figure 3).

DISCUSSION

Germination

Seed scarification is commonly used as a technique for inducing germination in seeds with a hard seed coat. This technique was not effective in *T. multiflorus*. In contrast, nicking a small piece of testa near the embryonic axis

produced a rapid germination response. Seed nicking has also proved beneficial in other species including *Tetrapleura tetraptera* (Odoemena 1988) and *Geleznowia verrucosa* (Paynter & Dixon 1991). The target area for nicking appears to be important, since seeds nicked at the 'bottom' did not germinate but produced swellings at the site of nicking: this could be a response to imbibition and wounding. Nicking partially removes the physical barrier which prevents imbibition, gaseous exchange, and/or the leaching of chemical inhibitors. The extent to which each of these contributes to preventing germination is difficult to assess.

The optimum temperature for germinating *T. multiflorus* was not established in the experiment. Of the three temperatures tested 15°C gave the best germination response in terms of percentage germination, (Table 1) and speed (Figure 2). It is probable that the optimum germination temperature is in the vicinity of 15°C. It is reasonable to surmise that germination in nature - although sporadic - occurs predominantly in the cooler times of the year, from autumn and spring .

Germinant Survival

Germination as a physiological process may be considered from different perspectives, namely, the transformation of the embryo to a seedling, or as resumption of the embryo's metabolism and growth which have been temporarily suppressed or suspended (Jann & Amen 1977). In *T. multiflorus*, the elongation and emergence of the radicle in top-nicked seeds appears to be an obligatory response after imbibition, but most germinants become necrotic and succumb. This 'apparent' germination - if radicle emergence is used as the sole criterion for germination - has also been noted in *G. verrucosa* (Paynter & Dixon 1991).

The washing of nicked seeds was shown to enhance seedling survival. This suggests the presence in the seed of some water-soluble compound(s) that is detrimental to seedling development. Nicking the seed will induce premature germination whatever the endogenous level of this compound(s).

Under natural conditions the seed is subjected to environmental stresses such as fire, fluctuating temperatures, and cycles of wetting and drying. A gradual degradation and leaching of this hypothetical compound should take place under these conditions. As its level diminishes concomitant with testa weathering or fracturing, germination resulting in a normal seedling may take place.

The results of this study raise the question whether poor germination in the fringe lily can be ascribed more to low germinant survival than germination failure *per se*.

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SALT TOLERANCE IN *RHAGODIA EREMAEA*

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SUMMARY

Rhagodia eremaea is a species of potential value for revegetation of gold mine waste dumps. The suitability of *R. eremaea* seedlings for revegetation purposes was tested using a salinity tolerance trial. Seedlings were subjected to one of four salinity treatments: irrigation with 25, 50, 100 or 200 mM NaCl. Irrigation with deionised water only was used as a control. Plants were grown under glasshouse conditions for 6 weeks, to observe effects on seedling survival and growth.

Rapid root growth is of great ecological significance for halophytic species and appears an essential adaptation to saline conditions. Increased growth at the lower salinity levels provided evidence confirming that *R. eremaea* is a halophyte. An increase in leaf production, followed by enhanced root growth was observed in seedlings subjected to 25 and 50 mM NaCl. Significant differences in plant height or shoot biomass between treatments were not detected. Higher levels of salinity (100 and 200 mM) inhibited root growth before shoot growth, while the highest level used depressed both shoot and root growth. Seedling deaths of 75% occurred in the most saline treatment, and losses in the next most severe treatment were 15% over the experimental period. In these treatments characteristic symptoms of salt injury developed, including necrosis of leaf margins and incurving of leaves. Thickening and succulence of leaves were also observed in the higher salinity regimes.

Seedlings irrigated with 50 mM NaCl solution had more numerous side shoots and branches, as well as a more vigorous root system. This treatment was the most favourable salinity regime in respect of growth of *R. eremaea* seedlings. From the data provided in this investigation revegetation of saline mine waste dumps with *R. eremaea* should be possible on materials with soil conductivity between 5 and 9 dS m^{-1}, which is equivalent to applying NaCl concentrations >25 mM and < 100 mM over a 6 week period. Research into germination response and tolerance of larger *R. eremaea* plants, over a longer time period, is required to confirm suitable establishment procedures.

INTRODUCTION

Rock waste dumps resulting from open cut goldmines consist of overburden materials characteristically of high salinity. Hart (1972) summarised processes that can lead to salt accumulation. Two of these are of particular importance to gold mining (Stephens & Osborne, 1988). First, removal of natural vegetation results in reduced evapo-transpiration and therefore a rise in the water table. The water then evaporates, causing salts to come out of solution and deposit on the soil surface. Second, internal drainage patterns of some dump sites may also result in salt accumulation on the soil surface.

Revegetation of saline dumps requires plants that can tolerate the salinity levels present. Discussion of salinity tolerance in plants often draws comparisons between halophytes and non-halophytes (Haswell, 1987), but differentiation between these categories is often difficult to establish (Munns, Greenway & Kirst, 1983). Salinity levels may vary between dump sites, requiring differing management strategies dependant on salinity differences. Investigations into the tolerance of species used in the revegetation of saline land in the goldfields have been conducted on a variety of *Eucalyptus* species (Sands, 1981; Pepper & Craig, 1986; Fox, Neilsen & Osborne, 1990) and *Acacia* species (Fox, Barrett & Osborne, 1987; Stephens & Osborne, 1988; Craig, Bell & Atkins, 1990).

Salinity affects several aspects of plant metabolism and induces changes to anatomy and morphology. These changes can be considered as adaptations which increase the chance of plants surviving under salinity related stress, or as an indication of cellular damage and disruption of normal metabolic activities (Poljakoff-Mayber, 1975). Suppression of plant growth is the main effect on non-halophytic plants subjected to a saline environment. As salinity increases, so does the effect until eventually the plant dies. Growth decreases linearly as salinity increases beyond a threshold salinity level (Hoffman & Shannon, 1986). The salt level at which growth is affected varies among species and may also vary to some extent within a species (Waisel, 1972). Some halophytic genera (*Atriplex* and *Salicornia*) respond to relatively high salinity levels, which stimulate plant growth (Greenway, 1973; Poljakoff-Mayber, 1975). Salinity may also affect both the time and rate of germination, plant size, branching, leaf size, and overall plant anatomy (Poljakoff-Mayber, 1975). Long-term exposure to highly saline environments causes salt injury, due to the accumulation of inorganic ions in the apoplastic region between plant cells (Oertli, 1968), causing a decrease in cell volume and turgor as a result of osmotic loss of water from the cells.

Plants that have adapted to saline environments *via* ion uptake can either avoid or tolerate high internal concen-

trations of salt. Salinity avoiding plants reduce the amount of salt-induced stress by regulating uptake and internal transfer of ions. Haswell (1987) described three important ways in which such plants avoid high internal salinities:

1) controlled salt uptake by roots and redistribution to the shoot;
2) excretion of salt from salt glands and bladders (Thomson, 1975); and
3) increases in shoot volume (*i. e.* succulence) due to the uptake of inorganic ions increases cell turgor and hence promotes cellular expansion (Jennings, 1976).

Translocation of inorganic ions *via* the phloem, removing excess ions from the shoot, may also be an adaptive mechanism for salinity avoidance (Haswell, 1987). Salinity tolerant plants are characterised by plant cells accumulating organic solutes which restore cell turgor and hence facilitate osmotic adjustment (Greenway, 1973). Internal water deficits may occur in plants that restrict the uptake of inorganic salts. These can cause degradation of cell membranes, cell injury and protein denaturation.

The family Chenopodiaceae contains a number of genera many of which *(e.g. Atriplex, Maireana),* have been demonstrated to be halophytic (Sharma, 1982). Chenopods are also valuable species for livestock fodder in arid and semi-arid areas because of their tolerance to drought and salinity (Leigh, 1986; Mitchell & Wilcox, 1988). *Rhagodia eremaea* Paul G. Wilson (Chenopodiaceae), commonly known as the tall (thorny) saltbush, is a species we consider to have potential for mine dump revegetation. *R. eremaea* is found in the Pilbara District and northern goldfields of Western Australia, and also in central Australia. The species occurs on a variety of soil types and ecotypes of *R. eremaea* exhibit a range of geographical variations (Wilson, 1983). Although plants of the genus *Rhagodia* are referred to as saltbush, there is no salinity tolerance data to support this claim. Overall there has been little work conducted on this genus, except for an autecological study of *R. baccata* (Labill.) Moq. (Hellmuth, 1986).

The study reported here sought to investigate the suspected halophytic nature of *R. eremaea* seedlings by examining seedling growth and survival in response to increasing levels of salinity. Such an investigation will provide guidelines relating to optimum salinity levels for maximising seedling growth.

MATERIALS AND METHODS

Seed was collected from mature *Rhagodia eremaea* plants in the garden of Lake Way homestead (26°57' S, 120°28' E), Murchison, Western Australia. The seeds were germinated in the week beginning 17 June 1990. Three weeks later, 100 seedlings of similar height and leaf number were transplanted to small plastic pots (70 x 70 x 100 mm) containing a soil mixture of coarse sand, red loam and peat moss in the ratio of 1:1:1. Seedlings which died within a few days of being transplanted were replaced. Plants were grouped according to size and dispersed among the 5 trays (20 pots per tray) to ensure an even distribution of plant sizes. Trays were randomly allocated to one of five solution treatments.

Stock saline solutions of 25, 50, 100 and 200 mM NaCl were made up with deionised water to fill 20 l black polydrums, fitted with taps. Deionised water was used in the control treatment. Pots were watered twice a week with the appropriate solution. Liquid was added until the first drops began to leach out of the pots, as an indication that field capacity had been reached. Care was taken not to allow salt solutions to come in contact with foliage, to avoid salt absorption by leaves and possible salt injury.

Seedling growth

Measurements of seedling height, and observations of leaf number, leaf senescence and death, plant health and appearance were made on a weekly basis commencing from 31 July 1990. On the death of a seedling, a small soil sample was taken in a plastic vial before the plant was harvested. Seedlings were harvested, separated into shoot and root components, dried in an oven at 105°C and weighed. Surviving plants were similarly harvested 6 weeks after the initiation of measurements. Total leaf area of three plants per treatment (all ~100 mm high) was measured using a portable LAMBDA leaf area planimeter. Harvest measurements were subjected to analysis of variance to determine if there were any differences in growth response between treatments. Tukey's pairwise comparison test was used to compare differences between means (Day & Quinn, 1989).

Soil chemistry

Five replicate pots per treatment were selected at random, prior to harvesting, for determination of soil pH and salinity. Soil:deionised water solutions (1:5) were prepared and mixed for approximately 2 hours on an automatic agitator at 100-200 rpm. A glass electrode Beckman pH unit was used for pH determination. Soil salinity ($EC_{(1+5)}$) was measured in dS m^{-1} with a Philips conductivity meter and used to estimate the conductivity of the saturated extract (ECe) using a multiplication factor of 6. In each case means were tested for significance by one-way analysis of variance. Effects on plant growth and soil chemistry for salinity regimes are discused below in relation to the NaCl concentration of the stock solutions applied, and not soil conductivity, which naturally increased over the duration of the trial due to the accumulation of salt in the soil.

RESULTS

No deaths were observed from the control or the two lower salinity level treatments (Table 1). Two weeks after the initiation of the experiment, deaths had occurred in both the 100 and 200 mM treatments. After three weeks no more deaths occurred in the 100 mM treatment. In the 200 mM treatment there was a gradual decrease in the number of living plants, until at the sixth week only five seedlings were alive. The first sign of death was wilting of the plant apex. This led to permanent wilting of the entire stem and foliage, followed by leaf fall.

In both the 100 and 200 mM treatments morphological changes in leaf structure were noticed that were not detectable on seedlings in the other treatments. These changes involved leaf incurving, reddening of leaf and petiole, and succulence of the leaf. Many affected seedlings did not display all these features. Both young and old leaves on plants developing succulence were often brittle. Reddening was initiated from the leaf tip, spreading down the margins and eventually engulfing the entire leaf. Despite independent occurrence of some features, a generalised pattern was as follows:

incurving of leaf→ thickening; and
succulence of leaf→ reddening.

Succulence was also observed at lower salinity treatments, but this was rare and less extensive.

Seedling growth

Mean seedling heights at harvest were not significantly different between salinity treatments (Table 2). Growth rates, based on mean height data, varied (Figure 1) with greatest growth in the 25 and 50 mM treatments (1.95 and 1.77 mm/day respectively). The rate was slowest in the 200 mM treatment (1.05 mm/day). The control and 100 mM treatments had similar rates of height growth (1.63 and 1.60 mm/day respectively), falling in between the two extremes.

Numbers of leaves at harvest differed with fewest leaves in the highest salinity treatment. Leaf counts from both the 25 and 50 mM treatment plants were significantly greater than plants grown in the 200 mM treatment (Table 2). Plants grown at 50 mM also had significantly more leaves than those in 100 mM. The number of green leaves present generally increased, with some variation between treatments, during the six week period (Figure 2). A sudden decrease in the number of leaves present on seedlings in 100 and 200 mM was associated with an increase in the number of fallen and dead leaves recorded (not shown).

Regression analysis was used to compare seedling architecture between treatments (Table 3). The leaf number:seedling height ratio, indicated by the regression coefficient (*b*), was highest in the 50 mM treatment. This was associated with an increased number of side shoots and branches found on seedlings. These regressions indicate that seedlings in the 25 mM treatment were closest to the control in overall plant architecture and that those in the 50 mM set differed most from control. Plants subjected to the 100 and 200 mM treatments were similar in leaf number:seedling height ratio, although this was lower than that of other treatments.

Salinity treatment affected both leaf colour and size. Leaves of plants in the control treatment were uniformly green (Royal Horticulture Society (RHS) colour 137B) and relatively small in size, with a mean of 9.63 mm^2/leaf. All seedlings subjected to salinity treatments produced leaves of yellow-green coloration (RHS colour 146B) but leaf size differed through the treatments. Largest leaves were observed on seedlings growing in 25 and 50 mM, (11.35 and 11.86 mm^2/leaf respectively), whereas smallest leaves were on plants in the 100 mM treatment (8.8 mm^2/leaf). Total leaf areas per plant at harvest were significantly different (Table 2) with smallest leaf areas occurring at higher salinity levels.

TABLE 1. Percentage survival of *R. eremaea* seedlings by salinity treatment.

Treatment	Number of days of treatment						
mM NaCl	0	7	14	21	28	35	42
0	100	100	100	100	100	100	100
25	100	100	100	100	100	100	100
50	100	100	100	100	100	100	100
100	100	100	95	85	85	85	85
200	100	100	70	45	40	30	25

Biomass allocation was also influenced by the application of NaCl (Table 2), with seedlings subjected to 50 mM NaCl producing the greatest total and root dry weights. Irrigation with deionised water produced the same root weights as did plants irrigated with 25 and 200 mM NaCl. In both the control and 50 mM treatments root biomass contributed more to total dry weight than did shoot biomass. No significant difference in shoot dry weights was observed throughout the treatments. Regression of shoot on root weight (Table 3) shows strong linear relationships between shoot and root mass for the 4 salinity treatments.

TABLE 2. Mean harvest values (with SD in parentheses) for *R. eremaea* after 6 weeks of experimental growth.

Parameter	Treatment mM of NaCl					Significance
	0	25	50	100	200	
n =	20	20	20	17	5	
Height	a	a	a	a	a	NS
(mm)	108.4	131.2	121.0	105.9	87.4	
	(32.5)	(36.9)	(30.8)	(34.3)	(33.5)	
Leaf	ac	ab	b	ac	c	p<0.001
number	73.6	102.1	104.8	71.9	43.8	
	(28.2)	(37.5)	(43.2)	(24.3)	(26.7)	
Total leaf	ab	a	ab	b	b	p<0.05
area	8.28	10.86	7.99	6.21	5.51	
(cm^2)†	(1.94)	(2.15)	(1.03)	(0.74)	(2.00)	
Dry weights (mg)						
Shoot	a	a	a	a	a	p<0.05
	237.4	380.5	379.5	255.3	163.5	
	(122.6)	(265.7)	(239.2)	(165.1)	(128.4)	
Root	bc	bc	a	d	cd	p<0.001
	268.5	295.0	425.0	141.5	114.3	
	(137.5)	(172.0)	(225.9)	(89.1)	(92.8)	
Totals	ab	ab	a	b	b	p<0.001
	505.9	675.4	804.5	306.9	277.8	
	(218.9)	(428.9)	(422.6)	(244.7)	(219.9)	
Shoot /root	0.88	1.29	0.89	1.80	1.43	

Values with similar letters were not significantly different with Tukey's pairwise comparison test. † Values represent means from 3 seedlings.

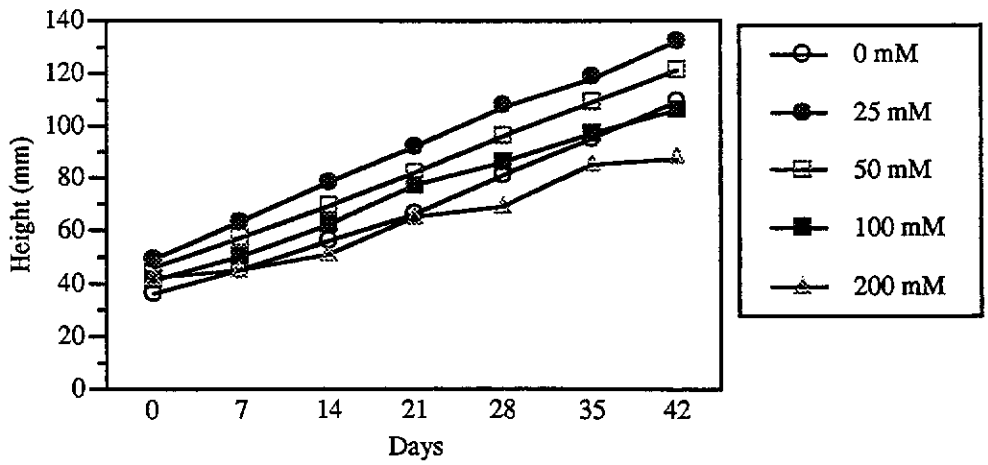

FIGURE 1. Mean height of *R. eremaea* seedlings in different salinity treatments, over a period of 6 weeks (31 July-10 October 1990).

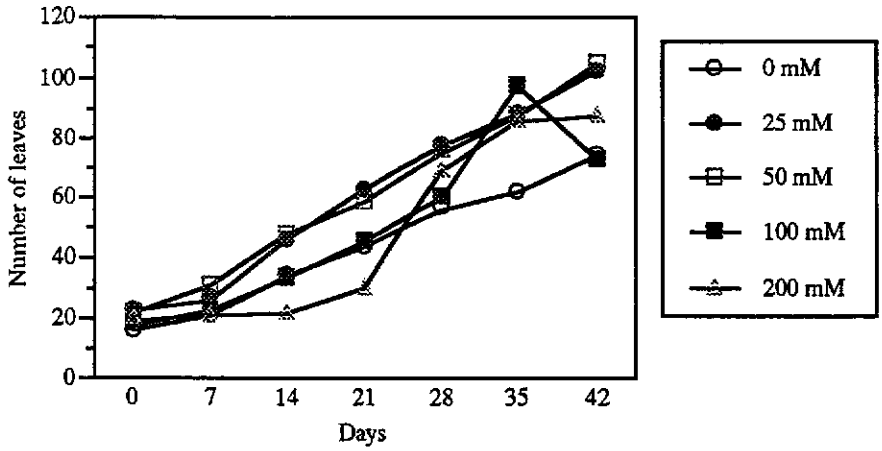

FIGURE 2. The average number of live leaves present on *R. eremaea* seedlings in the different salinity treatments, over the 6 week period.

TABLE 3. Regression (y = *b*x + *a*) and correlation (*r*) of harvest values for *R. eremaea* seedlings by treatment.

Treatment	Leaf no. & height†		Shoot & root weight†	
mM NaCl	Regression	r	Regression	r
0	L = 0.815 H - 14.719	0.939	S = 0.370 R + 0.138	0.415
25	L = 0.894 H - 15.251	0.879	S = 1.338 R + 0.007	0.938
50	L = 1.229 H - 43.906	0.876	S = 0.667 R + 0.102	0.642
100	L = 0.631 H - 5.084	0.891	S = 1.551 R + 0.036	0.837
200	L = 0.653 H - 13.217	0.818	S = 1.350 R + 0.009	0.976

† where L = leaf number, H = seedling height, S = dry shoot weight, R = dry root weight.

