MULGA RESEARCH CENTRE JOURNAL

VOLUME NO. 10

December 20, 1990

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 in typed format, with double spacing. References should follow the style contained in the current volume. Figures and Tables should be clearly presented. A page charge will be made depending on the level of sponsorship for a given volume.

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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 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 functions held in pursuance of Objective No. 1; and to appoint suitably qualified persons to advisory subcommittees for the purpose which may arise should funds generated by Objective 4 be required 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. His report shall be read at the Annual General Meeting. An annual report will be prepared to cover each calendar year, this 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 Biology, Curtin University of Technology, Kent Street, Bentley, W.A. 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 the School of Biology, Curtin University of Technology by Mrs Melody Best.

The following persons are thanked for reviewing papers in Volume 10: J. Van Schagen, F. McKinnell, J.P. Orsini, J. Bartle, D. Fox and S. Davies.

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ANT SUCCESSION IN PILBARA BORROW PITS

D Fletcher, School of Biology, Curtin University of Technology, Kent Street, Bentley, Western Australia, 6102.

Introduction

The concept of succession may be viewed as a series of stages through which a community passes, on its way to a climax state. The boundaries between these stages may be very indistinct and more like a mosaic model. As the community passes through the stages a number of the original pioneer species disappear only to be replaced by intermediate species which may in turn be replaced in a later stage. This continues until a climax community is reached (Krebs 1985).

This study aimed to document the earliest stage of succession within a set of borrow pits located in the Pilbara region of Western Australia. The pits had been abandoned 28 months earlier and were therefore still in the early stages of succession.

A borrow pit is any area from which either gravel, sand or other material for road making, building and fill is obtained (Walker 1986). Formation of a borrow pit causes alterations to the soil profile, leads to a redistribution of overland water flow, the destruction of vegetation and the migration of fauna from the area. In the short term dust generation and erosion may also result.

This paper presents an assessment of the ant fauna in and around five borrow pits. Ants were chosen because it was considered they would play an important role in the ecology of a pit, can be easily and rapidly collected and could presumably be expected to show successional characteristics parralleling the vegetation (Majer et al. 1984). Dunlop and Majer (1985) describe ant communities as ecological indicators for the assessment of minesite rehabilitation in the Pilbara region. They assessed the ant communities present and estimated the time for significant ecological recovery to take place.

Ants are one of the two most common types of soil surface insects in Australia's arid zone. They require high and fluctuating temperatures to maintain their high levels of locomotory activity and therefore are well suited to arid environments (Greenslade 1984). Arid zone areas characteristically have only few genera but these are rich in species. Genera which reach their greatest frequency in dry areas are **Cerapachys, Meranoplus, Tetramorium** and **Melophorus** (Greenslade 1979). Common pioneer species of the area belong to the genera **Iridomyrmex, Camponotus, Rhytidoponera** and **Melophorus**, with **Iridomyrmex** being the most dominant genus in most arid enviroments (Dunlop and Majer 1985).

The borrow pits examined in this study were located along a 50.6 km stretch of recently constructed road in the Pilbara region, approximately 120 km west of Newman (Figure 1). The section is called 'Munjina Packsaddle' and forms part of the National Highway between Newman and Port Hedland. This is a semi-arid region, with any significant rain usually being associated with summer cyclones. Maximum temperatures reach in excess of 45 ^{o}C in summer and winter frosts occur in most years (Start 1986).

The principal vegetation formations of the Pilbara region are hummock grasslands, mulga and other low woodlands, bunch grasslands and the succulent steppes (Beard 1984). The vegetation surrounding the borrow pits studied was a mixture of hummock grassland (with mixed **Eucalyptus** spp.), mulga woodlands and some mixed **Acacia** thickets.

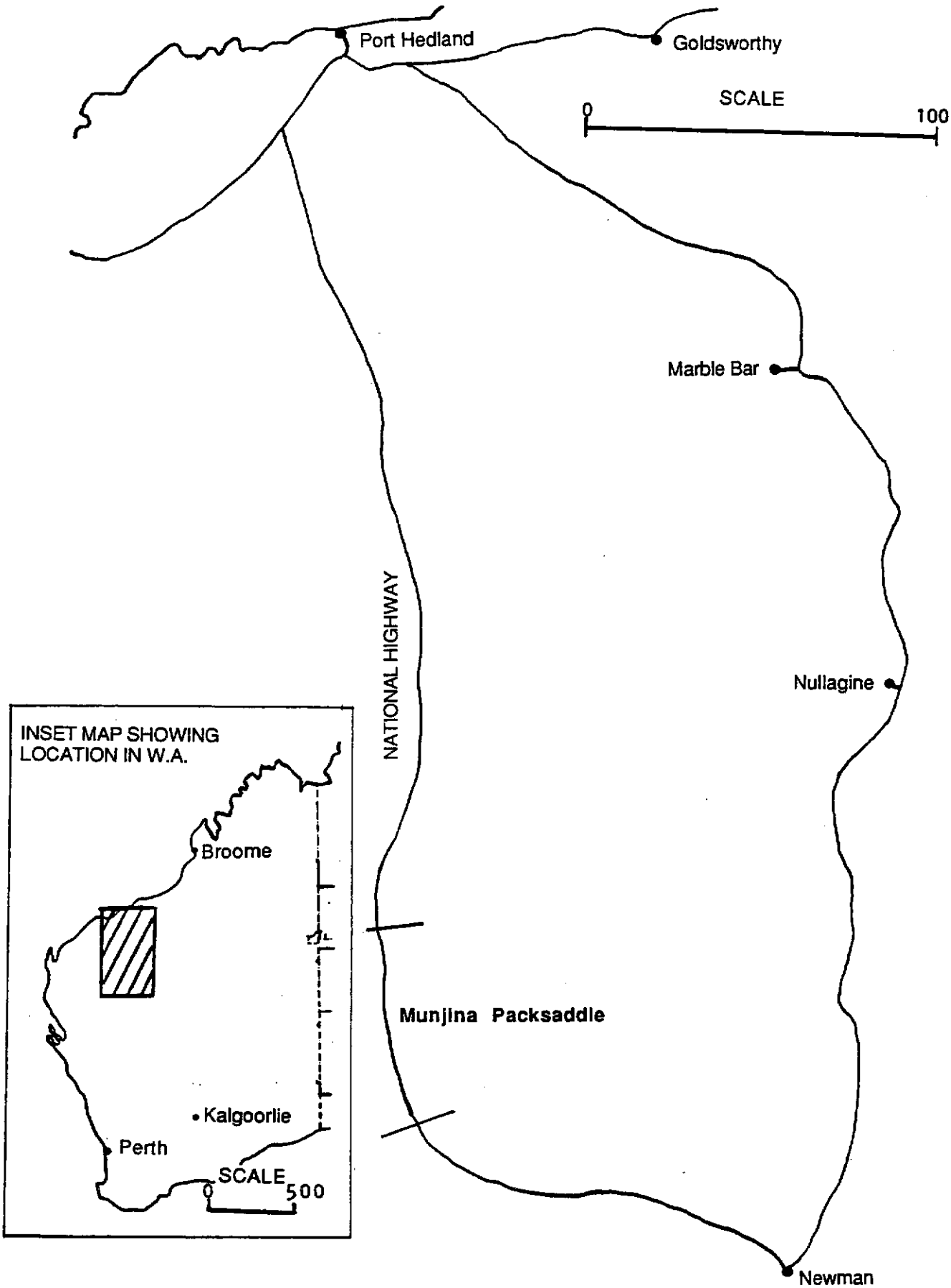
Materials and Methods

Field data was gathered during two field trips to the "Munjina-Packsaddle" section of road. The first of these field trips was conducted in February and the second in July 1988. Ants were surveyed in five pits (A, B, C, D and E) which ranged over the entire length of the section of road and encompassed the two major vegetation types of the area (mulga and spinifex). Table 1 displays the vegetation type surrounding each pit.

TABLE 1 : Pits surrounding vegetation

Pit	Dominant plant species in the surrounding vegetation
A	**Acacia aneura, Triodia pungens, Acacia pruinocarpa**
B	**Triodia pungens, T. basedowii, Eucalyptus gammophylla**
C	**Triodia basedowii, T. wiseana, Eucalyptus leucophloia**
D	**Acacia aneura, Triodia pungens**
E	**Triodia pungens, T. basedowii, E. gammophylla**

FIGURE 1 : A map showing the location of the National Highway and the 'Munjina Packsaddle' section.



In February ten pitfall traps were placed at even intervals across each pit. In addition to these, ten more traps were placed as controls in the surrounding vegetation at similar intervals. In the case of pit B two sets of 10 were used as controls. The control sites were used to give an indication of what the ant community of the area may have been like prior to excavation of the pit.

The hardness of the ground made it necessary to use an auger to put the traps into the ground. The traps were approximately 100ml in volume and were filled with a 70:30 mix of alcohol to glycerol. The threat of impending rain, which could easily have flooded the traps, led to the decision to only leave the traps out for approximately 28 hours. All pits were sampled simultaneously.

In July, twice the number of pitfall traps were used, with twenty traps placed inside and outside each borrow pit. The traps were placed along vegetation transects at 5 m intervals and were left open for 48 hours at each pit. All pits were again sampled simultaneously. The method employed for sampling was that used by Dunlop and Majer (1985).