Soil Chemistry

Saline solutions tended to progressively raise both estimated conductivity (ECe) and pH (Table 4). pH values of the 200 mM treatment were significantly higher than the lowest salinity level and the control, which were of similar acidity. All salinity treatments developed higher conductivities than the control, but only the 50 and 200 mM treatments produced conductivity levels of any signif

DISCUSSION

Growth enhancement shown under applied salinity provides evidence that *Rhagodia eremaea* can be classed as a halophytic species. In the present trial, growth at the two lower salinity levels points to an initial response, presumably to sodium (Sharma, 1982), in stimulation of shoot growth, particularly of foliage production. Similarities in overall height and shoot biomass suggest a compromise between foliage production and stem mass between treatments, as indicated by plants in the 25 and 50 mM salinity regimes. Under these two salinity regimes shoot growth was then followed by an increase in root growth. Higher salinity levels have the reverse effect, inhibiting root growth prior to shoot growth. The build up of salt in the soil at the highest level applied was clearly too toxic for this species, causing depressed shoot and root growth. For halophytes, rapid root growth is of great ecological significance, and appears to be an essential adaptation to saline conditions (Waisel, 1972).

Seedlings grown in the 50 mM treatment had high root weights and a shoot/root ratio similar to that of control seedlings. Plants in this treatment also exhibited an increased number of side shoots and branching, not observed in the other treatments. NaCl has been shown to stimulate intense sprouting of lateral leaf buds in halophytes (Waisel, 1972) although Troughton (1960) claimed that high water stress (induced by NaCl) promotes an increase in root growth and subsequently decreases in shoot growth,

TABLE 4. Mean values for estimated conductivity of saturated extract (ECe) and pH of soil (n=5).

Parameter		Treatments (mM of NaCl)					Significance
		0	25	50	100	200	
Conductivity	Mean	2.4a	5.2ab	8.9bc	5.2ab	10.6c	p<0.005
ECe (dS m^{-1})	SD	1.5	2.1	5.1	0.5	2.4	
pH	Mean	4.4a	4.4a	4.5ab	4.9ab	4.7b	p<0.001
	SD	0.2	0.2	0.1	0.2	0.2	

Values with similar letters not significantly different with Tukey's pairwise comparison test.

with total growth of the plant reduced. Seedlings which died in the 200 mM treatment had lower shoot/root ratios (close to 1, data not shown) indicating root stimulation prior to succumbing. Surviving plants in the 100 and 200 mM treatments had highest mean shoot/root ratios, suggesting that gradual accumulation of salt eventually leads to inhibition of root growth in *R. eremaea.*

Morphological characteristics associated with halophytes grown in the absence of salt (NaCl), include development of a spindly or etiolated appearance, reduced branching, small leaves and an overall small photosynthetic area (Poljakoff-Mayber, 1975). These characteristics were observed in the control treatment, and suggest that growth of *R. eremaea* may be limited in the absence of NaCl. Plants subjected to the 100 mM treatment had considerably less root growth than the control, suggesting that this salinity regime provided conditions close to the upper limit of salt tolerance for *R. eremaea* seedlings. Waisel (1972) suggests that most halophytes can endure long periods in relatively high salt concentrations, with little or no additional growth.

At salt concentrations above the tolerance limit for individual seedlings, salt injury and eventual death of the plant occurs. The results obtained from the 200 mM treatment showed this regime had an inhibitory effect on all aspects of growth, including large numbers of dead and fallen leaves. Most seedlings that died (55%), did so within 3 weeks of initiation of the experiment. Of the seedlings that survived 6 weeks in this treatment, all had symptoms consistent with those of salt injury; necrosis of the leaf margin and curling of the leaves are characteristic of salt injury in plants (Levitt, 1980). The frequent reddening observed on some leaves and petioles of seedlings in the 100 and 200 mM treatments may have been an expression of nutrient deficiency. Decreased growth in saline conditions has been explained in terms of suppression of nutrient absorption due to the selective uptake of NaCl, however, there is insufficient evidence to suggest that salt injury is a direct result of nutrient deficiency (Levitt, 1980). Soil pH also affects nutrient availability, and hence growth, although pH values obtained in this investigation (ranging from 4.5 [0-25 mM] to 4.7-4.9 [100-200 mM]), were well within the pH range for optimal nutrient uptake by plants (Russell, 1961).

Leaf succulence is considered a good indicator of salt stress, and is normally stimulated by an accumulation of Cl^- in the leaves (Black, 1958). Greenway, Gunn & Thomas (1966) noted that halophytes often respond to high concentrations of NaCl with increased succulence of mature leaves. Thickening and succulence of the leaves is due to the development of larger cells in the spongy mesophyll and the presence of a multi-layered palisade tissue. Salinity inhibits cell division in halophytes, with cell extension remaining unaffected (Poljakoff-Mayber, 1975). This suggests salt uptake is the primary cause of leaf succulence in *R. eremaea* seedlings subjected to relative high levels of salinity.

The control of intercellular electrolyte concentrations to promote osmotic adjustment and reduce the effect of inorganic ion toxicity is an important adaptation for halophytic plants. It is hypothesised that *R. eremaea* may avoid high internal ion concentrations in soil of low salinity by the re-distribution of ions from the shoot and older leaves to younger leaves (*cf* Greenway *et al.*, 1966), and to a limited extent by increased stem and leaf succulence. At levels up to 9 dS m^{-1}, ion export may continue to occur, as the plants remained healthy while seedling growth was reduced (Black 1956, 1960). Higher levels of soil conductivity were clearly too toxic for *R. eremaea* seedlings, resulting in cessation of growth and an increase in leaf succulence. A continual build up of internal salt concentrations may occur in high levels of salinity, unless ion uptake is stopped or export becomes appreciable (Greenway & Thomas, 1965).

CONCLUSION

Rhagodia eremaea is a halophyte and showed noticeable differences in growth as a response to salinity treatments in glasshouse trials over a six week period. Applications of low salt levels stimulated leaf production and root growth. Significant differences were not observed in

plant height or shoot biomass between treatments. Seedlings in the 50 mM salinity treatment had more numerous side shoots and branches, as well as a more vigorous root system. Under the conditions used, weekly irrigation of 50 mM NaCl, was the most favourable salinity regimes in respect of growth of *R. eremaea* seedlings. The control treatment seedlings were spindly and showed relatively less growth. Control seedling results were similar to those for the 100 mM treatment, suggesting that salt-induced growth inhibition occurs between 50 and 100 mM NaCl for *R. eremaea.*

Seedling losses only occurred in the two stronger treatments (100 and 200 mM), with survivals of 85 and 25%, respectively, over the six week experimental period. Effects of salt injury were prominent in these two treatments, characterised by necrosis of the leaf margins and incurving of leaves. A distinct reddening of the leaves and petioles may be due to a nutrient deficiency. This may be caused by a suppression of nutrient absorption due to the selective uptake of NaCl. Thickening and succulence of leaves was a response to the higher salinity regimes, and is not considered to be an avoidance mechanism of high internal salt concentrations. Avoidance of salt injury is probably due to the re-distribution of salt from the older leaves and shoots and to a limited extent succulence.

Indications are that revegetation of saline mine waste dumps with *R. eremaea* will be possible on materials where the soil solutions have conductivity values between 5-9 dS m^{-1}. This is equivalent to NaCl solution concentrations of > 25 mM and < 100 mM as used in this investigation. Research into germination response and tolerance of adult *R. eremaea* plants, over a longer time period, is required to confirm suitable field establishment procedures.

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EARLY GROWTH OF *EUCALYPTUS PATENS* ON COAL WASTE IN RESPONSE TO NITROGEN AND PHOSPHORUS FERTILISATION

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SUMMARY

This paper reports the response of *Eucalyptus patens* seedling growth to fertiliser nitrogen and phosphorus, when grown on two waste materials from the Muja open - cut coal mine. The reported acid tolerance potential of this species rates it high on the list for potential rehabilitation use. Seedlings were grown in pots for 75 days and harvested. Observations were made on height growth, leaf development, dry matter production and on foliar nitrogen and phosphorus.

Significant differences in height became detectable earlier in over - burden from above the Ate coal seam than in the inter - burden from between the Ceres and the Diana coal seams. In the absence of phosphorus there was no response to nitrogen. At all levels of P there was always a response to increased nitrogen, consistently greater in Ate over - burden than in Ceres - Diana inter - burden.

At the highest nitrogen level the best total dry weight response to P was at 30 kg ha^{-1}. Response to N peaked at this level of P in Ate but was lower in Ceres - Diana. At low fertiliser levels the greatest relative response was to phosphorus, with root weight benefiting more in Ate over - burden and shoot growth in Ceres - Diana inter - burden. Shoot growth response to nitrogen was consistently greater than root growth. The response to P was proportionately greater than to N at the lowest fertiliser level. At 0 - 15 kg ha^{-1} P shoot response was lower than root response in Ate over - burden, but the reverse was true in Ceres - Diana. At the higher P levels resources went to shoots at the expense of roots, with shoots responding to P up to 30 kg ha^{-1} P in Ate over - burden. With high nitrogen levels root growth response to P was greater at low P and least at high P. Root growth showed no response at all N levels from 30 to 60 kg ha^{-1} P.

It is concluded that initial fertilisation of 30 kg ha^{-1} P and 40 kg ha^{-1} N would be appropriate for securing good early growth and establishment of *E. patens*.

INTRODUCTION

Species of plants used in coal - mine dump restoration must be able to tolerate the conditions characteristic of the dump material. These materials are typically low in nutrient elements and are of poor structure. Chemical and physical properties of the strata above and between coal seams at Muja, Collie, are described in Dames & Moore (1983). A classification of the layers of materials removed in the course of winning coal, mainly based on acidity, has led to the use of preferred (Class 1) materials for the surface dressing of dumps around the open - cut Muja mine. Class 1 materials are from the upper levels of Ate over - burden, and from Ceres - Diana and Galatea - Hebe inter - burdens between the coal seams of those names.

The inter - burden materials are mainly derived from sandstones. They have varied amounts of acidic coal fragments / sulphides and tend to be coarse textured with no organic matter or nitrogen. They are prone to compaction when dry. Bulk density values lie in the range of 1.4 - 1.7 g l^{-1} and field capacity between 13.4 and 17.6% for the Class 1 materials (Fox, Colquhoun & Owens 1988). Waste dumps at Muja have a low clay fraction, of kaolin. This provides typically low cation exchange and low water holding capacity (McKissock & Gilkes 1991). The soil reaction is acidic and phosphorus is not detectable.

It is assumed that those species which are represented in natural regeneration on abandoned dumps at Collie have a degree of tolerance to the extreme conditions. Successfully established individuals can be assumed better suited to dump conditions than the run of the species within the near - by forest. For managed restoration the preferred end - point species are of the genus *Eucalyptus*, due to original dominance in the area, height and life-span. It is advantageous for these to be grown with associate understorey species, including *Acacia* spp., and other legumes, which have the capacity to symbiotically fix atmospheric nitrogen.

Eucalyptus patens Benth. has been found growing on acidic material at Collie, W. A. (Bartle & Riches 1978). It has been reported as present on at least three old abandoned dumps (Koch 1984). Commonly called the Western Australian blackbutt, Swan River blackbutt or yarri, it is a large jarrah forest tree found from Wooroloo to Esperance (Marchant *et al.* 1987) within a latitudinal range of some 500 km, from 32 - 35°S. *E. patens* occurs within some 120 km of the coast from sea level to 300 m altitude. In the north it is mostly found in valleys or depressions in the Darling Range and further south on deeper alluvial loams or clay soils in hilly areas. With this amount of geographic spread in occurrence it is reasonable to assume some degree of variation within the species

(Fox *et al.* 1988). In its natural forest habitat, *E. patens* grows best on moist, sandy loams, with well developed clay subsoil, on lower slopes, valleys and creek margins (Boland *et al.* 1984). It can grow to a height of 46 m, with a large straight bole up to 1.8 m in diameter.

The potential of *E. patens* as a suitable tree for reafforestation of coal mine dumps in the Collie area depends on presumed natural tolerance of acidity and on its ability to utilise and respond to applied nutrients. Bartle & Riches (1978) recorded *E. patens* on inter - burden material with pH down to 4.5 and Koch (1984) noted *E. patens* growing where the pH was as low as 2.7. Earlier experiments demonstrated that several *Eucalyptus* species grown on coal spoil from Collie can respond to fertilizer treatments (Fox, Colquhoun & Leone 1987). Eighty two day seedling growth of *E. patens* was better than both *E. camaldulensis* Dehnh. and *E. wandoo* Blakely in inter - burden materials. *E. patens* also produced greater dry matter as a percentage of initial weight than did *E. calophylla* Lindley, a species with large cotyledons. Subsequently *E. patens* was included in a field trial at Muja (Doronila & Fox 1990).

Eucalyptus plantations are often routinely fertilized although under some conditions there is no response to fertilizer (Cotterill, Moran & Grigg 1985). Combined application of N and P may give a dramatic growth response where little response follows either N or P alone (Anon. 1981). A number of studies indicate nitrogen as the primary limiting factor for eucalypts (Crane 1978, Ellis *et al.* 1985). Eucalypts rarely respond to P alone and generally may have a low requirement for phosphorus (McKimm & Flinn 1979). A previous pot trial in which seedlings were grown for 20 months on the three Class 1 materials (Fox *et al.* 1988), indicated phosphorus was the primary limiting factor to growth in the Muja materials.

The present experiment was designed to seek responses in early seedling growth of *Eucalyptus patens* to different levels of applied nitrogen and phosphorus fertilisers in two of the Class 1 materials. The objectives of this work were to provide evidence that fertiliser application enhances growth and to quantify the levels of N and P fertilisers required.

METHODS

Two strata from the Muja open - cut mine, namely Ate over - burden and Ceres - Diana inter - burden, were used as growth media. Both materials were passed through a 3 - 4 mm mesh to remove large rock fragments and ensure uniformity of materials within fertilisation treatment sets. Ate over - burden is light brown in colour with a sandy - loam texture, pH (1:5 H_2O) of 4.5 and mean electrical conductivity of 980 mS cm^{-1}. Ceres - Diana is white, coarser than Ate over - burden, but contains more fine particles, has a lower conductivity, of 107 mS cm^{-1}, and similar pH (Table 1).

Seeds of *Eucalyptus patens* were collected from the abandoned Black Diamond coal mine at Collie (33°20' S, 116° 06' E) in October 1988 and sown in sterilised coarse sand germination trays in March 1989. Four hundred seedlings were selected at the 2 - 4 leaf stage (7th May) and potted out into 7 by 7 by 10 cm plastic pots, half into Ate over - burden and half into Ceres - Diana inter -

TABLE 1. Chemical and physical characteristics of the waste materials.

Stratum	Ate Over - burden	Ceres - Diana Inter - burden
*Elemental composition (mg kg^{-1})		
Nitrogen	-	-
Phosphorus	-	-
Potassium	0.57 - 5.3	2.0 - 5.6
Magnesium	1 - 37	4.8 - 37
Sodium	7.2 - 310	58 - 120
+Other characteristics		
Conductivity (mS cm^{-1})	980	107
pH (1:5 H_2O)	4.5	4.6
Texture	Sandy loam	Sandy loam
Colour	Light brown	White
Bulk density (g l^{-1})	1.4	1.5
Field capacity (%)	14.9	17.6

** after Dames & Moore (1983); + after Fox, Colqhoun & Owens (1988).*

burden. Seedlings were measured (to 0.5 mm) from cotyledon nodes to the base of the uppermost bud. Pots were then allocated to 16 treatment groups, with twelve replicate pots for each treatment and material. Initial heights were tested by one - way analysis of variance to ensure equal heights amongst treatment groups for each material. Pots were shifted between treatments to achieve low variance. After assignment to treatment groups the Ate seedling treatments had means between 16.4 and 16.6 mm, and the Ceres - Diana set means lay between 9.8 and 11.4 mm.

Four levels of nitrogen, supplied as Agran™, ammonium nitrate, containing 34% N, and four levels of phosphorus, as single superphosphate, containing 9.1% P were used (Table 2). Fertiliser was weighed out and applied to individual pots (14th May). Drained pots were used and plants were hand watered to field capacity every three days. Care was taken that water did not escape through the base of the pots. Seedlings were maintained under glasshouse conditions at the Field Trial Area on the Bentley Campus of Curtin University, with pots held in sets in plastic trays. Trays were shifted in position within the glasshouse on a systematic basis.

Plant heights were measured at 25, 50 and 75 days and leaf numbers were counted at 10, 60 and 75 days after fertiliser application. Seedlings were harvested at 75 days, washed free of soil, rinsed and placed between newspaper and cardboard in plant presses and then dried in an oven at 105°C for 24 hours. Weights of roots and shoots were recorded.

Leaves from each replicate of the Ate over - burden set were pooled into treatments (16) and foliar N and P levels were determined by Kjeldahl digestion and the molybdenum blue method respectively (Allen 1974).

RESULTS

During the course of the experiment there were only 3 seedling deaths in Ate over - burden but 35, or 18%, of those in Ceres - Diana did not survive. Of the losses in Ceres - Diana 80% died within the first 25 days after potting out. Chi - square analysis indicated no difference in survivals between the 16 individual treatments, but when treatments were pooled there were significant differences due to both the N - level and the P - level. Most deaths occurred at the highest nitrogen level (20, or 42%), and in zero phosphorus with nitrogen (15, or 31%).

Height growth

Significant differences in height between treatments had become apparent by 25 days from fertiliser application (Table 3).

The Ate over - burden treatments with either no nitrogen or no phosphorus were significantly shorter than those with both added. Differences were not as significant in Ceres - Diana, but the pattern was similar for both the upper and lower mean heights. At 25 days the N1 P2 treatment was tallest in Ate and second in Ceres - Diana, where N1 P1 was tallest. By 50 days the three treatments with the highest nitrogen level and phosphorus, had become tallest in Ate over - burden, in the order N3 P2 >

TABLE 2. Treatments used in *Eucalyptus patens* trial.

Treatment No.	Treatment code	Nutrient (kg ha^{-1}) N	Nutrient (kg ha^{-1}) P	Fertiliser (g per pot) Agran (34 % N)	Fertiliser (g per pot) Super P (9.1 % P)
1	N0 P0	0	0	0	0
2	N1 P0	20	0	0.03	0
3	N2 P0	40	0	0.06	0
4	N3 P0	80	0	0.12	0
5	N0 P1	0	15	0	0.08
6	N1 P1	20	15	0.03	0.08
7	N2 P1	40	15	0.06	0.08
8	N3 P1	80	15	0.12	0.08
9	N0 P2	0	30	0	0.16
10	N1 P2	20	30	0.03	0.16
11	N2 P2	40	30	0.06	0.16
12	N3 P2	80	30	0.12	0.16
13	N0 P3	0	60	0	0.32
14	N1 P3	20	60	0.03	0.32
15	N2 P3	40	60	0.06	0.32
16	N3 P3	80	60	0.12	0.32

N3 P3 > N3 P1; this continued until the harvest. At 75 days the tallest treatments in Ceres - Diana were N3 P1 > N3 P2 > N2 P2.

Figure 1 illustrates the progression of mean height growth for all plants in treatments and the tallest 5, for Ceres - Diana. Treatment numbers 4 (N3 P0) and 16 (N3 P3) had 5 survivors each. The overall trend for all and best 5 replicates was similar, indicating a generally uniform pattern of height response to fertiliser treatment. Aggregation of heights at harvest by nitrogen and phosphorus levels (Table 4) indicates a much greater response to fertiliser by *E. patens* grown in Ate over - burden than in Ceres - Diana.

Plant height more than doubled with the lowest level of phosphorus (15 kg ha^{-1}) in both materials compared to nil phosphorus. Height was not significantly increased by additional phosphorus, apart from 30 kg ha^{-1} in Ate over - burden. Incremental responses to nitrogen occurred in both materials, with significant differences in height between all levels supplied. The incremental response to nitrogen was greatest in Ate over - burden. Both leaf area and leaf number tended to increase with increased plant height.

TABLE 3. Mean heights of *Eucalyptus patens* by fertiliser treatment.