The ant specimens were sorted by recording the type and density of each species in each trap. Identification of specimens was to species level and was done using the keys of Greenslade (1979). Each individual species was designated its genus name, then a number allocated in numerical order upon its discovery. For example, the second **Iridomyrmex** species recorded would have been called **Iridomyrmex** species 2.

Ant species density, ant species richness inside pits and in controls, plant density, percentage plant cover and plant species richness values for each of the five pits were entered into Minitab (Ryan, Joiner and Ryan 1985). Correlations were then tested between ant density, richness and all other factors (Zar 1984).

Dendrograms were also computed for the ant data based on the presence and absence of species. This was done to establish any patterns related to species distribution. These two methods of analysis were performed on data from both field trips.

Results

The genera with most species recorded for February were, **Melophorus** with thirteen species, **Iridomyrmex** with eight species, **Camponotus** with six species and **Monomorium** with five species. The genus with the highest density of ants was **Iridomyrmex**, with most of these being **Iridomyrmex purpureus**. The next most abundant was the **Melophorus** ants. Species richness ranged from nine to fifteen species in the controls and from four to ten species inside the pits. In every case ant densities and species richness were lower inside the pits than in the control (see appendix 1).

The genera with most species in July were **Iridomyrmex** with thirteen species, **Camponotus** with five species and **Monomorium** with five species. The highest densities of ants were again recorded for **Iridomyrmex**. This was mainly due to the abundance of **Iridomyrmex purpureus** and **Iridomyrmex** sp. 7. The next most abundant genus was **Rhytidoponera**. The species richness in the controls ranged from nine to sixteen species while inside the pits it ranged from six to ten species. In the majority of the pits, ant densities were higher outside the pit than inside. The exceptions were pit E, where 1321 **Iridomyrmex** sp. 7 were recorded inside the pit along with 35 **Monomorium** sp. 6, and pit D with 413 **Iridomyrex purpureus** and 48 **Iridomyrmex** sp. 4 (see appendix 2).

The only significant correlation was between plant cover and ant density inside the pits, during the July sampling period (Table 2).

TABLE 2 : Correlations tested for ant data.

Factors Correlated	Ant species richness inside pits		Density of ants inside pits	
	February	July	February	July
Ant species richness outside pits	N.S.	N.S.	N.S.	N.S.
Plant density inside pits	N.S.	N.S.	N.S.	N.S.
Plant cover inside pits	N.S.	N.S.	N.S.	*
Plant species richness inside pits	N.S.	N.S.	N.S.	N.S.

where N.S. not significant and * significant at $p < 0.05$.

Figures 2 and 3 are dendrograms, on the ant data collected in both February and July. The February dendrogram shows ants on pit A, pit B and pit D were most similar to the ants recorded in their corresponding controls. The July dendrogram continues this trend with pits A, B, C and E coming out most similar to their controls. Pit D was the only exception, but even so it came out most similar to the combination of its control and pit B and its control.

FIGURE 2 : Dendrogram based on the presence and absence of ant species in February 1988.

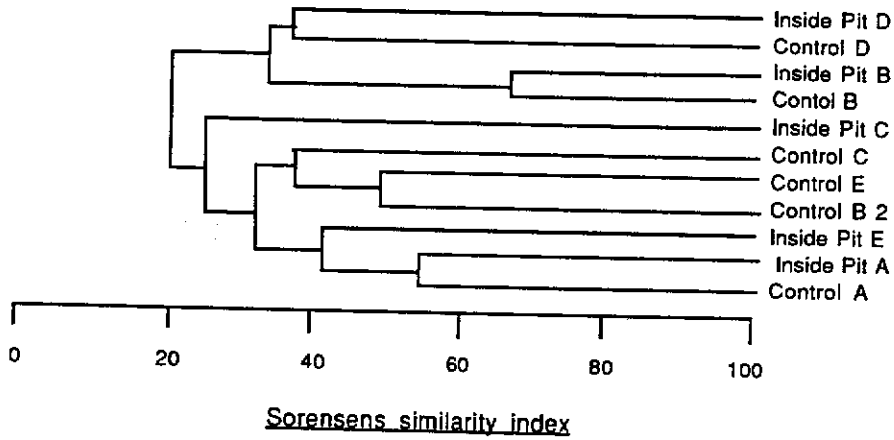


FIGURE 3 : Dendrogram based on the presence and absence of ant species in July 1988.