Material	Ate over - burden				Ceres - Diana inter - burden			
Treatment	Days after fertiliser application							
code	0	25	50	75	0	25	50	75
N0 P0	16.46 a	25.55 a	31.77 abc	32.55 ab	10.83 a	17.29 f	20.92 f	22.86 d
N1 P0	16.38 a	25.04 a	28.58 a	29.50 a	10.79 a	18.05 f	22.33 f	22.17 d
N2 P0	16.46 a	25.71 a	27.17 a	27.25 a	10.75 a	18.64 ef	24.14 f	26.50 d
N3 P0	16.50 a	26.46 a	29.29 ab	29.00 a	10.71 a	15.93 f	20.17 f	19.80 d
N0 P1	16.46 a	30.58 a	38.63 c	41.42 b	10.71 a	20.38 cdef	26.25 f	29.42 d
N1 P1	16.50 a	40.71 b	56.04 d	61.46 c	10.29 a	25.88 a	44.50 bcd	54.12 c
N2 P1	16.54 a	41.46 b	67.75 e	81.63 d	10.75 a	25.73 ab	49.23 abc	63.45 b
N3 P1	16.46 a	43.09 b	73.27 e	103.32 e	9.79 a	25.09 abcd	55.09 a	90.23 a
N0 P2	16.50 a	30.21 a	37.50 c	39.88 b	10.25 a	16.92 f	24.13 f	26.50 d
N1 P2	16.46 a	45.42 b	59.50 d	66.38 c	10.46 a	25.80 ab	47.45 abc	60.25 c
N2 P2	16.38 a	40.67 b	68.21 e	86.17 d	10.96 a	23.92abcde	48.37 abc	64.17 b
N3 P2	16.29 a	40.17 b	82.17 f	127.88 f	11.00 a	24.71abcde	53.57 ab	81.64 a
N0 P3	16.42 a	29.17 a	36.83 bc	39.62 b	11.04 a	20.32 cdef	28.65 ef	30.89 d
N1 P3	16.42 a	40.21 b	55.92 d	67.13 c	11.21 a	25.50 ab	44.00 cd	52.54 c
N2 P3	16.42 a	40.63 b	67.50 e	85.71 d	11.17 a	21.08bcdef	47.67 abc	63.25 b
N3 P3	16.58 a	39.91 b	73.50 a	106.23 e	11.38 a	19.91 def	37.50 def	60.30bc
F values	0.03	8.31	30.64	56.88	0.11	2.72	11.99	25.93
p =	1.000	<0.0001	<0.0001	<0.0001	1.000	<0.001	<0.0001	<0.0001

Treatments with the same letter at a measurement are not significantly different (LSD test at p<0.05).

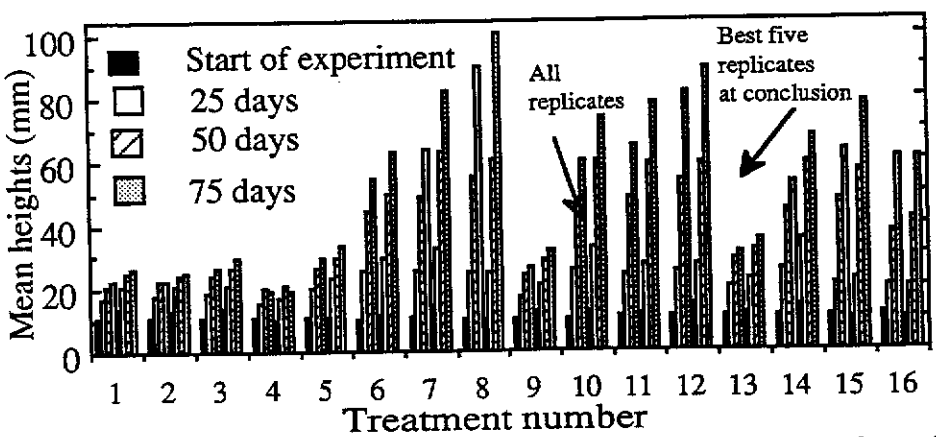


FIGURE 1. Comparison of heights of survivors and the best 5 at the end of the experiment for plants grown in Ceres - Diana material. (Treatment numbers as in Table 2).

Leaf numbers

At ten days after fertilisation the majority of plants had 4 leaves and there were no significant differences in leaf numbers between treatments in either material. At 60 days the 7 treatment sets without either of nitrogen or phosphorus had significantly fewer leaves than other treatment sets. The N3 P3 and N3 P2 sets had highest numbers in each waste material. These patterns continued through to harvest. One replicate in the N3 P3 treatment using Ate over - burden had 32 leaves, the maximum number attained. Some plants in the high nitrogen Ceres - Diana sets showed stunted height growth and rosette leaf development.

Aggregation of leaf numbers by nitrogen and phosphorus levels at harvest (Table 5) indicates that plants grown in Ate over - burden had become more leafy than those in Ceres - Diana.

The pattern of response to nitrogen was similar in both materials, mirroring the height changes, and there was a much greater difference between plants with no phosphorus than those with phosphorus. It was observed that treatments with zero phosphorus in both soils had a number of plants with reddish - purple coloration from mid - vein to leaf edge. Several plants in Ceres - Diana had chloritic and red patches on leaves.

Dry matter production

Analysis of variance using data for all 16 treatments, for each material separately, indicated significant differences in each of shoot, root, and total dry weights, and in the shoot/root ratios. Ate over - burden treatments had greater mean total dry weights than Ceres - Diana (Figures 2 & 3).

TABLE 4. Mean heights of *Eucalyptus patens* by nitrogen and phosphorus levels at 75 days.

Treatment kg ha $^{-1}$	Ate over - burden	Ceres - Diana inter - burden
N 0	38.4 a	27.3 a
N 20	56.1 b	48.4 b
N 40	70.2 c	57.4 c
N 80	91.6 d	70.2 d
F - value	118.95***	30.31***
P 0	29.6 a	23.0 a
P 15	71.9 b	58.5 b
P 30	80.1 c	55.2 b
P 60	74.7 b	51.8 b
F - value	126.86***	20.29***

Means with the same letters in sets do not differ significantly with the least significant difference test.
****All F - values, $p<0.001$.*

In both materials the greatest growth response was to the first level of phosphorus addition, although biomass tended to rise steadily with increasing levels of nitrogen at each of the P levels. Nitrogen responses peaked at either 15 kg ha^{-1} P (zero N, 40 kg ha^{-1} N) or 30 kg ha^{-1} P (20 kg ha^{-1} N, 80 kg ha^{-1} N). Within nitrogen levels, zero N had the best yield at 15 kg ha^{-1} P, both the 20 and 80 kg ha^{-1} N sets peaked at 30 kg ha^{-1} P and at 40 kg ha^{-1} N all P treatments were similar.

Aggregation of harvest weights by N and P levels (Tables 6 and 7) also indicated significant differences in each of shoot, root and total dry weights, and in shoot / root ratios of *E. patens* grown in both materials. F - values were lower in Ceres - Diana and survival of inadequate replications prevented use of two - way analysis of variance for this medium.

Shoot and root weights

The least significant difference test showed that the seven treatments without either of N or P (numbers 1 - 5, 9 and 13 of Figure 1), had significantly least mean shoot weights. All were < 0.16 g in Ate over - burden and < 0.10 g in Ceres - Diana. This set did not exceed 0.42 g total weight in Ate or 0.21 g in Ceres - Diana.

Heaviest shoot weights were attained in treatment N3 P2. The four zero P treatments had lowest mean root weights of 0.08 g or less in Ate and 0.06 g or less in Ceres - Diana.

Ratios for shoot / root were similar for both materials (Figure 4).

Absence of nitrogen was associated with reduced ratios.

TABLE 5. Mean leaf numbers at 75 days of *Eucalyptus patens* by nitrogen and phosphorus levels.

Treatment kg ha $^{-1}$	Ate over - burden	Ceres - Diana inter - burden
N 0	6.4a	5.6a
N 20	8.3b	7.6b
N 40	8.9b	8.1b
N 80	11.3c	9.9c
F - value	24.03***	19.68***
P 0	5.0a	4.8a
P 15	9.1b	8.6b
P 30	10.3c	8.3b
P 60	10.5c	8.0b
F - value	36.83***	17.38***
Interaction	N x P	
F - value	4.26***	5.49***

Means with the same letters in sets do not differ significantly with the least significant difference test.
****All F - values, $p<0.001$.*

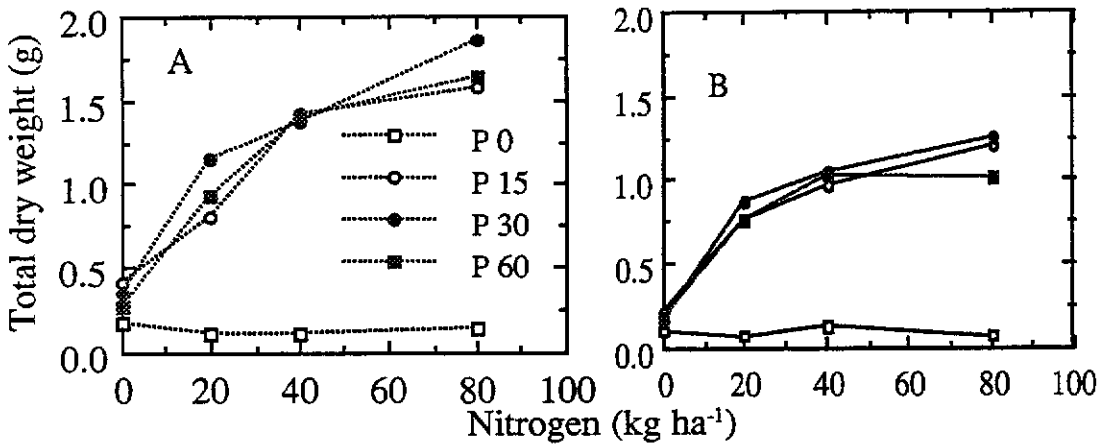


FIGURE 2. Total dry weights indicating phosphorus response by nitrogen levels. A. Ate over - burden; B. Ceres - Diana inter - burden.

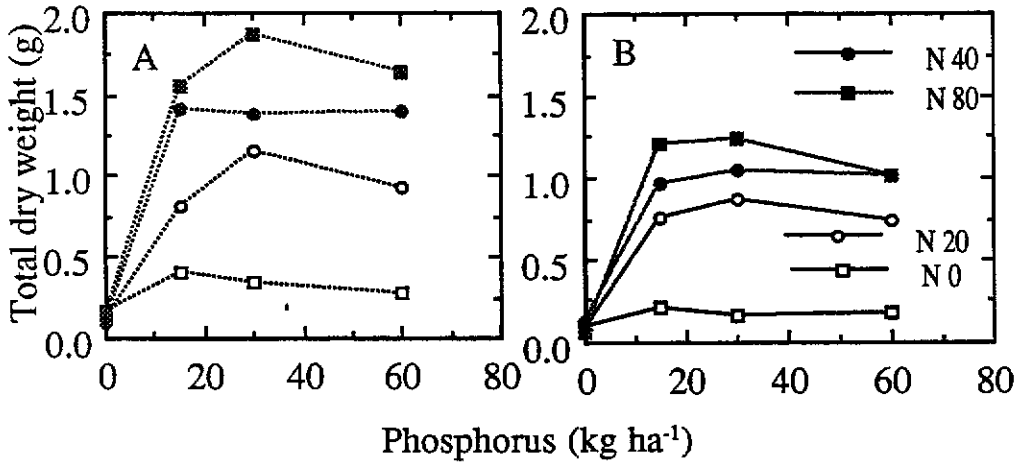


FIGURE 3. Total dry weights indicating nitrogen response by phosphorus levels. A. Ate over - burden; B. Ceres - Diana inter - burden.

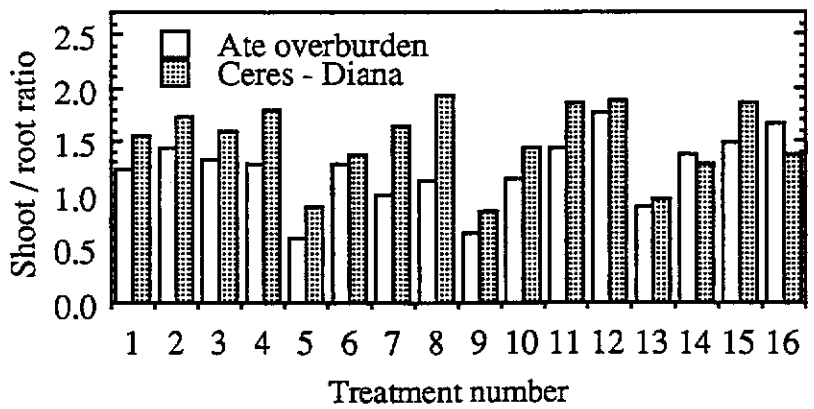


FIGURE 4. Comparison of shoot / root ratios by treatment for two coal mine waste materials (Treatment numbers as in Table 2).

TABLE 6. Mean weights of *E. patens* grown in Ate over - burden, by N and P levels.

Treatment Element	Weight (g) Shoot	Root	Total	Shoot / root ratio
Nitrogen (kg ha^{-1})				
0	0.124a	0.176a	0.300a	0.70a
20	0.414b	0.335b	0.749b	1.24b
40	0.601c	0.472c	1.073c	1.27b
80	0.776d	0.527c	1.303d	1.47c
F - value	68.9	41.4	63.7	27.4
sig. p =	< 0.001	< 0.001	< 0.001	< 0.001
Phosphorus (kg ha^{-1})				
0	0.072a	0.061a	0.133a	1.18b
15	0.526b	0.524c	1.050b	1.00a
30	0.685c	0.499c	1.184c	1.37b
60	0.633c	0.426b	1.059bc	1.49b
F - value	68.9	78.2	79.5	9.68
sig. p =	< 0.001	< 0.001	< 0.001	< 0.001
Interaction N*P				
F - value	9.18	6.82	8.35	4.42
sig. p =	<0.001	<0.001	<0.001	<0.001

Means within columns with the same letter do not differ significantly (p< 0.05 using LSD test).

TABLE 7. Mean weights of *E. patens* grown in Ceres - Diana inter - burden, by N and P levels.

Treatment Element	Weight (g) Shoot	Root	Total	Shoot / root ratio
Nitrogen (kg ha^{-1})				
0	0.074a	0.085a	0.160a	0.87 a
20	0.359b	0.280b	0.638b	1.28 b
40	0.535c	0.333bc	0.867c	1.61 c
80	0.613c	0.363c	0.976c	1.69 c
F - value	29.74	17.83	27.10	14.19
sig. p =	< 0.001	< 0.001	< 0.001	< 0.001
Phosphorus (kg ha^{-1})				
0	0.055a	0.042a	0.097a	1.31a
15	0.467b	0.303b	0.771b	1.54a
30	0.469b	0.307b	0.776b	1.53a
60	0.416b	0.317b	0.733b	1.31a
F - value	14.99	17.12	17.31	0.99
sig. p =	< 0.001	< 0.001	< 0.001	0.40

Means within columns with the same letter do not differ significantly (p< 0.05 using LSD test).

Ratios in Ate over - burden were lowest in N0 P1 (0.59) and N0 P2 (0.61). These were followed by N0 P3 (0.83) and N2 P1 (0.94), no others were lower than 1.00.

In Ceres - Diana ratios below 1.00 were also found in the three zero nitrogen plus phosphorus treatments, *viz* : N0 P2 (0.79), N0 P3 (0.80) and N0 P1 (0.86).

Response to nutrients

The Muja materials differ from most growing media reported in the literature, being devoid of any significant nitrogen or phosphorus. The results indicate that in the absence of phosphorus there is no response to nitrogen. At all levels of P there was always a response to increased nitrogen, consistently greater in Ate over - burden than in Ceres - Diana. The onset of significant differences in height growth was also observed sooner in Ate over - burden. At the highest nitrogen level the best total dry weight response to P was at the 30 kg ha^{-1} level (Table 6), and response to N tended to peak at this level of P (Figure 3A). The peak in Ceres - Diana was somewhat lower, perhaps at 20 kg ha^{-1} (Figure 3B).

At zero nitrogen mean root weight was greater than shoot weight in both materials. Both shoot and root weight increased with nitrogen fertiliser. The proportional response to N from zero for each N level was greater in Ceres - Diana than in Ate over - burden, although less significance was observed in shoot weight increase than for Ate. For both materials the response to nitrogen was consistently greater in shoot than in root growth. Increased root weight was not significant in Ate over - burden between 40 and 80 kg ha^{-1} N nor was it between 20 and 80 kg ha^{-1} in Ceres - Diana. As the shoot weight increased with nitrogen so did the shoot / root ratio, reaching its highest value at 80 kg ha^{-1} N. Values for shoot/ root ratio were higher in Ceres - Diana for all nitrogen levels.

The response to P was proportionately greater than the response to N at the lowest fertiliser level, albeit from a lower zero base for P. The P response in shoot weight was less than that for root weight from zero P to 15 kg ha^{-1} P in Ate, but in Ceres - Diana the shoot response was greater. At higher P levels root weight did not increase with additional P whereas shoot growth continued to show a response up to 30 kg ha^{-1} P in Ate over - burden. In Ceres - Diana the small increases in both shoot and root weight with increased P beyond 15 kg ha^{-1} P were not significant.

At 15 kg ha^{-1} P shoot and root weights were similar in Ate over - burden, whereas at zero P and higher P levels the shoot/root ratio exceeded 1.00. It was > 1.00 in Ceres - Diana at all levels of P.

For total dry weight the nitrogen response was progressive with increased nitrogen, whereas there was little or no phosphorus response above the lowest P level (15 kg ha^{-1}). In the absence of phosphorus there was no response to nitrogen in either total dry weight (Figure 2) or root growth (Figure 5). At all levels of P there was always a response to increased nitrogen, this was greater in Ate over - burden than in Ceres - Diana (Figure 2). At the highest nitrogen level the best total dry weight response to P was the intermediate (30 kg ha^{-1}) level and response to N tended to peak at this level of P. The peak in Ceres - Diana may have been between 15 and 30 kg ha^{-1} (Figure 3B).

Root growth response to high nitrogen in Ate over - burden was best at 15 kg ha^{-1} P, but the response to lowest nitrogen was best at 30 kg ha^{-1} P (Figure 6,A). In Ceres - Diana root growth increased slightly with high nitrogen across the range of P, but most response to P came at 15 kg ha^{-1} P for all N levels (Figure 6,B). In the absence of nitrogen the initial response to P at 15 kg ha^{-1} declined consistently with increased P for both total (Figure 3) and root weight (Figure 6) in Ate over - burden, but was more static in Ceres - Diana. At higher nitrogen levels root growth response to P was best at lowest P and least to highest P in Ate over - burden; differences were less obvious in Ceres - Diana (Figure 5) but more losses had occurred at high nitrogen levels in this medium. Root growth generally declined at all N levels from 30 to 60 kg ha^{-1} P in Ate but was stationary in Ceres - Diana.

Shoot growth response to nitrogen was consistently greater than root growth. The response to P was proportionately greater than to N at the lowest fertiliser level. At 0 - 15 kg ha^{-1} P shoot response was lower than root response in Ate over - burden, but the reverse was true in Ceres - Diana. At higher P levels resources went to shoots at the expense of roots, with shoots responding to P up to 30 kg ha^{-1} P in Ate over - burden. With high nitrogen levels root growth response to P was greater at low P and least at high P. Root growth showed no response at all N levels from 30 to 60 kg ha^{-1} P.

Foliar nutrient levels

Nitrogen content of foliage was never less than 1%, even for those treatments which received no fertiliser nitrogen (Figure 7).

With added nitrogen the leaf concentration was highest at zero phosphorus, although there was no increase in the foliar concentration beyond that attained with the lowest level of applied nitrogen. For all levels of phosphorus there was no increase in foliar nitrogen with the lowest level of nitrogen. Beyond 20 kg ha^{-1} of nitrogen the intermediate phosphorus level resulted in the greatest gain in foliar nitrogen. At both low (15 kg ha^{-1}) and high (60 kg ha^{-1}) phosphorus there was a steady increase in foliar nitrogen with additional amounts of nitrogen fertiliser over the range of 20 - 80 kg ha^{-1}.