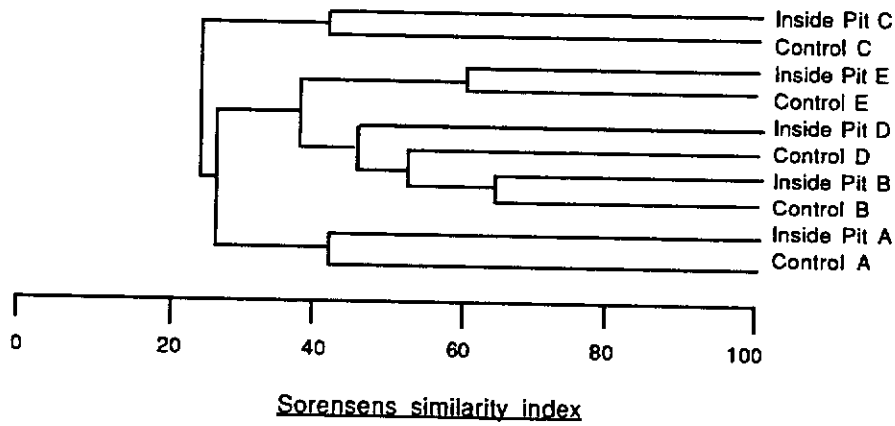
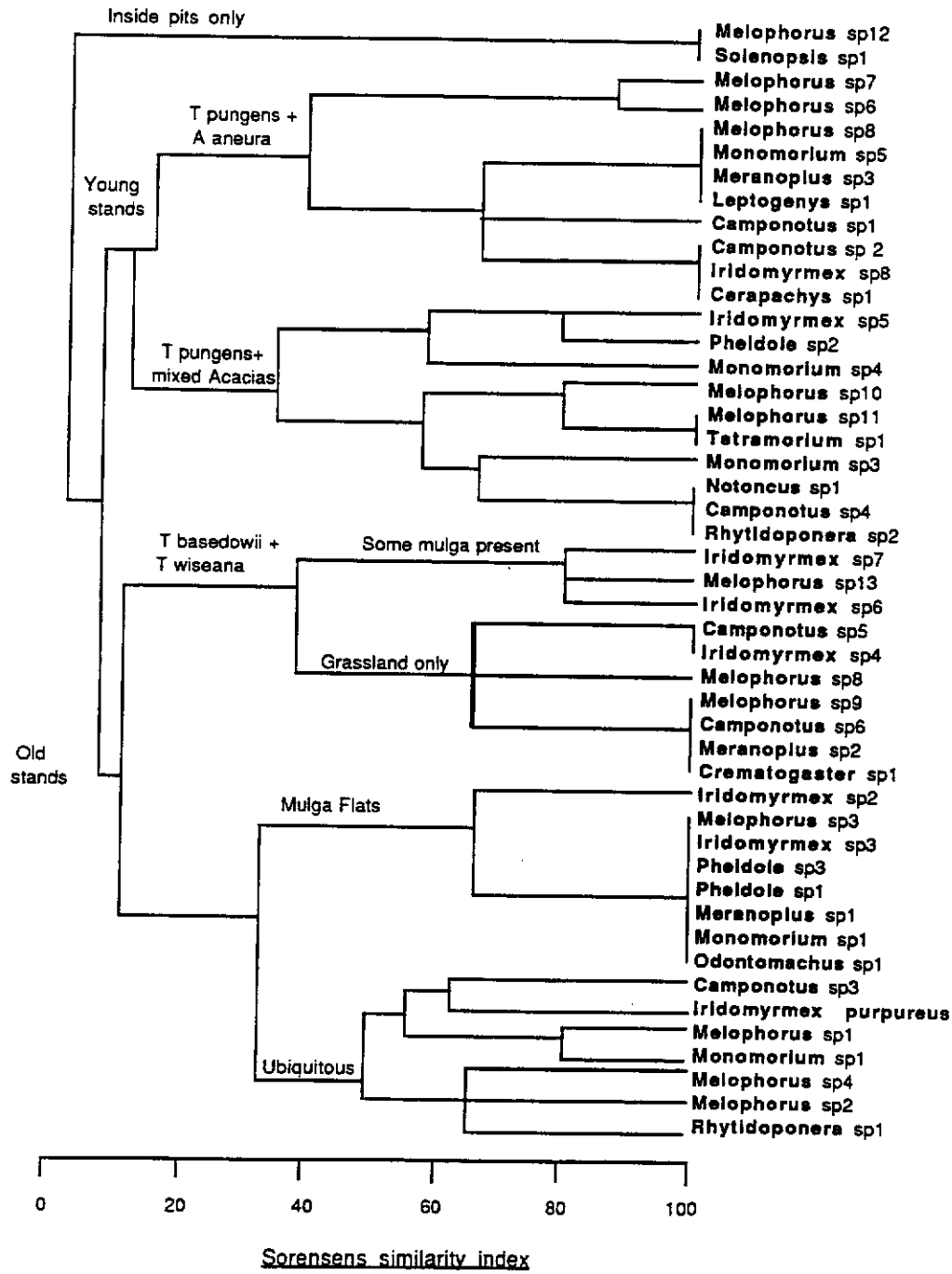