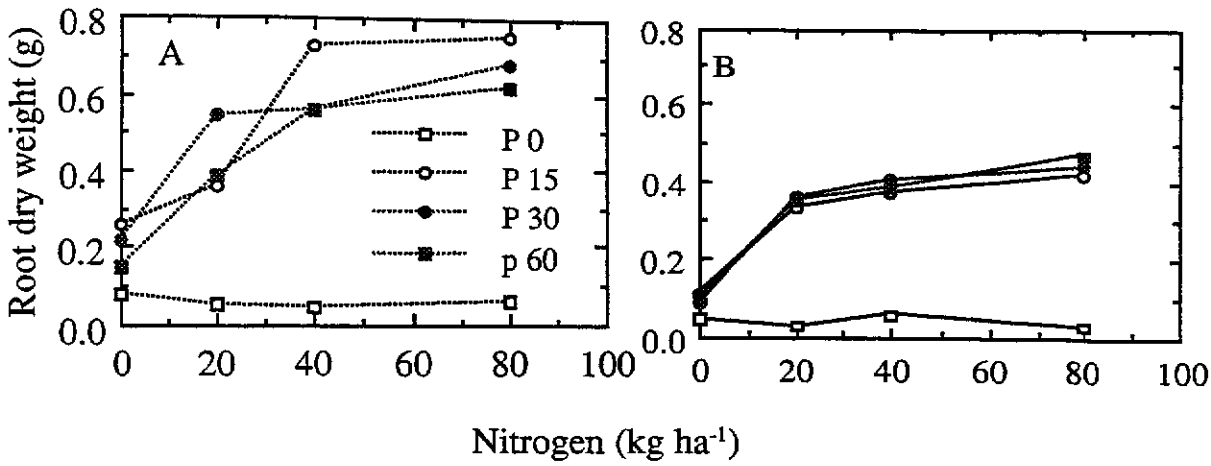


FIGURE 5. Root dry weights indicating phosphorus response by nitrogen levels. A. Ate over - burden; B. Ceres - Diana inter - burden.

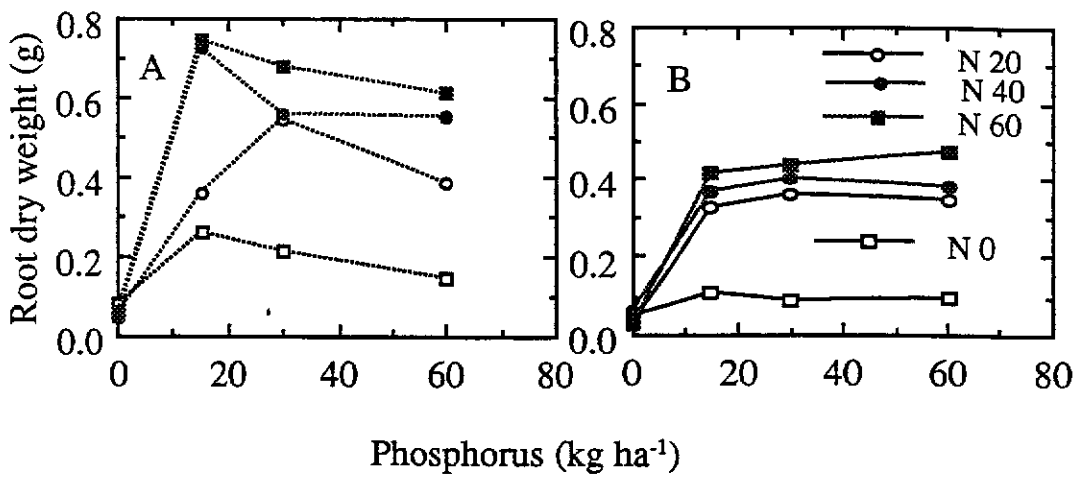


FIGURE 6. Root dry weights indicating nitrogen response by phosphorus levels. A. Ate over - burden; B. Ceres - Diana inter - burden.

In the absence of phosphorus fertiliser foliar P ranged between 102 ppm, at 80 kg ha^{-1} nitrogen, and 360 ppm, at zero nitrogen (Figure 8). In the absence of nitrogen, foliar phosphorus reached the highest levels with added P fertiliser. However, foliar P peaked at 30 kg ha^{-1} of added phosphorus, not only for zero nitrogen, but also at 40 and 80 kg ha^{-1} levels of nitrogen. Only in the case of the lowest level of added nitrogen did foliar phosphorus levels show a consistent increase with added phosphorus fertiliser.

The ratio of foliar N / P was low, at less than 10, in all P 30 kg ha^{-1} treatments, and with zero and low nitrogen at P 60 kg ha^{-1}. Ratios were very high in all zero phosphorus treatments and in the high P / high N combination. Ratios were between 11 and 22 in the P 15 kg ha^{-1} set.

Aggregation of single treatment values for foliar nitrogen and phosphorus by N and P applications (Table 8) indicated that seedlings grown at the highest nitrogen rate had significantly more foliar nitrogen (almost 2 x) than those which received no nitrogen. There was a progressive increase in foliar N with the amount of N applied, and differences were significant.

Nitrogen levels were significantly greater in plants without phosphorus compared to those at 15 and 60 kg ha^{-1} levels. The intermediate P application (30 kg ha^{-1}) did not differ from all other levels.

There was a wide variation in foliar phosphorus content. As a consequence no significant differences were observed between nitrogen treatments. At zero nitrogen foliar P was 2 - 3 times as great as in nitrogen treatments. The higher phosphorus fertiliser levels had significantly greater foliar P than the zero and 15 kg ha^{-1} sets.

DISCUSSION

The results presented confirm that a basal dressing of phosphorus is essential to secure eucalyptus growth in these nutrient deficient coal mine materials. Phosphorus was necessary to obtain a growth response above that obtained with zero levels of N and P. Deficiency symptoms became evident in the absence of phosphorus. Growth of *E. patens* in zero P treatments was presumably constrained by seed resource. It is estimated that an *E. patens* seed has a phosphorus content of the order of 67 mg (Fox *et al.* 1988). The amount of P estimated in zero P treatments, assuming an even spread through all tissues, ranged from 14 to 61 mg, confirming the lack of any utilisable phosphorus in the materials used.

Seedlings in Ate over - burden had not only greater growth than those in Ceres - Diana inter - burden, but also fewer losses. These results confirm some earlier findings with a fertiliser omission trial in which *Eucalyptus patens* survived better in Ate over - burden, with responses to fertiliser N and P. However, in Ceres - Diana individual responses to N and P could not be detected (Fox *et al.* 1988). It is possible that the phosphorus responses at zero nitrogen may have been associated with the calcium component of superphosphate (20%). Seed used in the earlier trial came from a forest source and in the present case from trees growing on an acidic, old coal mine dump west of Collie. In the earlier work with *E. patens*, some degree of genetic/ecotypic variation within this species had been postulated (Fox *et al.* 1988) and it is possible that the contrast in growth responses to different interburdens may have been associated with seed origin.

In a comparative study of several eucalypts Olsen & Bell (1990) found that the N requirement to achieve 50% of maximum yield was up to 60 kg ha^{-1} whereas no P was required. Our observed response to nitrogen by *Eucalyptus patens*, in essentially barren substrates, was progressive with increased nitrogen, whereas there was little or no phosphorus response above the lowest P level (15 kg ha^{-1}). Maximum yields were obtained in both materials at 80 kg ha^{-1} N and 30 kg ha^{-1} P. 50% of these maximum yields were attained with 15 kg ha^{-1} P in both materials, but whereas 40 kg ha^{-1} N was required in Ate over - burden only 20 kg ha^{-1} N was required in Ceres - Diana.

The only treatments in which root growth was consistently heavier than shoot growth were those with zero nitrogen and added phosphorus. Part of the reason for this may have been due to the use of pots. The short trial duration sought to minimise any constraint of pots on root development but by harvest many replicates had one or more roots protruding. This was less evident in Ceres - Diana. The overall poorer root development in this material was associated with a greater degree of surface hardening in this material, whereas Ate over - burden did not exhibit crusting to the same degree.

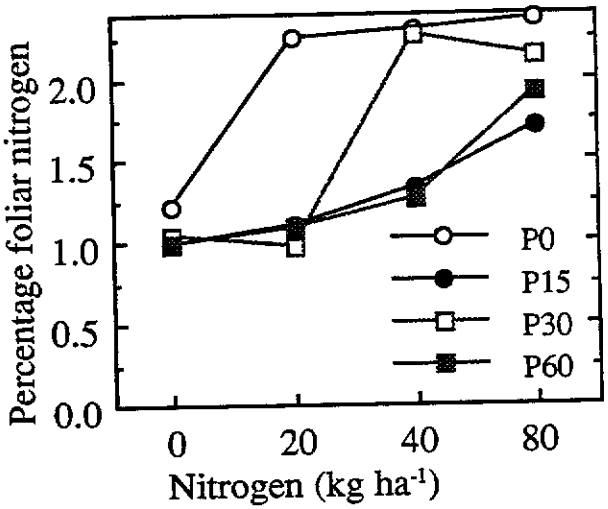
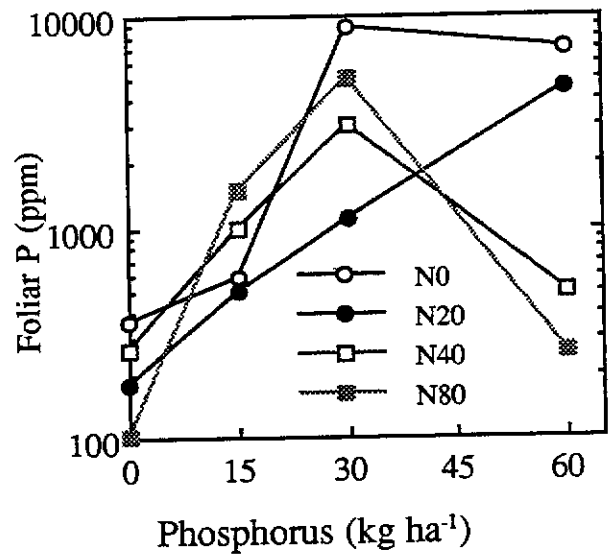


FIGURE 7. Foliar nitrogen as % leaf tissue.



(Note log scale).

FIGURE 8. Foliar phosphorus expressed as parts per million of leaf tissue.

Fast growing eucalypts require critical levels of nutrients to maintain growth. *E. globulus* is a typically fast growing species. It is more sensitive to low levels of P than *E. microcorys*, for example (Ward 1983). It follows that differences between species in growth rates will be associated with nutrient demand. In the present study foliar nitrogen did not fall lower than 1%, and this may

TABLE 8. Foliar phosphorus and nitrogen contents of *E. patens* plants grown in Ate over - burden.

Treatment (Fertiliser kg ha^{-1})	Foliar analysis	
	N(%)	P(ppm)
Nitrogen		
0	1.05a	4160a
20	1.36ab	1570a
40	1.79bc	1188a
80	2.03c	1714a
F - value	3.90	1.00
sig.	0.037	0.43
Phosphorus		
0	2.04b	222.5a
15	1.28a	892.5a
30	1.60ab	4450b
60	1.31a	3064ab
F - value	1.86	3.23
sig.	0.191	0.075

Means within columns with the same letter are not significantly different using the LSD test, p 0.05.

represent a lower critical level. Higher leaf nitrogen levels (> 2.2%) were associated with lack of phosphorus, and best yielding sets had 1.3 - 2.1% nitrogen.

It has been suggested that there may be some optimum ratio of foliar N / P for maximum growth. In *E. globulus* when this ratio is > 15, there is a response to P, and when < 15, there is a response to N (Cromer *et al.* 1981). Critical N / P ratios for a selection of Queensland eucalypts lie in the range of 4 - 10 (Olsen & Bell 1990). The values for the fastest grown sets of *E. patens* in the present study lay between 4 and 13 and plants denied phosphorus had high values. Very low ratios were noted for some sets denied nitrogen.

Early rehabilitation efforts on the Muja dumps concentrated on establishing pastures. For that procedure, potassium was included in initial fertilization. Analysis of inter - burden materials indicates the presence of this element in most *in situ* non - coal strata materials (Dames & Moore 1983). Earlier trials with *Eucalyptus patens* indicated that supplying potassium and trace elements may be detrimental to early eucalypt seedling growth (Fox *et al.* 1988). Potassium was not therefore included in the trial described in this paper. More recently McKissock & Gilkes (1991) report that potassium is present in very low quantities in both extant dumps and interburdens at Muja, but that the levels present are low in comparison with other Australian soils. The relatively high rainfall in the area may well be responsible for leaching out potassium.

The role of potassium fertilisation in eucalypts has not received a great deal of attention, presumably because it is rarely deficient. McKimm & Flinn (1979) reported no response to potassium or trace elements. Potassium, with or without trace elements, provided no additional response to that obtained by N or P (Cromer *et al.* 1981). An inter - active, positive response to potassium was reported by Ellis *et al.* (1985) and significant, but small, responses to eucalypt growth by potassium fertilizer are reported by Ward (1983).

High seedling mortality is a well documented response to high rates of fertilizer application (Christensen 1974, McGrath 1979). Fox *et al.* (1988) provided some evidence for *Eucalyptus patens* that lack of calcium or phosphorus may enhance early mortality with soluble fertilizers containing potassium and trace elements. In the present study most losses were within the first three weeks and were associated with either high nitrogen or nitrogen unbuffered by phosphorus. This suggests that small seedling roots are susceptible to damage by nitrogen fertiliser. Losses were mainly in Ceres - Diana and may have been associated with greater difficulty in root establishment in that medium. It can be recommended that direct seeding should not be associated with the use of soluble fertilisers *per se*. A field trial at Muja, which used potassium at the rate of 36 kg ha^{-1}, incorporated with superphosphate, gave an establishment rate at two years for *E. patens* of 1.4% of seed sown. *E. patens* was the only one of five eucalypts sown to establish on the relatively inhospitable Class III material (Doronila & Fox 1990).

CONCLUSIONS

The results presented confirm that phosphorus is the primary limiting factor to growth of the potentially useful *Eucalyptus patens* in both Ate over - burden and Ceres - Diana inter - burden. These are two of the three preferred Class I materials for the surface dressing of over - burden dumps at the Muja mine. As it is not generally practicable to segregate individual Class 1 materials for dump surface dressings the overall results are of more import than attempting to distinguish a prefered surface material. Nitrogen is ineffective in stimulating early seedling growth in the absence of phosphorus. The greatest fertiliser growth response is to the first level of phosphorus addition (15 kg ha^{-1} P), although biomass tends to rise steadily with increasing levels of nitrogen at each level of P. Nitrogen responses peak at 15 - 30 kg ha^{-1} P.

The main conclusion of this experiment is that the most appropriate fertilizer application is considered to be 30 kg ha^{-1} P with 40 kg ha^{-1} N at the season of sowing. This would minimise early seedling losses and avoid excess top growth at the expense of roots, both of which may be associated with higher levels of initial nitrogen fertilisation. As *Eucalyptus patens* appears able to make use of higher levels of nitrogen, a possible additional dressing of 40 kg ha^{-1} N could prove beneficial in the second growing season. Vigorous legume understorey growth may provide additional nitrogen. It is suggested that a useful field trial could test this supposition and also distinguish between the potential contribution of nitrogen from the legume understorey component currently included as standard practice.

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PHYTOTOXIC EFFECTS OF ALUMINIUM ON *PARASERIANTHES LOPHANTHA* AND *ACACIA DECURRENS*

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SUMMARY

Old abandoned dumps in the Collie coal field have been colonised by both native and introduced species and these indicate a potential for rehabilitation. The coal waste dumps are more acidic than the surrounding forest soils. Soil pH of less than 5.0 indicates a potentially high toxic presence of aluminium. Tolerance to aluminium is a feature of some plant species, and these may accumulate, avoid or exclude aluminium. Excluders are reported to include legumes, such as *Acacia* and *Paraserianthes*, by restricting translocation of the metal which is bound in root cells. Excluders may have reduced plant nitrogen levels generally but nitrogen is also higher in roots than shoots. Evolution of tolerance to metal stress may be comparatively rapid and although aluminium has many adverse affects on growth, plants tolerant of its presence also tend to be drought tolerant, an advantage for survival on old dumps which may be very dry in summer.

Seedlings of *Paraserianthes lophantha* and *Acacia decurrens* from dump and non-dump seed sources were subjected to varied levels of aluminium solution in pot culture. Hydrated aluminium chloride was dissolved in de-ionized water to provide concentrations of 200, 500, 700, 900, 1000 and 1100 ppm. Aluminium solutions were applied three times a week and a nutrient solution once a week. Surviving plants were harvested at ten weeks.

A. decurrens appeared better able to tolerate low levels of applied aluminium than *P. lophantha*, but at 500 ppm both species exhibited severe effects on growth. *P. lophantha* had a number of deaths in aluminium treatments. Plants of dump origin were not able to produce greater plant biomass over the trial period than non-dump plants. However there were distinct effects of treatment in relation to aluminium distribution within plants. Dump origin plants contained less total aluminium than non-dump plants indicating a possible avoidance mechanism. Dump progeny held more aluminium within the roots, transporting lower quantities to the foliage, suggesting an exclusion mechanism. Non-dump plants were more susceptible to wilting and it is hypothesised that dump progeny may have a denser structure than plants from non-dump sites. With increased aluminium applied plant nutrient contents decreased: calcium decline was the most severe. Trace element and manganese concentrations increased with added aluminium, and differences were noted in foliar levels between plants of dump and non-dump origin.

It is possible that the degree of apparent adaptation to aluminium levels may be related to the history of prior exposure. Whereas the *P. lophantha* seed came from plants established three years earlier by direct seeding onto a restored dump, the seed of *A. decurrens* was taken from self-sown trees established many years earlier on an un-restored waste dump. The trial conditions should be viewed as extreme cases of exposure. It is recommended that collection of seed for use on coal-mine rehabilitation sites should be made from plants growing on dumps already colonised successfully.

INTRODUCTION

The Collie coal field, located in the south west of Western Australia, has been exploited for nearly a century, but large scale operations commenced only forty years ago. Rehabilitation was not undertaken on the waste dumps, which were located in sparsely populated areas, until relatively recently. Unrestored dumps became severely eroded and were compacted in places. Some ingress of both native and introduced species is evident on old dumps and these provide a guide to species suitability for rehabilitation. The majority of the materials that make up coal waste dumps were originally below the water table and had not been previously exposed to surface conditions before excavation. When exposed to the atmosphere, such materials undergo rapid oxidation. Because of a high presence of pyrites (FeS_2), sulphuric acid is generated and coal waste dumps have low pH relative to the surrounding surface soils. Of eighty two samples taken at the Western Collieries No. 5 mine, pH ranged from 2.2 to 5.6 with a median value of 4.1 (Bartle & Ritches, 1978). In addition dumps are typically of low moisture and fertility and heavy metals may be present.

Rehabilitation operations can be costly due to correction of pH, ripping the surface and generally preparing the dump spoil for planting. Costs of rehabilitation can be reduced if certain procedures are eliminated. Revegetating with plants able to grow in the conditions provided by the dump is such a technique (Fox *et al.*, 1988). If the mechanisms by which the major constituents of the coal spoil dump prevent healthy growth of plants are understood, steps can be taken to reduce these effects instead of

treating the symptoms. A wide range of materials, including clay sub-soil, crushed sand-stone, ripped rock and fine quarry wastes has been shown to be satisfactory for the growth of plants (Temple & Bungey, 1979). Natural succession on abandoned mine dump sites accounts for less than 10 percent of the area and significant cover occurs only where the pH is greater than 5.0 (Bartle & Ritches, 1978). Legumes possess advantages for establishment on these sites (Koch & Bell, 1985). A screening technique for acidity tolerance indicated that dump progeny of *Acacia extensa* are more tolerant of acidic conditions than non-dump progeny (Fox *et al.*, 1988). Soil pH of less than 5.0 is associated with a high toxic presence of aluminium (Brady, 1991).

Aluminium affects the length of the primary root and, at toxic levels, alters root system architecture. Lateral branching is increased whereas root hair density and length of the root hair zone are decreased. A more compact and dense root system results (Barcelo & Poschenreider, 1990; Paganelli *et al.*, 1987; Thornton *et al.*, 1987; Wong & Bradshaw, 1982). Aluminium phytotoxicity has not been studied extensively. Tolerance to this metal would require an extremely adaptable plant species or a complex system of inheritance. Whereas Aniol & Gustafson (1990) suggest that in grasses a single recessive gene may be responsible for the inheritance of aluminium tolerance, Barcelo & Poschenreider (1990) argue that because the effects of aluminium are wide ranging, the inheritance of tolerance to aluminium stress would be more likely to be controlled by more than one recessive gene as it is rare for a useful trait to be controlled by a single recessive gene. Evolution of tolerance to metal stress can be very rapid. Although aluminium has many adverse affects, plants tolerant of its presence are also drought tolerant but do not produce as many seed or fruit (Smith & Bradshaw, 1979).

Rehabilitation is essential for abandoned coal mine sites or spoil dumps as the accumulation of unusable waste land is not acceptable in the current political and social climate (Bartle & Ritches, 1978). Use of plants tolerant to conditions on coal mine waste dumps for revegetation would save costs associated with changing the waste dump environment. If plant species are found that can tolerate the harsh conditions, there would be less need for liming, fertilisation and time and energy could be saved. The work reported here examined the effects of aluminium on two legume species, *Paraserianthes lophantha* and *Acacia decurrens*, with respect to growth, nutrient uptake and concentration in plant tissue. *P. lophantha* is a West Australian native, tall shrub or tree growing to 10 m in winter-wet depressions near creeks or swamps (Marchant *et al.*, 1987). *A. decurrens* is an eastern states native tree reaching 10 m with a 5 m crown (Blomberry, 1971), and now commonly seen in south-west Western Australia as semi-naturalised. Both species can tolerate drought and frost at maturity and can adapt to most soil conditions. Both species grow well on coal-mine dumps at Collie. These traits of hardiness render *P. lophantha* and *A. decurrens* prime candidates for mine dump rehabilitation.

METHODS

Seeds were obtained of *Paraserianthes lophantha* and *Acacia decurrens* from two locations for each, representing a dump source (Marron Pool, Muja and Stockton, Collie) and a non-dump source (Curtin University campus and Westralia Block, Collie). 300 seeds were randomly chosen from each of the dump and non-dump sources and weighed. Seeds were scarified and sown into separate trays. Tray bases were lined with moistened paper towels then covered with a layer of moist, coarse, sterile sand. Seeds were distributed over the moist sand then covered with a thin layer of dry, coarse, sterile sand.

Seedlings were held in the trays until the first leaf opened and then transferred to 150 mm tall, cylindrical pots containing coarse, sterile sand as a growth medium. Seedlings from each of the four groups, *i.e. A. decurrens* dump, *A. decurrens* non-dump, *P. lophantha* dump and *P. lophantha* non-dump, were then divided into seven treatments consisting of one control and six aluminium treatments. The aluminium treatments were applied to the plants by dissolving hydrated aluminium chloride into 20 litre quantities of de-ionized water. Concentrations of 200, 500, 700, 900, 1000 and 1100 ppm were used with fifteen replicates per treatment. Aluminium solutions were applied three times a week, using 50 - 100 ml per application per plant in hot months and 30 - 50 ml during cold months. Hoagland's nutrient solution No. 2 (Jones, 1983) was added once a week to all treatments on a day that aluminium was not supplied. The control group received only the nutrient solution. This nutrient level was used to ensure that plants did not suffer from lack of any essential nutrients, and that any effects on growth could be attributed solely to aluminium treatments. The pot trials were run for ten weeks.

At harvest each plant was divided into roots, shoots and leaves and dried at 105°C in an oven for 24 hours. After drying, the plants were weighed by parts. This enabled root shoot ratios to be calculated and compared between treatments and groups. The experiment was designed to examine the effects of aluminium treatment level on dry matter production for each of the four accessions separately. Analysis of variance was used for each accession and the Duncan test was used to distinguish significant differences between treatment means.

After weighing, plant parts, including seed samples, were ground into fine powder using a rotary mill with a 40 mi-

cron mesh. Available phosphorus was extracted from the samples using a Na_2CO_3 solution. The concentration of phosphate was measured colorimetrically (Colwell, 1965). 1M KCL was used to extract ammonium nitrogen and concentration was measured colorimetrically using the indol-phenol blue reaction (Cawse, 1967). Al was determined by dissolving samples in concentrated HNO_3 and oxidising with H_2O_2. Filtrates were directly used for Al determination without further processing. Al concentrations in the prepared samples were measured by AAS (Zhang & Taylor, 1990). Potassium and trace metal concentrations were determined using flame AAS.

Insufficient plant material allowed incomplete nutrient determinations as follows :

Dump plants	*roots*	no analysis at 1000 & 1100 ppm Al, no Al at 200 & 700 ppm;
	shoots	no analysis at 900 - 1100 ppm Al, also no Al at 700;
	leaves	only N at 1000 ppm Al, also no Al at 900 - 1100;
Non - dump	*roots*	no Al at 900 - 1100 ppm Al;
	shoots	no analysis at 900 & 1100 ppm Al, also no Al at 700 & 1000;
	leaves	no analysis at 1100 ppm Al, also no Al at 900 & 1000.

RESULTS

Acacia decurrens

There were no deaths over the trial period. Non-dump plants appeared to show signs of wilting sooner than dump plants between watering events. Wilting on the latter was only observed just prior to watering. Plants of dump origin did not grow as well as those of forest origin in the absence of added aluminium. Dump plants in the control treatment yielded 81% of the dry weight of non-dump plants but were only 2% shorter in height. After 10 weeks *A. decurrens* plants from both dump and non-dump origins differed in mean heights and dry weights (Table 1). Control plants were significantly taller and heavier than all Al treatments, except for dump origin plants at the lowest aluminium level. 200 ppm did not significantly reduce growth in plants of dump origin, but there was a 10% weight reduction. At 500 ppm dump plants attained only 49% of the height and 42% of the weight of the control. Non-dump sets between 200 and 700 ppm were significantly shorter and lighter than the control, and taller and heavier than plants subjected to 900 - 1100 ppm.

Non-dump plants were heavier than dump plants over the range 500 - 900 ppm. The lowest level of aluminium depressed height and weight to two thirds of the control in non-dump plants. Dump plants were consistently taller than non-dump plants at 900 ppm and more; non-dump plants were not exceeded in total weight by dump plants until 1000 ppm.

The contribution to dry weight by leaves was consistently more than that of roots and shoots in both sets (Figure 1). Foliage was in the range of 55 - 64% of total dry weight. Each component generally declined with increased Al in a similar pattern to that of total dry weight. Roots of dump plants were heavier than non-dump plants at 900 ppm and greater, but leaves and shoots did not become heavier in the dump set until 1000 ppm. The % of dry weight in roots declined in non-dump plants from 26% in control to 15% at 900 - 1000 ppm Al. Dump plant roots increased from 21% of total dry weight to 27% at 500 and 900 ppm and then declined to 20%.

TABLE 1. Mean heights and plant dry weights after 10 weeks growth of *Acacia decurrens* by treatment.

Aluminium (ppm)	Height (mm)		Dry weight (g)	
	Dump	Non - dump	Dump	Non - dump
0	360 a	383 a	6.33 a	7.57 a
200	361 a	260 b	5.68 a	4.97 b
500	175 b	220 b	2.69 b	4.59 b
700	160 bc	217 b	2.30 bc	4.09 bc
900	120 bc	73 c	1.57 c	2.06 cd
1000	93 c	75 c	2.06 bc	1.86 cd
1100	94 c	67 c	1.89 c	1.44 d

Values in columns with the same letter are not significantly different at $p<0.05$ using the Duncan test.

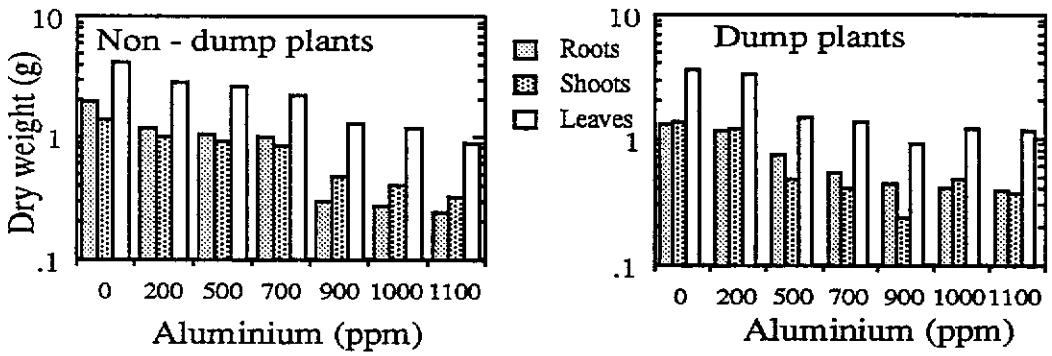

FIGURE 1. *Acacia decurrens* harvest dry weights by plant parts.

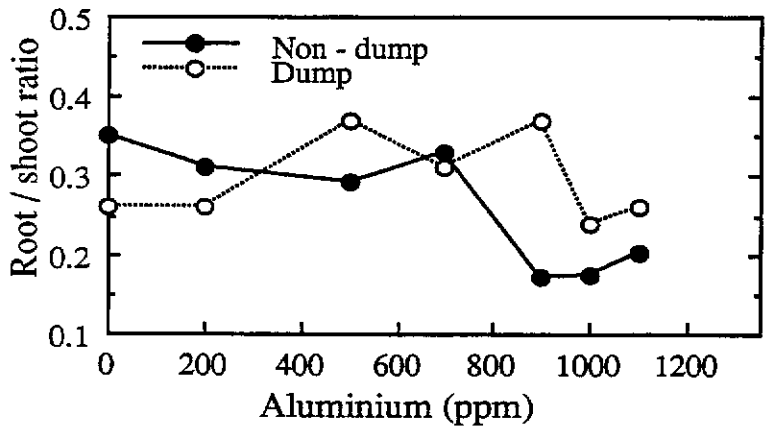

FIGURE 2. *Acacia decurrens* root/shoot ratios.

In the non-dump set the decline in weight as a proportion of control was steady and consistent with increasing aluminium. Root growth declined most rapidly and was only 15% of control at 900 ppm, when leaves and shoots were still more than 30%. At 1100 ppm non-dump root weight had declined to 12% of the control and leaves and roots were 21% and 23% respectively.

Plants of dump origin did not differ as markedly as dump plants between control and the first aluminium level. Dump plant dry weights fell markedly between 200 and 500 ppm with shoot and leaf contributions to dry weight at 35 and 41% of control weights respectively. Roots declined less as a proportion of control weight such that they were 55% of control at 500 ppm. At 1100 ppm the roots were 29% of the control, more than twice the proportion in non-dump plants. Dump plant root dry weights declined consistently and were at higher proportions of control than non-dump plants over the range 900 - 1100 ppm. Dump plant shoots and leaves did not differ over the range of 700 - 1100 from that attained at 500 ppm. At 900 ppm both shoots and leaves in the dump set were of lower weight than at the two more severe levels of aluminium.

Root - shoot ratios for dump plants varied inconsistently with the amount of Al applied (Figure 2), whereas the ratio for non-dump *A. decurrens* generally decreased with increasing concentrations of Al.

In the absence of aluminium both dump and non-dump *A. decurrens* had similar elemental composition in each of roots, shoots and leaves for nitrogen, phosphorus and calcium. Calcium and nitrogen were highest in leaves and lowest in shoots (Table 2). Phosphorus was highest in roots and least in shoots. Root contents of manganese, iron and copper were similar for dump and non-dump plants. However, dump plant roots had higher concentrations of zinc and aluminium than non-dump plants.

Dump plants had lower shoot and leaf concentrations than non-dump plants for all metallic elements, except aluminium. Higher quantities of manganese, iron, zinc and copper entered tissues of non-dump plants and formed a higher percentage of plant dry weight than in dump plants (Table 3). Dump plants retained more in roots and had less in the leaves for all elements than non-dump plants. The difference was most dramatic in respect of zinc where non-dump plants held only 6% of all zinc in the roots.

TABLE 2. Elemental concentration in *Acacia decurrens* not subjected to aluminium treatments.

Source / Tissue	Element							
	%			ppm				
	N	P	Ca	Mn	Fe	Zn	Cu	Al
Dump								
Root	2.21	0.60	0.45	205	1065	57	66	2071
Shoot	1.37	0.34	0.28	19	91	13	9	165
Leaf	2.93	0.41	0.56	26	52	6	6	267
Non - dump								
Root	2.20	0.63	0.41	219	1126	6	63	1000
Shoot	1.42	0.30	0.32	126	424	95	51	101
Leaf	2.80	0.45	0.57	54	100	14	20	279

TABLE 3. Allocation of elemental uptake in *Acacia decurrens* not subjected to aluminium treatments.

Element / Source	Uptake (mg)	% dry weight	% distribution		
			Root	Shoot	Leaf
Zinc					
Dump	0.117	0.0018	65	15	20
Non - dump	0.207	0.0027	6	65	29
Aluminium					
Dump	3.949	0.0624	69	6	25
Non - dump	3.236	0.0427	60	4	36
Manganese					
Dump	0.395	0.0062	69	7	24
Non - dump	0.829	0.0109	51	22	27
Iron					
Dump	1.725	0.0272	82	7	11
Non - dump	3.201	0.0423	68	19	13
Copper					
Dump	0.122	0.0019	71	10	19
Non - dump	0.277	0.0037	44	26	30

Dump plants held more aluminium in total, with more in roots and less in the leaves than the non-dump plants. This indicates that less aluminium was translocated within the dump plants.

Elemental uptake in experimental plants may be compared with the "normal" ranges for plants. Allen (1974) gives the following:

	%		ppm
N	1 - 3	Al	100 - 10,000
P	0.05 - 0.3	Mn	50 - 1,000
Ca	0.3 - 2.5	Fe	40 - 500
		Zn	15 - 100
		Cu	2 - 25

None of the values of Table 3 lay outside these limits except for copper in the non-dump set. Iron and copper levels, in both accessions, were higher in roots (Table 2) than the normal limits.

Aluminium treatments were associated with a reduction in *Acacia decurrens* nitrogen concentration (Figures 3a & 4a). Nitrogen levels were < 1% in shoots of dump plants treated at 200 - 500 ppm aluminium. All other levels for dump plants were > 1%, and roots and leaves were never < 1% N. In contrast non-dump plants had very low leaf nitrogen contents at all levels of aluminium. Roots were not deficient but shoots were so at the two highest aluminium levels. Collectively, nitrogen levels rose slightly over the range of aluminium applied to dump plants but the pattern was more obscure with non-dump plants.

Phosphorus and calcium levels declined with increased aluminium. P - levels were not below the critical level for any tissues in either accession. The greatest change in P content was from zero to 200 ppm aluminium (Figures 3b & 4b). Non-dump plants had a two stage decline and after 500 ppm aluminium P concentrations stabilised at around 0.2%. Foliar P in dump plants progressively declined over the range of added aluminium. In dump plants calcium levels were below the critical limit at all alu-

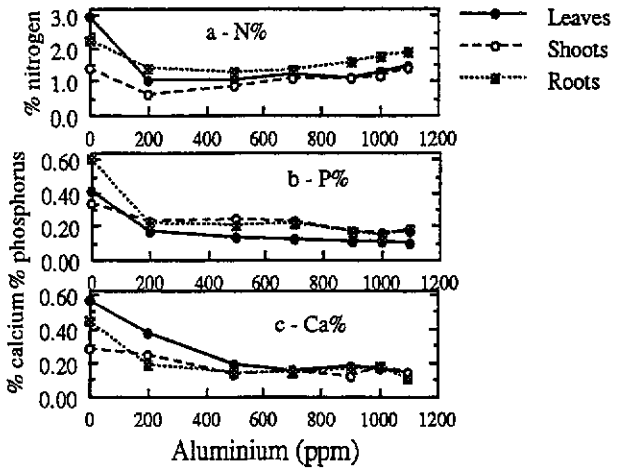

FIGURE 3. Nutrient % in dump plants of *Acacia decurrens*.

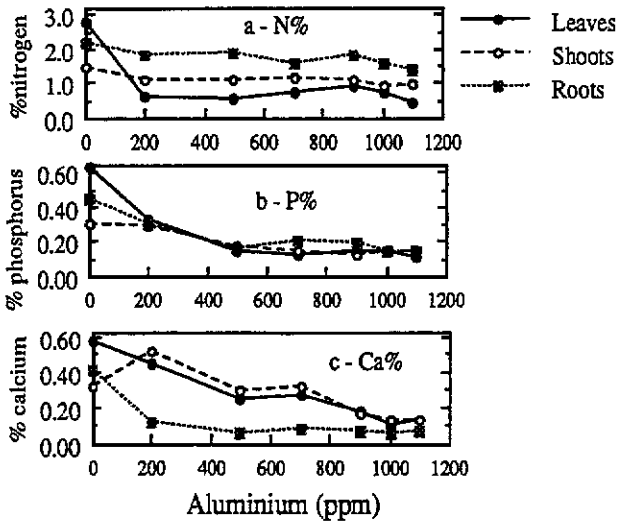

FIGURE 4. Nutrient % in non-dump plants of *Acacia decurrens*.

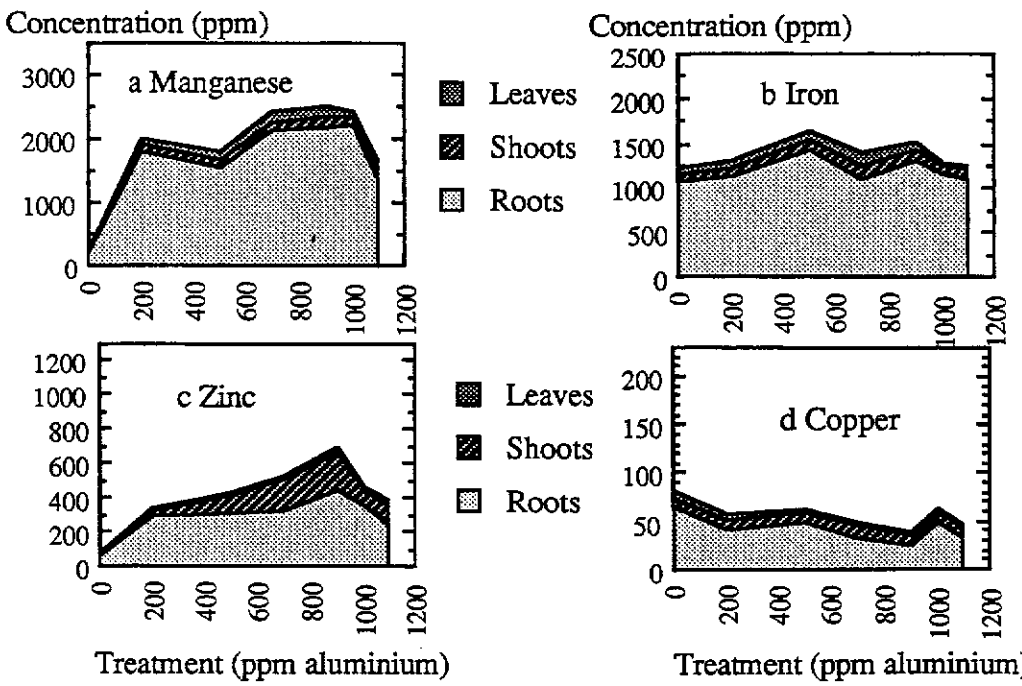

FIGURE 5. Trace elements in tissues of *Acacia decurrens* (dump origin).

minium levels for all tissues (Figure 3c). Levels stabilised after 500 ppm aluminium. Calcium levels were low at all levels in the non-dump plant roots (Figure 4c). Leaf and shoot calcium levels were adequate until after the 700 ppm aluminium level.

Most of the trace elements taken up by dump plants remained in the roots (Figure 5). The levels of copper and iron in roots were higher than normal (Allen, 1974), reflecting the high levels of nutrients supplied, although these levels were not affected by aluminium treatment. For each of roots, shoots and leaves, concentrations of copper and iron were similar to the zero aluminium levels, or less (in the case of copper in roots). Aluminium treatment was associated with increased levels of manganese and zinc in all plant parts. Manganese levels in roots were well over the normal limit of 1000 ppm, but in shoots and leaves, where levels also increased with aluminium, compared to the control, concentrations were between 100 and 160 ppm. Zinc levels were high in all roots (> 250

ppm) and shoots (> 100 ppm) at aluminium concentrations of 500 ppm or more.

Non-dump plants of *A. decurrens* (Figure 6) took up greater quantities of trace elements with the addition of aluminium, but retained smaller proportions in the roots, than did dump plants. Whereas root levels of manganese, iron and copper were similar between accessions each of these and zinc had much higher shoot and leaf concentrations in non-dump compared with dump plants. For both manganese and copper non-dump plants had similar root and shoot concentrations. Higher than normal levels (Allen, 1974) of manganese, iron and copper were present in non-dump roots and shoots. Very little zinc was retained in roots of non-dump plants (Figure 6c) with highest concentration in shoots (above normal levels), and leaf levels also generally higher than in roots. Shoot zinc peaked at 1300 ppm in the 700 ppm aluminium treatment along with aluminium (Figure 7). Insufficient plant material was available to analyse aluminium content of dump plant roots at 700 and 1100 ppm Al and shoots at 900 - 1100 ppm Al. Similarly no values are available for non-dump plant roots at 1100 ppm and shoots at 900 - 1100 ppm.