Figure 4 is another dendrogram based on a species by species comparison. It shows the species could be divided into some distinct groupings on the basis of the age of vegetation (since fire) aswell as the type of vegetation. A set of ubiquitous species were also identified as being present in a wide range of habitat. These included species from the genera **Rhytidoponera, Melophorus, Camponotus, Iridomyrmex** and **Monomorium.**

FIGURE 4 : A species by species dendrogram based on the presence or absence of ant species in February 1988.



Discussion

A large number of ant species was recorded both in and outside the five pits which were surveyed in February and July. The most common genera found occurring inside the pits were **Iridomyrmex, Melophorus, Camponotus** and **Rhytidoponera**. This is consistent with the results obtained by Majer et al. (1984) in a study of minesite rehabilitation in the south-west of Western Australia. They suggested that species of these genera which occurred on pits were generalists with a broad food base, which allowed them to colonise the disturbed areas more easily.

The major change in species composition between February and July was in the number of **Melophorus** species, which fell from thirteen to four. This can be explained by the fact that **Melophorus** ants are adapted for foraging in the extremely hot temperatures of summer and are often inactive in the cooler dry season (Greenslade 1984).

Species richness in the controls in both February and July had very similar ranges, and was virtually identical to the estimate of the range of values for ant species richness in summer for mature hummock grasslands (Dunlop and Majer 1985), namely ten to eighteen species. The species richness inside two pits fell into this range. Pit D in February had ten species and pit A in July had twelve, indicating that some of the pits are perhaps approaching a climax species richness level.

The July correlation data showed a significant positive correlation between percentage vegetation cover and ant density. This may be explained by percentage vegetation cover being viewed as an approximation of plant biomass on a pit, which in turn reflects the basic food resource base of each pit. The larger the food resource, the more ants that can be supported on a pit (Majer **et al.** 1984). This correlation was not found in February probably because less traps were set, and traps were not placed along transects.

The species dendrogram revealed that most of the ant species were restricted to distinct vegetation types. The exceptions were the seven ubiquitous species which occupied a wide variety of vegetation types. Each identifiable vegetation type usually contained at least one species representative of each ecological ant groupings described by Dunlop and Majer (1985). These groups were, dominant **Iridomyrmex** species, subordinate camponotine Formicinae, taxa whose occurrence depends on physical properties such as climate (ie **Melophorus**), opportunist (ie **Rhytidoponera**), generalised myrmicines and large solitary foragers (ie **Camponotus**). The only group described which was not represented was the cryptic soil and litter ants, a group which has been acknowledged as being rare in hummock grasslands (Dunlop and Majer 1985).

The ant species composition of each pit was usually most similar to the ant fauna in its corresponding control. This suggests that the ant fauna of the surrounding area is having an influence on which ant species are colonising the pits and also the final climax community which will be formed. This community should be very similar to the climax community in the surrounding area.

In summary, ant establishment on the pits is progressing well, with the ant fauna of surrounding areas having the most significant influence on which species are becoming established. The ant communities of these surrounding areas are quite distinct in terms of their species but have similar structure in terms of ecological groups. Succession appears to be heading towards a climax community which is most similar to that present in the surrounding vegetation.

Acknowledgements

I would like to acknowledge the supervision of the project by Assoc. Prof. J.E.D. Fox. Assistance in ant identification and data interpretation was given by Dr. J. Majer and Mr J. Van Schagen. Finally, the dendrograms were produced with the help of Jan Henry and Ken Youngson. The input of all the forementioned is greatly appreciated.

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APPENDIX 1 : Ant species densities for pits from the sampling in February.