Roots of both dump and non-dump plants had increased levels of Al (Figure 7) with increased aluminium. Root aluminium contents in dump plants tended to stabilise after the 500 ppm treatment at around 6000 ppm aluminium. Dump plant foliar aluminium increased with

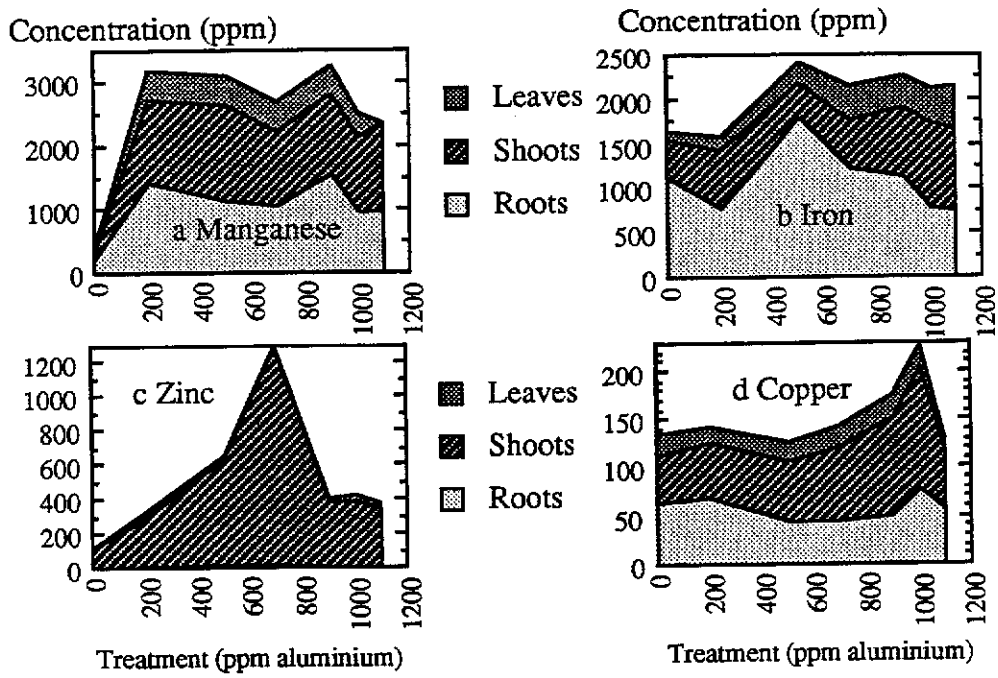

FIGURE 6. Trace elements in tissues of *Acacia decurrens* (non-dump origin).

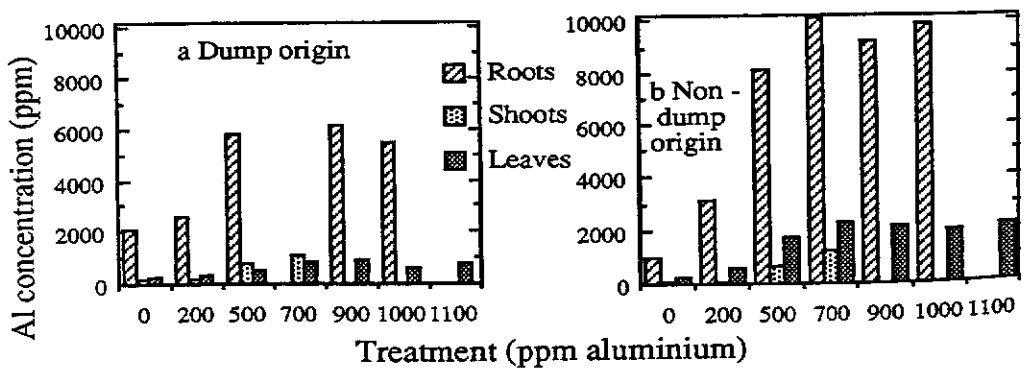

FIGURE 7. Aluminium concentration in tissues of *Acacia decurrens*.

treatment level from 300 ppm at the lowest Al treatment to > 900 at 900 ppm Al, and then fell away to 6 - 700 at higher treatment levels. Both roots and leaves of non-dump plants had much higher concentrations than dump plants, with root levels between 8000 and 10000 ppm after the 500 ppm treatment. Non-dump plant foliar aluminium rose from 600 ppm at the lowest Al treatment to around 2000 ppm for all other treatment levels.

Absolute levels of aluminium in tissues (mg Al) were higher in non-dump plants (Table 4).

With no supply dump plants accumulated more Al in total (4.0 mg versus 3.2 mg). With aluminium supplied both sets took up increased amounts with increased treatment levels, at least to the 700 pm treatment, but non-dump plants absorbed considerably more than dump plants, reaching in excess of 10 mg at the 700 ppm treatment (Table 4). The non-dump plants consistently translocated a higher proportion to foliage, in excess of 30%. Non-dump plants had higher contained total aluminium (mg) in foliage. Dump plants consistently retained a higher proportion of contained aluminium within roots, but for all treatments dump roots had lower amounts of total aluminium than non-dump plants.

Paraserianthes lophantha

In contrast with *Acacia decurrens* where there were no losses, a number of *Paraserianthes lophantha* seedlings died in higher aluminium treatments during the trial. By 10 weeks non-dump plants subjected to the three higher treatment levels had lost three plants each. Dump plant progeny at 700 and 900 ppm had one death each during the fifth week and the 1000 and 1100 ppm treatments both had six deaths in total.

Plants of dump and non-dump origin reached similar mean heights in the absence of aluminium. The dump batch had 16% heavier yield at harvest than plants of non-dump origin (Table 5). Control plants were significantly taller and heavier than all Al treatments : even the lowest Al level significantly reduced both height and weight in both accessions.

P. lophantha plants of dump origin were consistently lighter in weight (but taller) than non-dump plants over the range of applied aluminium level from 200 - 700 ppm. Yields at 200 ppm aluminium were 58% and 70% of the controls for dump and non-dump origin plants respectively. Dry weight yields fell away with more aluminium more quickly in plants of dump origin. At 500 ppm dump plants attained only 30% of the control weight, whereas non-dump plants reached 50%. Plant heights fell away more rapidly in the non-dump treatment plants. Differences associated with treatment levels were significant between treatments at up to 500 ppm aluminium. Between 900 and 1100 ppm aluminium, dry weights had fallen to < 20% of the control in both batches of seedlings. Differences between height and yields were not significant at these levels.

Shoot weights as a proportion of control declined more rapidly in *P. lophantha* than in *A. decurrens*. The most dramatic change in resource allocation was a decline in non-dump shoot weight from 24% of total weight in control to 6% at 1100 ppm aluminium. In contrast dump plants had 28% of mass in control shoots and 23% at 1100 ppm. Of the three components measured, leaves generally contributed most to dry weight (Figure 8). However the non-dump set had heavier roots than leaves between 900 and 1100 ppm aluminium. This coincided with a major decline in both shoot and leaf weight.

TABLE 4. Absolute levels of aluminium in tissues of *Acacia decurrens* (* = no data available) and percentage distribution of aluminium between the plant parts for those treatments with sufficient material for analysis.

Treatment ppm Al	Uptake	Dump			Non - dump		
		Root	Shoot	Leaf	Root	Shoot	Leaf
0	mg Al	2.75	0.22	0.98	1.94	0.14	1.15
	% Al	69	6	25	60	4	36
200	mg Al	3.05	0.19	1.04	3.63	0.12	1.72
	% Al	71	5	24	66	2	32
500	mg Al	4.24	0.34	0.70	8.52	0.59	4.53
	% Al	80	6.5	13.5	63	4	33
700	mg Al	*	0.43	2.00	10.26	1.03	5.09
	% Al	-	-	-	63	6	31
900	mg Al	2.64	*	1.43	2.76	*	2.77
1000	mg Al	2.21	*	1.26	2.71	*	2.34
1100	mg Al	*	*	1.37	*	*	1.86

TABLE 5. Mean heights and plant dry weights after 10 weeks growth of *Paraserianthes lophantha*.

Aluminium ppm	Height (mm)		Dry weight (g)	
	Dump	Non - dump	Dump	Non - dump
0	383 a	390 a	6.81 a	5.85 a
200	294 b	217 b	3.94 b	4.12 b
500	183 c	130 c	2.03 c	2.94 c
700	122 d	106 c	1.19 cd	2.47 cd
900	78 e	60 d	1.10 d	0.71 d
1000	60 e	57 d	0.64 de	0.72 d
1100	60 e	53 d	0.56 e	0.48 d

Values in columns with the same letter are not significantly different at $p<0.05$ using the Duncan test.

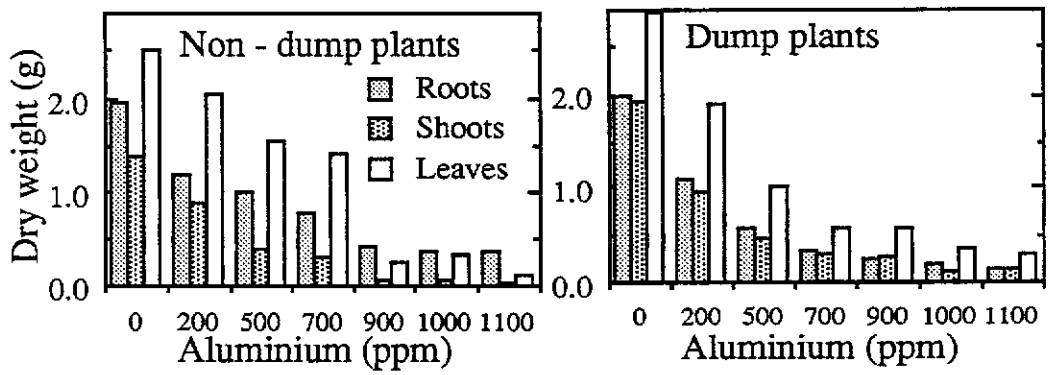

FIGURE 8. *Paraserianthes lophantha* harvest dry weights by plant parts.

The proportion of dry weight in leaves increased consistently in dump plants from 42% in control to 54% at the highest Al level. Non-dump progeny foliage increased to 56% of biomass at 700 ppm and then fell away to 21% of mass at 1100 ppm aluminium. The proportion of dry weight in roots of non-dump plants increased with increasing aluminium to a high of 73% at 1100 ppm whereas the dump root proportion was similar for all treatments at between 23% and 29%.

Root-shoot ratios were higher for all Al levels in *P. lophantha* plant sets than for *A. decurrens*, except for dump progeny at 900 ppm. Dump plants had a consistently lower, and more constant (0.42 - 0.29), root-shoot ratio than the non-dump sets (Figure 9). Non-dump plants in treatments greater than 700 ppm Al had very high root-shoot ratios reflecting the major declines in leaf and shoot contributions to dry weight.

In *Paraserianthes lophantha* plants grown with nutrient solution only, calcium and nitrogen concentrations were highest in leaves and least in shoots for plants of both sources (Table 6). Leaf nitrogen was above the normal range (Allen, 1974). Root phosphorus content was slightly higher than other tissues.

Non-dump plants had higher concentrations of these three elements in all tissues, with the exception of shoot calcium for which there was no difference between accessions. Dump plant roots had higher concentrations for each of Mn, Fe, Zn, Cu and Al. Non-dump leaves had higher Fe, Zn and Al than dump plants, and non-dump shoots had slightly more zinc. Apart from these all other shoot and leaf samples had higher levels of metallic elements in the plants of dump origin.

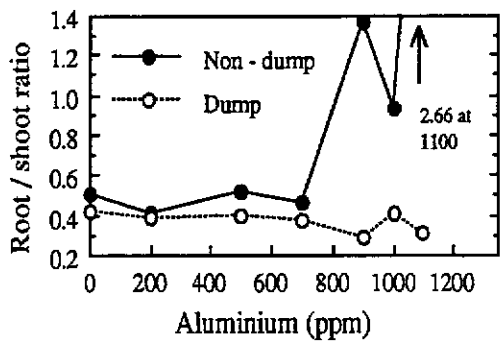

FIGURE 9. *Paraserianthes lophantha* root/shoot ratio.

The absolute quantities absorbed for each of zinc, aluminium, manganese, iron and copper in controls were higher in dump plants. Except for zinc, each of these elements made a higher contribution to percent dry weight in dump plants (Table 7). Dump plants retained higher percentages in roots and translocated less to foliage. Non-dump plants translocated in excess of 80% of the absorbed zinc and manganese to leaves with only 4 - 5% retained in roots. Dump plants retained 92% of aluminium in roots and sent only 4% to leaves. Both iron and copper in dump plants were above the normal range (Allen, 1974) and this was due to the elevated root levels (Table 6).

Leaf nitrogen content in dump plants remained at > 3% with increased aluminium until the highest treatment level (Figure 10a). The nitrogen content of roots increased with increasing Al addition. In non-dump plants foliar nitrogen remained high at the lowest aluminium level but then declined to < 3% (Figure 11a); root nitrogen generally declined with increased aluminium. Shoot nitrogen was consistently higher in the non-dump plants.

Phosphorus in the leaves of dump plants remained above 0.2% up to an addition of 500 ppm Al and then declined steadily to a minimum of 0.12% in the 1100 ppm treatment (Figure 10b). Both shoots and roots had increased phosphorus percentage with more Al. Phosphorus concentration in non-dump plants decreased with Al (Figure 11b). The decline of phosphorus in the leaves was not as great as in the roots, and both were irregular beyond 500 ppm Al.

The percentage of calcium decreased in the roots, shoots and leaves of the *P. lophantha* dump progeny (Figure 10c). In both sets the greatest change was at the first level of aluminium for root calcium, which was generally lower

TABLE 6. Elemental concentration in *Paraserianthes lophantha* not subjected to aluminium treatments.

Source / Tissue	Element							
	%			ppm				
	N	P	Ca	Mn	Fe	Zn	Cu	Al
Dump								
Root	1.46	0.24	0.58	188	1406	32	94	3650
Shoot	1.25	0.21	0.21	37	429	14	11	152
Leaf	3.87	0.22	0.83	203	125	47	25	108
Non - dump								
Root	2.38	0.33	0.69	8	101	4	7	1229
Shoot	1.93	0.28	0.20	16	115	17	8	105
Leaf	4.05	0.24	0.98	113	225	67	22	160

TABLE 7.
Allocation of elemental uptake in *Paraserianthes lophantha* not subjected to aluminium treatments.

Element / Source	Uptake (mg)	% dry weight	% distribution		
			Root	Shoot	Leaf
Zinc					
Dump	0.228	0.0034	28	12	60
Non - dump	0.199	0.0034	4	12	84
Aluminium					
Dump	7.905	0.1161	92	4	4
Non - dump	2.950	0.0505	81	5	14
Manganese					
Dump	1.032	0.0152	36	7	57
Non - dump	0.319	0.0055	5	7	88
Iron					
Dump	3.999	0.0587	70	21	9
Non - dump	0.921	0.0158	22	17	61
Copper					
Dump	0.280	0.0041	67	7	26
Non - dump	0.078	0.0013	17	13	70

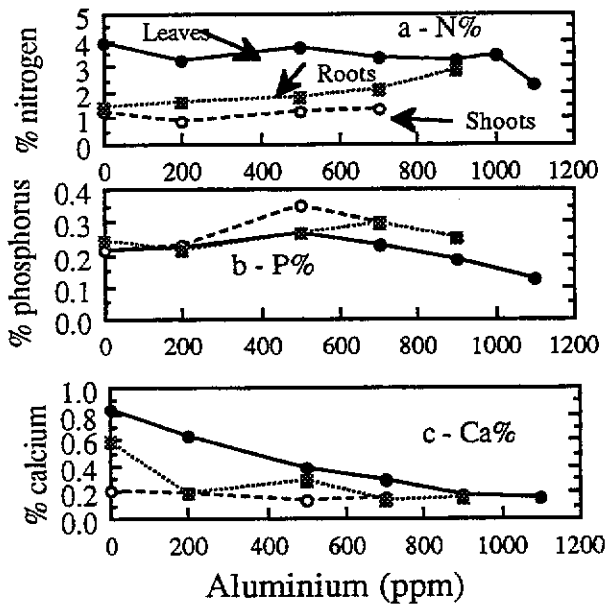

FIGURE 10. Nutrient (%) in dump plants of *Paraserianthes lophantha.*

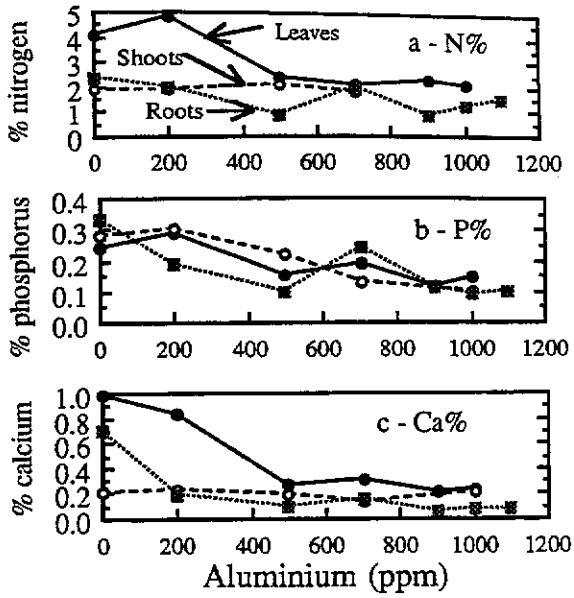

FIGURE 11. Nutrient (%) in non-dump plants *Paraserianthes lophantha.*

than 0.2% with any aluminium level. Leaf calcium remained comparatively high (> 0.6 %) at the first level of aluminium.

Aluminium addition increased the uptake of manganese and zinc in dump plants (Figure 12). Iron content, however, declined steadily and copper uptake was somewhat irregular, with highest root concentration (106 ppm) attained at 500 ppm Al. Most of the uptake of these elements was confined to the roots, and except for zinc, leaf contents were higher than shoots.

All root concentrations for each of manganese, iron, zinc and copper were beyond the normal levels of Allen (1974). Leaf and shoot levels were within normal limits, with the exceptions of stem zinc (128 ppm) at 700 ppm Al, leaf iron at 1100 ppm Al and leaf copper at 500 ppm Al.

In the non-dump set much less manganese, zinc and copper was absorbed than in dump plants. Root zinc levels were in fact below the accepted range with a low in control of 3.8 ppm and a high at 700 ppm Al of 23 ppm Zn. In no treatment were any of manganese, iron, zinc or copper levels in roots higher than the normal range of Allen (1974). Iron levels were similar in both sets of plants at higher aluminium concentrations. Very little remained in the roots and leaves held the bulk of these elements in non-dump plants (Figure 13). Leaf iron levels were above the normal range with aluminium treatments of 500 ppm and greater. Shoot and leaf zinc (150 ppm) and copper (30 and 40 ppm) exceeded the normal upper limits in the 200 ppm Al treatment. Leaf iron content exceeded 800 ppm from 500 ppm Al onwards.

Despite the lack of data points for aluminium contents of tissues there was a trend for higher uptake by non-dump plants when aluminium was supplied, and for these to have higher leaf levels (Figure 14). Non-dump roots in the 500 ppm treatment had 7000 ppm Al and 8000 ppm in the 700 ppm treatment. Foliar aluminium at the 500 ppm Al treatment exceeded 2000 ppm. Dump progeny foliar and root aluminium was lower than in non-dump progeny for each comparable treatment level. Dump root content reached 4000 ppm and foliar content was 700 ppm at the 500 ppm Al treatment level. Shoot Al was similar for both sets.

The ability of dump plants to accumulate aluminium in the control may not have been consistent in the presence of added aluminium (Table 8). Non-dump plants contained more aluminium with increased application and translocated higher proportions to the foliage. Both sources translocated considerably more Al to foliage than remained in shoots.