SPECIES	A con	B1 con	B2 con	C con	D con	E con	A pit	B pit	C pit	D pit	E pit
PONERINAE											
Rhytidoponera sp 1	10	1					2	2			
Rhytidoponera sp 2			1								
Cerapachys sp1										4	
Leptogenys sp 1					5						
Odontomachus sp 1	1										
MYRMICINAE											
Monomorium sp 1	11		1	2			2				
Monomorium sp 2	1										
Monomorium sp 3			2					1			
Monomorium sp 4						6					
Monomorium sp 5					1						
Crematogaster sp 1				9							
Meranoplus sp 1	3										
Meranoplus sp 2				2							
Meranoplus sp 3					1						
Pheidole sp 1	3										
Pheidole sp 2			6			4					
Pheidole sp 3	1										
Solenopsis sp 1									1		
Tetramorium sp 1			1							1	
DOLICHODERINAE											
Iridomyrmex purpureus	18	148	439	213	122	779	6	34	82	38	21
Iridomyrmex sp 2	3										
Iridomyrmex sp 3	1										
Iridomyrmex sp 4		3									
Iridomyrmex sp 5			1		14	4					
Iridomyrmex sp 6				1	3				1		
Iridomyrmex sp 7				41	1						1
Iridomyrmex sp 8										1	
FORMICINAE											
Camponotus sp 1					22					3	
Camponotus sp 2										1	
Camponotus sp 3		1	1	1		1			1		
Camponotus sp 4			1								
Camponotus sp 5		1									
Camponotus sp 6				2							
Melophorus sp 1	5		1	1		2	2				1
Melophorus sp 2	1						1				
Melophorus sp 3	5										
Melophorus sp 4	13	1	10	1				1			
Melophorus sp 5					20						
Melophorus sp 6		1	1		2			2		5	
Melophorus sp 7		1			1			1		1	
Melophorus sp 8		1		2							
Melophorus sp 9				3							
Melophorus sp 10			3	4						1	
Melophorus sp 11			2							1	
Melophorus sp 12									1		
Melophorus sp 13				2	2						
Notoncus sp 1			2								
Paratrechina sp 1	1										
TOTAL NO. SPECIES	15	9	15	14	12	6	5	6	5	10	4

APPENDIX 2 : Ant species densities for pits from the sampling in February.

SPECIES	A con	B1 con	C con	D con	E con	A pit	B pit	C pit	D pit	E pit
PONERINAE										
Rhytidoponera sp 1	127	15		11		11	5		10	
Rhytidoponera sp 2	1									
Rhytidoponera sp 3	9	5		21	9		3			2
Rhytidoponera sp 4				1				1		
Leptogenys sp 1		2		3						
Opisthopsis sp 1			1							
MYRMICINAE										
Monomorium sp 1	7							1		
Monomorium sp 3				1	1					
Monomorium sp 4		3								
Monomorium sp 5					1					3
Monomorium sp 6					2					35
Meranoplus sp 1	2									
Meranoplus sp 2			1		2					
Meranoplus sp 4						19				
Pheidole sp 1						5				
Pheidole sp 2		1								
Pheidole sp 4				2						
Tetramorium sp 1				1			1		1	3
Tetramorium sp 2				1						
Tetramorium sp 3	7					14				
DOLICHODERINAE										
Iridomyrmex purpureus	194	501	13	249	146	41	66	206	413	
Iridomyrmex darwiniensis		6	7	1						
Iridomyrmex sp 2		15								
Iridomyrmex sp 4		8		2	2	33			48	3
Iridomyrmex sp 5			5	2				2		
Iridomyrmex sp 6	7		13	25				13		
Iridomyrmex sp 7			383		90					1321
Iridomyrmex sp 9		13		42			67			
Iridomyrmex sp 10		57					4			13
Iridomyrmex sp 11									3	
Iridomyrmex sp 12	131					2				
Iridomyrmex sp 13						3				
Iridomyrmex sp 14		1			32					
FORMICINAE										
Camponotus sp 1		1		1	15		4	3	1	9
Camponotus sp 3	7	1	1				2			
Camponotus sp 7					3					
Camponotus sp 8						2				
Camponotus sp 9									1	
Melophorus sp 1	1									1
Melophorus sp 4						1				
Melophorus sp 7				1						
Melophorus sp 9			2							
TOTAL NO. SPECIES	11	14	9	16	11	10	8	6	7	9

FACTORS CONTRIBUTING TO THE PRESENCE AND PERSISTENCE OF BARE PATCHES IN PASTURE ON A REHABILITATED COAL MINE DUMP AT CHICKEN CREEK AREA 4, COLLIE

A I Doronila and J E D Fox, School of Biology, Curtin University of Technology, Kent Street, Bentley, Western Australia, 6102.

Introduction

In May 1982 the area 4 dump of the Chicken Creek coal mine, Muja, 20 km east of Collie was sown to pasture. Three treatments were applied to restore the dump to grazing land. One area received a 10 cm layer of topsoil taken from previously cleared jarrah-marri forest. Another received topsoil from the flat flood plain area of the Chicken Creek stream, previously in pasture. A third area received no topsoil. All three areas were given the same fertilizer and seeding rates of pasture species. The basis of the pasture is a sown species component of ryegrass (**Lolium rigidum**) and sub-clover (**Trifolium subterraneum**) strains. The non-sown component includes a range of volunteer grasses and other herbage species. The growth of pastures has been monitored annually from 1983 onwards. Random quadrat sampling, established that 100 percent cover was achieved in all treatments by the third year (Turner and Fox 1985).

Over recent years bare patches have appeared within the pasture. Bare ground is more frequent in the no topsoil treatment (Fox and Owens 1988; Fox and Doronila 1989). This paper describes investigations undertaken in order to identify the possible causes of pasture failing to grow and persist in these bare areas.

Materials and Methods

In 1988 a set of bare patches (n = 16, 0.1 - 0.6 m^2) was observed for reduction in size over the growing season. Minimal grazing occurred over the period. Bare ground was measured within 50 quadrats of 25 x 25 cm placed randomly over the 1989 growing season, during the regular sampling programme to contrast pasture growth between topsoil treatments.

Soil samples were collected in 1989 from bare patches (n=16) and from the adjoining pastures (n=3) to be later analysed in the School of Biology, Curtin University. Nitrogen content was determined using the Kjeldahl method (Gianello and Bremner 1986). Phosphorus analysis was performed by colorimetry on a mixed acid digest (Colwell 1965). Sodium, potassium determinations were done with flame photometry (Hesse 1971).

Acidity and conductivity levels were determined on 1:5 solutions of soil to deionized water (Loveday 1974). The pH data were converted to the negative antilog then analysis of variance was done on the transformation. Mean and standard errors were derived from the reconverted data.

Percentage bare ground was converted to arcsine then analysis of variance was calculated. All other data were compared with one-way analysis of variance.

Soil surface resistance was measured with the aid of a penetrometer (Proctor EL~28-651).

Other parameters observed were soil texture and water runoff. The latter was measured by pouring 250 ml of water on the surface, then measuring the distance travelled until the water was absorbed.

Results

The mean percentages of bare ground on the three topsoil treatment areas over the 1989 growing season are summarised in Table 1.

TABLE 1: The percentage bare ground per quadrat on the three treatment areas (mean ± s. e.).

Sampling Date	Topsoil	TOPSOIL TREATMENT No (n = 6)	Uplands (n = 11)	Lowlands (n = 19)	F-value	Level of Significance
14/09/89		$12.5^{b} \pm 21.8$	$2.2^{a} \pm 1.5$	$3.8^{a} \pm 1.7$	4.18	*
20/10/89		5.0 ± 6.3	1.7 ± 1.5	0.9 ± 0.6	0.53	NS

* P<0.05. Values with the same superscript letters are not significantly different.

No bare patches were recorded in the topsoil treatments at the first two visits. No topsoil had 3.9 percent and 12.6 percent bare area respectively. The amount of bare ground was significantly greater in no topsoil at the third visit. At the last assessment no significant difference was shown for the three treatments.

16 randomly selected bare patches were assessed for reduction in surface area over a 4 month period from 2 June 1988 to 13 October 1988. Descriptive statistics and the paired t-test result is summarised in Table 2. A significant reduction (32 percent) in size was observed.

TABLE 2: Bare patch size (m^2) over a four month period.

	June 2	October 13
N	16.0	16.0
minimum	0.13	0.11
maximum	0.81	0.51
range	0.68	0.40
mean	0.37	0.25
std. error	0.04	0.03

mean difference	=	0.12
std. error of difference	=	0.03
DF	=	15
t-value (paired test)	=	4.08

$p < 0.01$

The results for the physical and chemical analysis of bare area and pasture in the no topsoil area are summarised in Table 3.

NUTRIENT LEVELS

Mean nitrogen, potassium, calcium and phosphorus levels were significantly higher in soil samples from pasture covered areas with over a ten fold increase in the expression of nitrogen compared to the bare areas.

Sodium levels were not significantly different between the pasture covered and bare areas, however, soil from bare areas tended to have a higher mean sodium concentration and a high standard deviation due to the large range of sodium levels (20.36-78.26 mg g^{-1}). Pasture soil samples did not show a large standard deviation.

TABLE 3: Comparison of bare areas with pasture in Chicken Creek Area 4 (1989), no topsoil treatment. (mean + s.e.).

CHEMICAL ANALYSIS	Pasture (n = 3)	Bare Area (n = 16)	F Value	Level of Significance
Nitrogen (%N)	0.140 ± 0.039	0.011 ± 0.001	80.5	***
Potassium K^+ (mg g^{-1})	10.33 ± 0.85	7.48 ± 0.47	6.13	*
Calcium Ca^{++} (mg g^{-1})	5.57 ± 1.62	1.59 ± 0.15	32.06	***
Sodium Na^+ (mg g^{-1})	15.42 ± 1.56	44.31 ± 5.99	2.04	NS
Phosphorus (mg $100g^{-1}$)	3.7 ± 1.79	0.5 ± 0.10	9.32	**
PHYSICAL ANALYSIS				
Conductivity (m S cm^{-1})	2.98 ± 0.57	5.25 ± 0.43	17.30	**
Penetrometer (kg cm^{-2})	43.22 ± 3.79	61.33 ± 4.03	17.30	***
pH	5.6 ± 0.29	4.7 ± 0.10	3.41	**
Run off (m)	0.24 ± 0.13	0.80 ± 0.05	55.80	***

SOIL PHYSICAL PROPERTIES

The soil texture was classified as fine sandy loam.