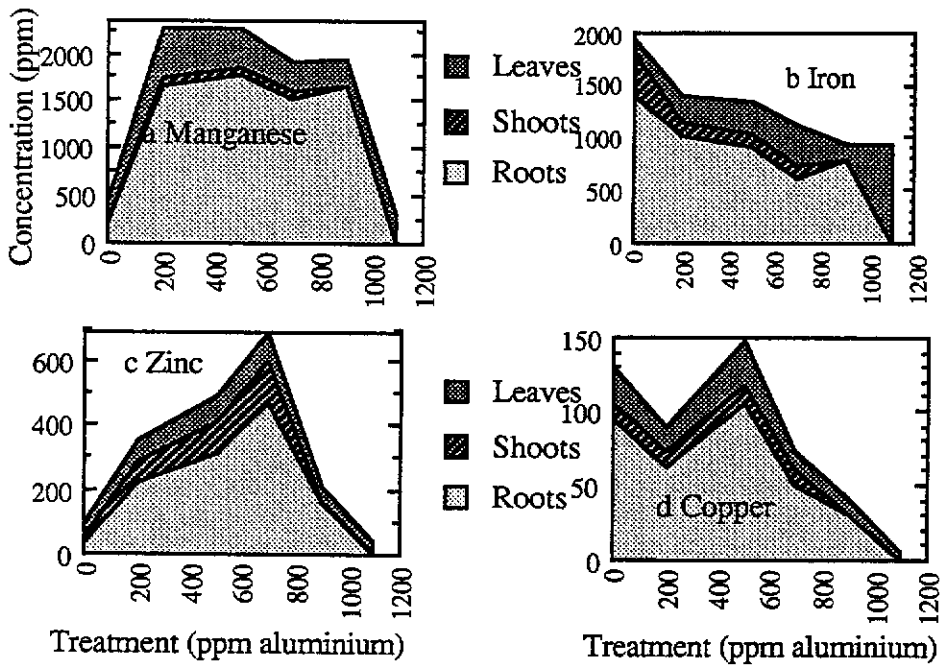

FIGURE 12. Trace elements in tissues of *Paraserianthes lophantha* (dump origin).

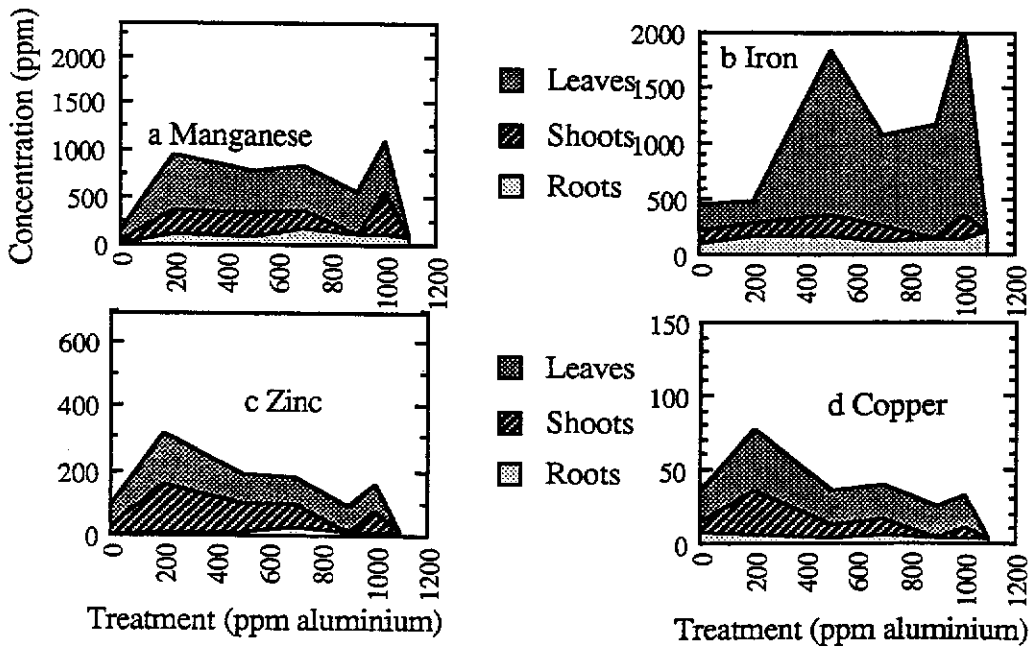

FIGURE 13. Trace elements in tissues of *Paraserianthes lophantha* (non-dump origin).

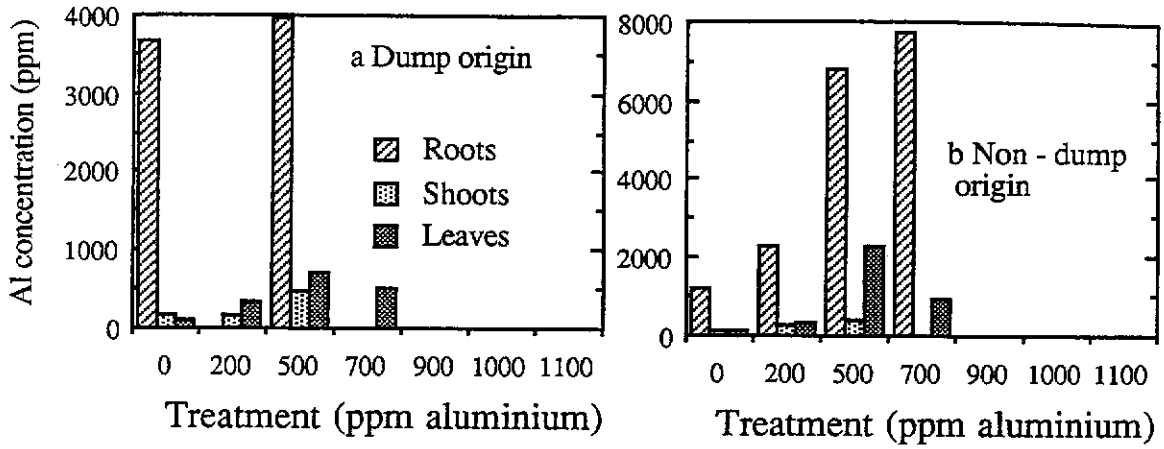

FIGURE 14. Aluminium concentration in tissues of *Paraserianthes lophantha*.

Despite the fact that addition of aluminium decreased growth and increased the death rate, plants subjected to aluminium treatments did not wilt as quickly as control plants. Two days after watering it was noted that all control plants showed signs of wilting while only three to four plants in the 200 ppm treatment appeared affected. At 500 ppm and above, none of the plants appeared to wilt but the leaves and stems were not as succulent as those of the control group.

Seed Analysis

For both species dump seed was significantly heavier than non-dump seed (Table 9). Dump seed had higher levels of nitrogen, phosphorus, manganese, zinc and aluminium whereas non-dump seed had more iron and copper. Non-dump seed of *Acacia decurrens* had most calcium and calcium levels in the two *Paraserianthes* accessions were similar. In terms of the normal ranges *Paraserianthes lophantha* dump seed had high nitrogen, and non-dump seed high copper. Manganese seed levels were an order of magnitude higher in *Acacia decurrens*. All seed was low in calcium.

DISCUSSION

The expected outcome was for an advantage in growth by plants from dump sources when exposed to aluminium, as well as decreased yield (Schaeffer & Walton, 1990) with aluminium concentration. An earlier study indicated a growth advantage for *Acacia extensa* with heavier dump seed, when plants were grown in acidic solution culture (Fox *et al.*, 1988). In the present trial no clear dry weight production advantages were obtained for plants of dump origin over those of non-dump origin. Whereas dump origin *A. decurrens* plants at the lowest level of aluminium had greater total dry weight than non-dump plants, this did not occur again until the two higher levels of application. Greater root mass was responsible. Growth in *P. lophantha* of dump origin was generally less than that of non-dump origin at all levels of aluminium, for both total biomass and root weights.

In nature plant roots would avoid locally high concentrations of potentially toxic elements so that the trial conditions must be viewed as extreme cases of exposure. Growth and life spans are considered important factors which may explain the observed differences. *A. extensa* is a species of relatively fast growth and short life - span, the seed used earlier (Fox *et al.*, 1988), were taken from plants long established on an old, naturally colonised dump and would represent a number of generations of growth on that dump. It is postulated that the degree of apparent adaptation to aluminium levels would be related to the history of prior exposure. Whereas the *P. lophantha* seed came from plants established three years earlier by direct seeding onto a restored dump, the seed of *A. decurrens* was taken from self-sown trees established many years earlier on an un-restored waste dump.

Acidity levels within treatments were not monitored. Al would have affected pH and this in turn influences the availability of nutrients to the plant, independently of aluminium concentration (Temple & Bungey, 1979). The comparatively strong nutrient solution applied sought to avoid any nutrient constraints to growth. In future work it would be useful to examine acidity levels to distinguish between pH and Al effects on growth.

In the absence of aluminium both sets of dump progeny took up more aluminium than the non-dump sets. With aluminium supplied the dump sets took up less than the non-dump sets. Within the plants aluminium was mainly held in roots with lower proportions in aerial parts suggesting an exclusion mechanism (Aniol & Gustafson, 1990). Non-dump *A. decurrens* plants accumulated more aluminium than dump progeny. Roots in dump plants took up to 6000 ppm Al and had < 1000 ppm in foliage, whereas non-dump plants accumulated up to 10,000 ppm Al in roots and > 2000 ppm in foliage after ten weeks of Al treatment. Although there was a lack of sufficient Al

TABLE 8. Absolute levels of aluminium in tissues of *Paraserianthes lophantha* (* = no data available) and percentage distribution of aluminium between the plant parts for those treatments with sufficient material for analysis.

Treatment ppm Al	Uptake	Dump			Non - dump		
		Root	Shoot	Leaf	Root	Shoot	Leaf
0	mg Al	7.30	0.29	0.31	2.40	0.15	0.40
	% Al	92	4	4	81	5	14
200	mg Al	*	0.15	0.61	2.75	0.22	0.67
	% Al	-	-	-	76	6	18
500	mg Al	2.27	0.22	0.72	6.80	0.16	3.52
	% Al	71	7	22	65	2	33
700	mg Al	*	*	*	6.00	*	1.31

TABLE 9. Seed characteristics of *Acacia decurrens* and *Paraserianthes lophantha*.

Elemental contents		*Acacia decurrens*		*P. lophantha*	
		Dump	Non - dump	Dump	Non - dump
Nitrogen	%	3.08	2.15	3.64	2.63
Phosphorus	%	0.30	0.14	0.21	0.09
Calcium	%	0.19	0.24	0.16	0.17
Manganese	ppm	467	369	38	31
Iron	ppm	108	210	120	229
Zinc	ppm	29	22	43	38
Copper	ppm	7.6	9.5	9.5	23.5
Aluminium	ppm	138	95	103	81
Seed weight	mg	19.09	12.64	67.60	54.49
S. D.	mg	4.02	5.35	6.64	11.58
Significance		$F = 942$, $p < 0.0001$		$F = 2190$, $p < 0.0001$	

analysis data for *P. lophantha*, similar levels were demonstrated with dump roots at c. 4000 ppm Al and foliage 700 ppm compared with non-dump plants at up to 8000 ppm Al and foliage > 2000 ppm Al. Above ~ 500 - 700 ppm application concentrations, tissue levels of Al remained constant or fell.

Within roots, aluminium may be precipitated in free space or bound in nucleic acids, cell walls and the calcium binding protein calmodulin, as well as binding directly with phosphorus and enzyme mediators of nitrogen (Clarkson, 1969; Webb & Sheehy, 1982; Gomes *et al.*, 1985; Taylor & Foy, 1985; Barcelo & Poschenreider, 1990; Schaeffer & Walton, 1990). Passive uptake of Al could depend on a concentration gradient and the electrical potential across the plasma membrane. If the pathways by which aluminium is absorbed are saturated, then the rate of binding in the roots will not increase. Passive uptake would not be effective once the concentration of Al bound in the roots is at equilibrium with the concentration in the rhizosphere. Uptake is then dependant on metabolic processes (Zhang & Taylor, 1990). These are likely to be the predominant method of uptake and binding of Al. Plants tolerant of aluminium may have the ability to maintain ion fluxes across the plasma membranes of root cells at an acceptable level (Miyasaka *et al.*, 1989). If plants from dump sites are tolerant to aluminium, they would also undergo other changes in physiology and morphology likely to improve their ability to survive and reproduce in the presence of Al (Baker, 1987).

Control plants of both species from dump sites were of shorter height at the end of the ten week trial than plants from non-dump sites. It is hypothesised that the heavier dry weights attained suggest that dump progeny may have a denser structure than plants from non-dump sites. Non-dump plants were the first to wilt and the last to recover turgidity. Dump plants did not wilt until two days without watering. Denser structure may be advantageous under water stressed conditions, whether this is related to either water retention *per se* or to mechanical support, presumably less water would be required to maintain turgidity. Aluminium within plants affects root pressure due to changes in distribution of potassium and hydrogen ions. Interaction between Al and root cell surfaces results in the

production of metal - sulphydryl bonds which determine the rate of leakage of ions from the cells. An increase in ion concentration in the rhizosphere alters the potential gradient between the root and soil by increasing the resistance to water entering the root. Root pressure is diminished, causing less water to be transported to the upper parts of the plant (Robinson, 1989; Barcelo & Poschenreider, 1990; Zhang & Taylor, 1990).

Root growth was inhibited in all plants exposed to Al treatments. Roots of dump plants exposed to Al, for both species, had inconsistent changes in the proportion of growth going to roots compared with shoots and leaves. Root - shoot ratios were not correlated with the concentration of Al. Non-dump progeny of *P. lophantha* had a proportional increase in the dry weight of the roots, as Al concentration increased, while *A. decurrens* non-dump progeny had a proportional decrease in dry weights relative to the shoots and leaves. Dry weight production in *A. decurrens* was not as severely affected by Al which suggests that this species has a greater ability to suppress the effects of Al by excluding it from upper parts of the plant. Dump origin plants of *A. decurrens* were consistently heavier than those of *P. lophantha*. *A. decurrens* (dump) accumulated approximately 500 ppm foliar Al after ten weeks of application of a 500 ppm solution, while *P. lophantha* (dump) had accumulated approximately 700 ppm over the same trial period of the same solution. With a higher concentration of Al in the upper parts of the plant than *A. decurrens*, Al would have more harsh inhibitory effects on the growth of the upper parts of *P. lophantha* (dump). This was reflected in lower leaf dry weight production by *P. lophantha*.

Manganese was not supplied to plants via the nutrient solution and presumably came from the sand medium used (not analysed) and seed. *A. decurrens* seed had a high level of manganese, whereas *P. lophantha* seed had relatively insignificant levels. Mn accumulated in high concentrations in roots of both *P. lophantha* and *A. decurrens*. Proportionally insignificant quantities of Mn were in shoots and leaves of *A. decurrens*, equivalent to the concentrations in the seeds collected from the dump site. A high concentration of Mn in the seeds of *A. decurrens* relative to *P. lophantha* suggests that the *A. decurrens* parent plants do not restrict this element as successfully as *P. lophantha*. Pot trials also indicated the inefficient binding of Mn by *A. decurrens*. Mn concentrations in the shoots and leaves of *A. decurrens* (dump) were twice those of *P. lophantha*. Al deteriorates cell membranes and alters enzyme activity (Barcelo & Poschenreider, 1990). This may also result in a partial breakdown of the passive ion control system. The ability of *P. lophantha* to maintain ion fluxes may indicate it is better adapted than *A. decurrens* to tolerate high levels of heavy metals.

Calcium deficiency is caused by aluminium (Schaeffer & Walton, 1990; Zhang & Taylor, 1990). Both non-dump and dump progeny exhibited dramatic falls in calcium, the most severely affected of the macronutrients. Al binds with the calcium binding protein, calmodulin which inhibits uptake of calcium into the plant. Calcium levels in the leaves dropped more than in the roots probably due to a decrease in root pressure which, in turn, would diminish the rate of translocation of nutrients from the roots to the upper sections of the plant. Therefore, any decrease in nutrient levels of the roots would be magnified in the leaves. Nitrogen and phosphorus levels, although significantly affected, were not as diminished as calcium in the roots. The high levels of P supplied in nutrient solution would have reduced the inhibitory activity of aluminium in the root environment. Al binds directly with phosphorus at the root surface and in the intercellular spaces, thus, it may still be absorbed into the roots but translocation is inhibited (Clarkson, 1969). Nitrogen levels in the roots were the least effected as Al does not bind directly with nitrogen but with an enzyme mediator of nitrogen absorption. Al also interferes with amino acid inter - conversion, changing the nitrogen forms translocated in the sap (Gomes *et al.*, 1985; Miyasaka *et al.*, 1989). Thus, while nitrogen content of the roots may increase slightly, as was the case with the *A. decurrens* and *P. lophantha* dump progeny, leaf nitrogen content was reduced significantly as a result of reduced root pressure and Al interference in translocation (Peterson, 1983; Gomes *et al.*, 1985).

Nodulation was not observed in either *A. decurrens* or *P. lophantha* seedlings in any of the Al treatments or control groups, despite the general occurrence of nodulation observed in other pot trials with leguminous species. Al inhibits nodulation because root hair numbers and density are reduced. At a concentration of 0.1 ppm, root hair density is reduced by 50% (Brady, 1991). Very little root hair growth would have occurred in this trial, preventing the establishment of rhizobial nodules.

Dump plant seeds were of heavier weight compared with seed from the non-dump plants, confirming trends noted earlier with species of *Acacia* (Fox *et al.*, 1988). Dump seeds have higher nutrient contents than seeds collected from non-dump sites. It has not been determined whether increased weight is related to seed coat thickness. It is hypothesised that dump plants may be subject to selective forces favouring increased seed coat thickness, this would enhance the regeneration potential of these species in the harsh environment of the dumps.

CONCLUSIONS

Addition of Al to the soil solution had significant inhibitory effects on the plants, as demonstrated by changes in morphology and physiology. Whether the symptoms displayed by the treated plants were a direct result of Al interaction with physiological activity or resulted from secondary toxic effects would require further, more de-

tailed, investigation. Al tended to accumulate in higher concentrations within the roots of all exposed plants while relatively small concentrations were translocated to the shoots and leaves. This supports the theory that legumes, such as *Acacia* and *Paraserianthes*, bind heavy metals in the roots, inhibiting translocation to the shoots and leaves (Aniol & Gustafson, 1990). Understanding the dynamics of Al toxicity and methods by which tolerance is achieved and inherited may lead to more efficient methods of revegetating environments, such as coal mines and waste dumps, in which heavy metals proliferate. Instead of treating the secondary effects of metal toxicity, such as nutrient deficiencies, steps can be taken to treat the problem at the source. Time, money and energy would be saved allowing resources to be distributed over a larger area of rehabilitation work, by the relatively simple expedient of collecting seed for use on coal-mine rehabilitation sites from those dumps already colonised successfully.

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PRELIMINARY OBSERVATIONS IN LABORATORY AND FIELD ON EFFECTS OF VESICULAR ARBUSCULAR MYCORRHIZAL ASSOCIATIONS AND PHOSPHORUS APPLICATION IN *HIBBERTIA HYPERICOIDES*

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INTRODUCTION

Vesicular-arbuscular mycorrhizae (VAM) colonize the roots of a vast number of vascular plants. The fungal symbiont is always a member of the primitive family Endogonaceae (Harley & Smith, 1983). Enhanced phosphorus uptake and improved phosphorus nutrition are the primary causes of growth and yield increases in mycorrhizal plants. VAM roots can receive up to five times the levels of phosphorus as those of non-mycorrhizal roots (Harley & Smith, 1983). Uptake experiments therefore have concentrated on phosphorus, generally considered the most limiting nutrient in sclerophyll vegetation (Beadle, 1966; Specht & Groves, 1966).

In addition to enhanced phosphorus uptake, VAM hyphae can readily transport zinc, copper and sulphate to the host roots (Marschner, 1986). Associations between plant and VAM fungi are also known to deter wilting and root pathogens, protect roots in adverse soil conditions such as low pH and high soil temperature (Harley & Smith, 1983; Bowen 1987; Daniels-Hetrick *et al.*, 1988), influence plant growth by the production of antibiotics and growth regulators (Malajczuk *et al.*, 1975) and provide organic carbon to the fungi (Wild, 1988).

The majority of Australian native plants are known to form symbiotic associations with vesicular-arbuscular fungi (Sward, 1978; Kummerow, 1977; Brundrett, personal communication). The fact that mycorrhizal associations occur in so many Australian native plant species is not unusual considering the generally low nutrient status, low water content, variation in soil pH and high soil temperatures of Australian soils.

The genus *Hibbertia* occurs in Malagasy, New Guinea, Australia, New Caledonia and Fiji, most of the species being endemic to Australia. About 60 species are endemic to the South West of Western Australia, occurring in many different habitats and mostly having yellow flowers. *Hibbertia hypericoides*, the "Yellow Buttercup" is a spreading shrub with narrowly oblong leaves and is widespread in the South West of Western Australia between Augusta and Northhampton (Marchant *et al.*, 1987). The species is very common in Banksia woodlands, but also inhabits dry sclerophyll forests of Jarrah, Wandoo and Marri. Plants flower profusely between April and November, and the shiny black seeds are dispersed by ants (Schatral, in preparation).

Vesicular-arbuscular mycorrhizae have been observed for several *Hibbertia* species (Titze *et al.*, 1980; Lamont, 1982; Brundrett & Abbott, personal communication). However, the effects of mycorrhizal associations on plant growth have not been studied for this genus. The present paper reports on preliminary experiments in which young plants of *H. hypericoides* were inoculated with VAM and supplied with phosphorus at different concentrations. The aims of the study were to provide evidence as to whether VAM can affect plant establishment and growth and to also examine mycorrhizal associations for mature plants in several different natural habitats.

MATERIAL AND METHODS

Glass house experiments

These experiments were designed to examine the effects of mycorrhizal inoculation and phosphorus on young plants. Young plants were obtained from cuttings or seedlings and grown in white sterilized sand at four different phosphorus levels (0, 3, 17 and 42 kg ha^{-1}). Plants were either inoculated with a vesicular-arbuscular mycorrhizal (VAM) inoculum or remained uninoculated (see below for details).

Plant material

Since germination of intact seed was slow and only a small number of viable seeds was available, young plants were mostly obtained from cuttings. Cutting material was collected in June 1991. At this time, extensive new growth and resprouting occurred as the result of the first winter rain. Cuttings were collected from the Casuarina Nature Reserve (situated about 10 km east of Kwinana, Western Australia) and rooted in a peat, coarse river sand and lateritic gravel medium (1:1:1).

Seedlings were obtained by planting fresh seed (collected at Cataby and in the Perth Metropolitan Area in December 1990), in glass house flats in a sterile sand mixture in January 1991. Eighty five percent of all young plants were grown from cuttings, the remaining young plants were seedlings.

Experimental procedure

At the beginning of the experiments both cuttings and seedlings were measured for initial shoot height and root

length. Each seedling and cutting respectively was transferred into a 500 ml plastic container lined with a plastic bag which was filled with the growth medium. All experiments were set up under shade cloth and under humid conditions in a glasshouse at the Field Trial Area of the School of Environmental Biology. Sets of 20 plants were used per treatment.

Responses to phosphorus and mycorrhizal infection

The growth medium was clean white sand, deficient in any soil nutrients and steam sterilized in an autocalve.

Responses to phosphorus only: Plants were grown in sterilized white sand at four different phosphorus levels. Potassium phosphate (KH_2PO_4) solution was used as a source of phosphorus and was applied to the surface of treated pots at the following rates: 0, 3, 17 and 42 kg ha^{-1}. These solutions also contained K_2SO_4 (100 kg ha^{-1}). Nitrogen was applied in the form of a NH_4NO_3 solution at a rate of 53.3 mg kg^{-1}. Finally two additional nutrient solutions were applied to the surface of each pot: solution 1. contained $CaCl_2$ (21.3 g l^{-1}), and solution 2. contained $CuSO_4.5H_2O$ (0.64 g l^{-1}), $ZnSO_4.7H_2O$ (1.49 g l^{-1}), $MnSO_4.H_2O$ (2.98 g l^{-1}), $CoSO_4.7H_2O$ $NaMoO_4.2H_2O$ (0.06 g l^{-1}) and $MgSO_4.7H_2O$ (5.96 g l^{-1}). The solutions were recommended by Chris Gazey, Dept. of Plant Nutrition and Soil Science, University of Western Australia.

Responses to phosphorus and mycorrhizal inoculation: Plants were inoculated with a vesicular-arbuscular mycorrhizal (VAM) inoculum and grown in sterilized white sand at four different phosphorus levels: 0, 3, 17 and 42 kg ha^{-1}. Nutrient solutions were as described above. The VAM inoculum was a *Glomus sp.* isolate, WUM 10 (1), obtained from a subterranean clover host supplied by the Department of Soil Science and Plant Nutrition of the University of Western Australia. The sand inoculum (50 g per pot) was thoroughly mixed with sterilized white sand. This VAM inoculum was used as it was known to colonize the roots of *Hibbertia amplexicaulis* and *H. commutata* (Brundrett & Abbott, personal communication).

Responses to natural soil

Plants were grown in either undisturbed natural soil (UNS), sterilized natural soil (SNS), or sterilized natural soil inoculated with *Glomus sp.* (SNS + VAM). Natural soil was collected from the root zones of adult plants at the Casuarina Nature Reserve. Seedlings and cuttings were transferred into 500 ml plastic containers as described above, and the experiments were set up under shade cloth and under humid conditions in a glass house at the field trial area of the School of Environmental Biology.

Harvesting

Plants were harvested 70 days after the experiments had commenced. At harvesting, cuttings and seedlings were handwashed over a sieve in a water basin to prevent loss of roots. Shoots and roots were separated and their length measured.

Root staining and mycorrhizae root counting technique

Root samples were stored in water at 5 °C for 2 days, then cleared with KOH and stained with 0.03% (w/v) Chlorazol black E using an autoclave for 15-20 minutes at 121°C (for details see Brundrett *et al.*, 1990).

The total length of primary roots and proportion of roots in a sample containing mycorrhizal associations was determined with a dissecting microscope and the gridline interception method used (Newman, 1966; Giovanetti & Mosse, 1980). The type of infection and morphology of the fungal symbiont *i.e.* presence of arbuscules, vesicles and hyphae, were recorded (Abbott, 1982).

Field Study

Mycorrhizal status and nutrient contents were examined for plants in seven natural habitats. All habitats are within 100 km of the Perth metropolitan area. Vegetation types are Banksia woodland (Casuarina Nature Reserve, King's Park), coastal mixed woodland (Swamp Star Reserve, Bold Park) and sclerophyll forest dominated by marri, jarrah and wandoo (Jarrahdale, Alcoa mine site, two areas examined; and Serpentine National Park).

Shoot material (leaves and branches) and fine root samples were collected from the same individuals in the various natural sites from the shoot material with four individuals per study site examined. The shoot material from each site was dried in a convectional oven at 105°C for 24 hours, ground and analysed for phosphorus, nitrogen and potassium. The fine root samples were scored for mycorrhizal colonization and were related to the amount of nutrients in the plant material.

Soil samples were collected from the root zones. Cores of 20 cm depth and 10 cm diameter were obtained and air dried at 30 °C. The soil was analysed for pH, total P, N and K.

RESULTS

Responses to phosphorus and mycorrhizal inocluation

Plant mortality

Plant mortality was high, and similar for VAM inoculated plants and plants which had not been inoculated. Only between 5 and 30% of the young plants survived (mean: 17.6, SE: 2.38, n = 8 treatments, 20 plants each).

Plant growth

No differences in growth were detected for plants inoculated with a *Glomus* inoculum and supplied with phos-

phorus and plants which were supplied with phosphorus only (Two way analysis of variance on log-transformed data: root, F = 2.87, df = 27, p = 0.104; shoot, F = 0.36, df = 27, p=0.55 (Table 1).

Mycorrhizal colonization

Mycorrhizal colonization of the roots decreased with increasing concentration of phosphorus supplied (Analysis of variance on arcsine transformed data : F = 39.25, p < 0.001, df = 9, followed by Neumann Keuls tests to separate means). The percentage of root length colonized decreased with increasing level of phosphorus applied (p <0.05)(Figure 1). External hyphae were very rare, and the infections were primarily in the form of internal vesicles joined by hyphae.

Responses to natural soil

Plant mortality

At the end of the experiment mortality was higher for plants grown in SNS (60 %, n = 20) and those grown in SNS+VAM (80 %, n = 20) than for plants grown in UNS (20 %, n = 20).

Plant growth

Root growth was significantly higher in plants grown in UNS than in plants grown in SNS only. The analysis of variance on log-transformed data, followed by Tukey tests, gave F = 6.99, df = 20, p = 0.004. No difference was found in root growth for plants grown in UNS and those

TABLE 1. Increase in root and shoot length (mm) for young plants of *H. hypericoides* grown in white sand treated with four different concentrations of phosphorus P (kg ha^{-1}). Young plants were either inoculated with VAM (+ VAM) or not inoculated (No VAM).

P (kg ha)	Increase in root length (mm)					
	No VAM			+ VAM		
	n	Mean	S E	n	Mean	S E
0	7	10.1	3.3	1	3.0	4.8
3	2	0		5	34.2	5.4
17	2	2.0		3	31.0	4.5
42	7	4.1	5.5	4	5.8	3.6

P (kg ha)	Increase in shoot length (mm)					
	No VAM			+ VAM		
	n	Mean	S E	n	Mean	S E
0	7	2.0	4.8	1	5.0	
3	4	12.5	4.2	5	21.0	4.8
17	2	29.0	9.0	4	24.5	8.0
42	8	16.4	3.3	4	20.8	7.3

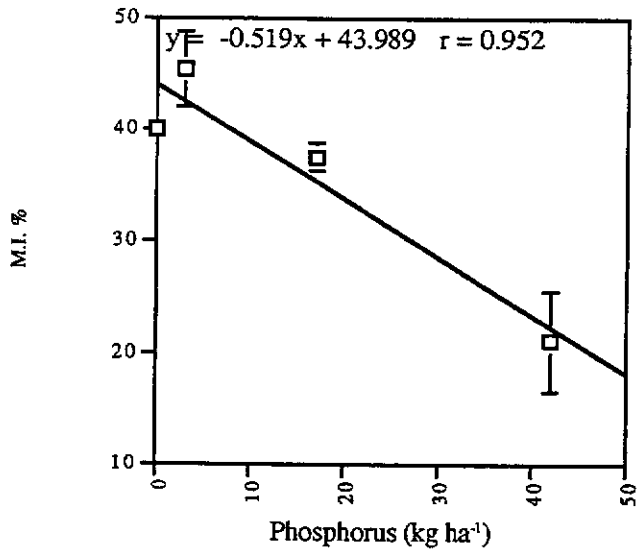

FIGURE 1. Mycorrhizal infectivity (M.I. %) as a response to phosphorus (kg ha^{-1}) in young plants of *H. hypericoides*.

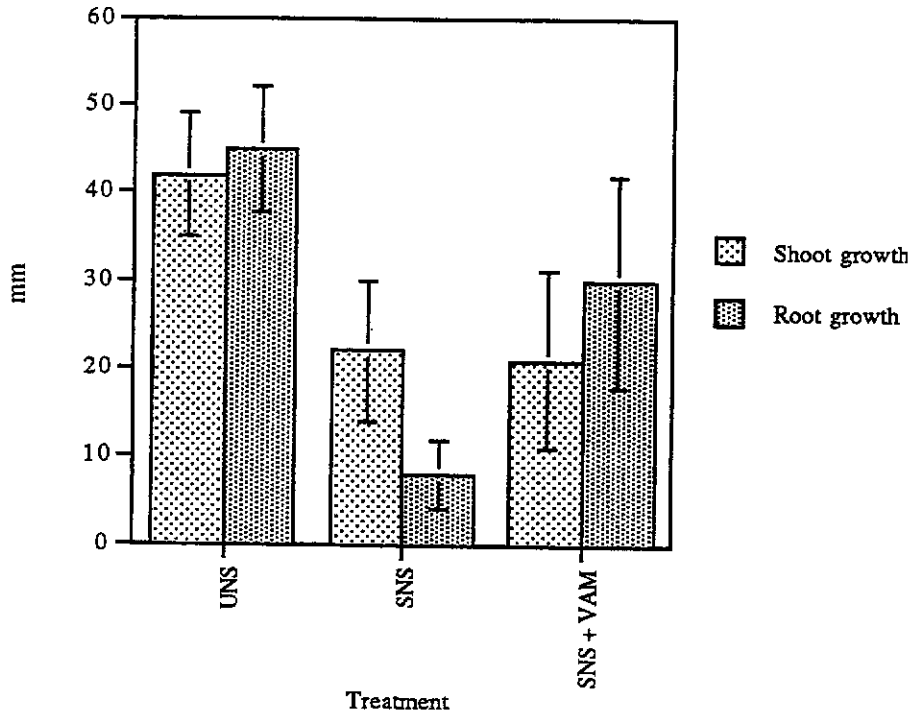

FIGURE 2. Root and shoot growth (mm) for young plants of *H. hypericoides* in response to three different soil treatments: UNS, SNS and SNS + VAM.

grown in SNS and VAM. The increase in shoot growth, however did not differ between the three soil treatments ($F = 3.27$, $df = 23$, $p = 0.054$) (Figure 2).

Mycorrhizal colonization

Mycorrhizal colonization of the roots was higher for plants grown in UNS than for plants grown in SNS + VAM. The t-test on arcsine transformed data was used and gave: $t = 2.89$, $df = 10$, $p<0.01$; mean $\pm$ SE %, UNS: 49.2 $\pm$ 2.39, SNS$\pm$VAM: 35 $\pm$ 4.2. The sterilized soil treatment (SNS) contained no mycorrhizal infection. Internal hyphae and masses of arbuscles within the host root cells were detected in the natural undisturbed soil treatment (UNS). External hyphae were occasionally observed in UNS but were very rare in SNS+VAM.

Mycorrhizal associations in natural habitats

Mycorrhizal colonization

The soil of all habitats was acidic, ranging from pH 3.8 to 5.9. The percentage of root length colonized ranged from 20 to 37% and was not related to the pH of the soil (mean infection rates for each of the seven habitats studied, 4 samples per site, $n = 7$, $r = 0.363$, n. s.). Internal hyphae, arbuscules and extensive external hyphae were found in all examined root samples.

Phosphorus content of the plant material and mycorrhizal colonization

The shoot material was analysed for phosphorus and related to the mycorrhizal colonization of the roots. The concentration of phosphorus increased with increasing percentage of colonization of the roots (data pooled for all seven habitats, $n = 27$, $r = 0.569$, $p<0.002$; mean values for $n = 7$ habitats: $r = 0.675$, $p<0.05$.)

Other nutrients and mycorrhizal colonization of the roots

Mycorrhizal colonization was neither related to the potassium concentration nor to the nitrogen concentration of the shoot material (for potassium: $r = 0.37$, mean values for seven habitats; and nitrogen: $r = 0.48$, $n = 6$.).

DISCUSSION

Mortality of the young plants was neither related to the level of phosphorus applied nor to the percentage of mycorrhizal infection of the roots in the white sand treatments. However, the survival rate was higher in undisturbed natural soil than in sterilised natural soil treatments (with and without VAM). These results indicate that the VAM inoculum used (*Glomus*) did not benefit plant survival. Soil micro-organisms other than *Glomus*, additional soil nutrients and/ or organic matter

may all have contributed to the better survival of the plants grown in the undisturbed natural soil treatment.

Mycorrhizal colonization appears to have been affected by the phosphorus level in the soil: high phosphorus concentrations (40 kg ha^{-1}) suppressed mycorrhizal colonization (Figure 1). It has also been observed for other plant species that beyond the optimum level of supplied phosphorus, the rate of infection will be depressed (Marschner, 1986).

In contrast to the findings of other phosphorus uptake studies (Gerdemann, 1975) neither root nor shoot growth of *H. hypericoides* increased in white sand treatments as a response to phosphorus and inoculation with VAM. Nutrients other than phosphorus may limit growth in young plants of *H. hypericoides* or the phosphorus concentrations used were not sufficient to affect growth. The absence of an effect of mycorrhizal inoculation on the growth of young plants of *H. hypericoides* may be due to the low infection rate of the roots. On average only 30% of the roots were infected during the experiments. Higher infection rates may be necessary to enhance plant growth, since the mycorrhizal infection of the roots leads to a larger adsorptive surface. Finally, a more detailed study with larger sample sizes may reveal effects of phosphorus and inoculation with VAM on the growth of young plants.

The VA mycorrhizal fungus used as inoculum was *Glomus* sp. Porter *et al.* (1987) found that some species of the genus *Glomus* were restricted to infection of roots in more alkaline soil (pH 6 - 8). The pH of the sterilized natural pot soil used in this study ranged from 3.7 - 4.0. Individual variation between plants was high, and this variability could have resulted from variability in the root activity of transplanted plants, with seedlings differing from cuttings and / or genetic variations between individuals (Brundrett & Abbott, personal communication).

In contrast to the results obtained for the white sand treatments the findings from the natural soil treatments suggest that mycorrhizal associations benefit root growth in *H. hypericoides*. However, shoot growth was not affected. Root growth would be expected to show rapid development as an adaptive growth form to ensure the survival of the young plants during the dry, hot summers typical of Western Australia. *Hibbertia hypericoides* has been assigned to a "Type 1" root morphology by Cannon (1949). The roots are characterized by well developed primary and lateral roots, with neither dominating over the other (Lamont, 1982).

Although mycorrhizal infectivity was significantly higher for plants grown in unsterilised sand than for plants grown in sterilised sand with VAM, root growth did not differ between the two treatments. Thus mycorrhizal infectivity does not appear to be simply related to root growth in *H. hypericoides*.

The infection of the roots in natural soil may be the result of acid tolerant fungi like *Acaulospora laevis* rather than *Glomus*. The acid tolerant *Acaulospora laevis* is a very common symbiont in Australian soils (Abbott & Robson, 1978). The morphology of the fungal symbiont also suggests that a fungus other than *Glomus* colonized the roots in the natural soil treatment: external hyphae were more common for the plant roots in the natural soil treatment than for the roots in the sterilized sand treatment inoculated with VAM. Infections were primarily in the form of internal vesicles, and the external hyphae were very rare. However, a more detailed study of the morphology of the fungal symbionts in natural soil treatments is necessary. Special care has to be taken when identifying fungal symbionts in natural soil since roots collected from field soil very often contain more than one species of fungus (Abbott, 1982).

Brundrett & Abbott (personal communication) demonstrated for a large number of jarrah forest species that seedlings inoculated with forest soil showed lower levels of VAM colonization than seedlings that had been exposed to a concentrated VAM inoculum. Our study on *H. hypericoides* however, did not find any difference in the rate of infectivity between plants inoculated with natural soil and those plants inoculated with pure *Glomus* inoculum. It was also found that after exposure to pure VAM or natural soil inoculum, the levels of VAM colonization were lower for *H. hypericoides* than for *H. commutata* and *H. amplexicaulis*, both species studied by Brundrett & Abbott (personal communication). Seasonal variations in VAM fungus infectivity in the natural soil, variability in the root activity of the young plants and/or genetic variations between individuals may have led to the different levels of VAM formation observed in the present study compared with that of Brundrett & Abbott (personal communication).

Mycorrhizal colonization of the roots in natural habitats varied between 30 and 40% for young plants of *H. hypericoides*. This rate of infectivity has been found for *H. amplexicaulis* and *H. commutata* after exposure to forest inoculum (Brundrett & Abbott, personal communication). Again it is *Acaulospora laevis* rather than *Glomus* that is likely to be the fungus infecting the roots of plants grown in the field, since (1) external hyphae are common for this fungus (see above) (see Abbot, 1982 for a description of the fungi) and (2) the examined habitats of *H. hypericoides* show an acidic soil.

Soil pH appears to be a major determinant in the distribution of VA mycorrhizae (Porter *et al.*, 1987). The present study did not find that mycorrhizal colonization of the roots was related to the pH of the soil. Thus the endomycorrhizal fungi colonizing the roots of *H. hypericoides* appear to tolerate a wide range of acidity. The most favourable conditions for plant growth are in

soil of pH 5-7 (Marschner, 1986). Soil acidity restricts the availability of nutrients essential to fungal growth in mycorrhizal plants (Abbott & Robson, 1981).

Our data show for *H. hypericoides* in its natural habitat, that the phosphorus level in the plant material increases with higher mycorrhizal colonization. A greater total uptake in essential nutrients is to be expected by infected plants because of the greater surface area resulting from the growth of hyphae, which may reach distances of several cm from the root surface (Sanders & Tinker, 1973).

No relationship was found between mycorrhizal colonization of the roots and potassium or nitrogen concentration in the shoot material of plants. VA mycorrhizal infection can aid in the uptake of nitrogen in the form of ammonium ions in other plants (Harley & Smith, 1983). However, VA mycorrhizas in general affect only those nutrients which have a very low mobility in soils and which are present in the soil solution in very low concentrations relative to the requirements of the plants. Therefore an increase in the levels of other mineral elements such as nitrogen or potassium may more often be an indirect effect (Harley & Smith, 1983).

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