The mean conductivity of the bare ground soil samples was significantly higher than that for pasture soils while the pH was significantly lower. Surface resistance was also significantly higher on bare ground. A surface crust was observed on bare ground and 18 kg cm^{-1} more force was required to push in the probe to the required depth compared to pasture areas.

Bare areas were also less absorptive of water as indicated by the water runoff data. The volume of water (0.25 l) travelled a significantly longer distance (80 cm) on bare ground in comparison to pasture (24 cm).

A correlation matrix of all 9 parameters investigated is summarised in Table 4.

TABLE 4: Correlation matrix of all parameters analysed for bare ground.

Test	ECU	Na	Ca	K	P	N	pH	Surface Resistance
ECO	1.0							
Na	0.718**	1.0						
Ca	0.213	0.600**	1.0					
K	-0.257	0.075	0.186	1.0				
P	0.031	-0.030	-0.253	-0.182	1.0			
N	-0.180	0.022	0.234	0.264	0.069	1.0		
pH	-0.269	0.079	0.155	0.625**	-0.085	0.611*	1.0	
Surface resistance	0.236	0.396	0.453	0.264	0.053	0.177	0.520	1.0
Run off	0.282	0.140	0.380	-0.032	-0.200	0.147	-0.052	0.410

Regression lines of significant correlations

y	=	b	x	+	a	F value	Sig.
ECU ($mS\ cm^{-1}$)	=	0.24 Na ($mg\ g^{-1}$)		+	0.006	14.9	**
Na ($mg\ g^{-1}$)	=	23.6 Ca ($mg\ g^{-1}$)		+	6.80	7.8	*
K ($mg\ g^{-1}$)	=	3.04 pH		-	7.03	8.9	**
N (%)	=	0.007 pH		-	0.023	8.3	*

note * $P<0.05$, ** $P<0.01$

The soil conductivity of bare areas was significantly correlated with sodium concentration ($r = 0.718$). Sodium concentration was also significantly correlated with calcium concentration ($r = 0.600$). Other soil nutrients (potassium and nitrogen) were also observed to have significant correlation with the pH of the soil (K: $r = 0.625$; N : $r = 0.611$).

DISCUSSION

Monitoring of pasture cover over the previous growing seasons (Fox and Owens 1988; Fox and Doronila 1989) showed that bare ground decreased progressively during the season. The start of each season had more bare ground on the no topsoil area (c. 30 percent), but the percentage gradually declined to approximately 5 percent towards the end of the season. The lower pasture cover may be related to the early seasonal decline of pasture species due to earlier drying out of this treatment compared with topsoiled areas (Fox et al. 1986). There may also be less pasture seed stored in the bare areas.

From the physical and chemical analysis of the bare areas, we can outline two major factors leading to the persistence of bare patches in the no topsoil area.

Firstly, higher surface resistance indicates an increase in compaction. This may impede seed from germinating in the bare area. Compacted coal spoil materials reduce grass yields and root growth (Rimmer 1979). Observations by Ayerst (1978) indicated that radicles of germinating seed fail to penetrate the more compacted spoil materials. The growth of clover species on bare patches would also be depressed due to the low calcium levels necessary for the function of nitrogen fixation. Nodulation of **Trifolium subterraneum c.v. Bacchus Marsh** roots are inhibited at a low pH (< 5.0) and low Ca concentration (10-100 µM). However, an improvement occurs at higher Ca concentration (10 µM) (Loneragan and Dowling 1958). Root nodules are pH sensitive and can only fix nitrogen

actively if the plant is adequately supplied with all the essential mineral elements (Russell 1988). Phosphorus uptake is also reduced in pasture species due to low pH levels (Kim et al. 1985).

Soil texture was observed to be a fine sandy loam. This is a soil where it is most difficult to maintain good structure (McKissock and Gilkes 1988). The absence of organic matter in bare patches reduces the permeability and aggregation of a poorly structured surface soil (Russell 1988).

The low porosity of bare areas can contribute to poor establishment of pasture seedlings, as there would be less moisture available when seedlings do germinate. Bare areas appear more subject to sheet erosion therefore increasing the loss of any seed present. The bare patches tended to be compacted. This is a condition that correlates with high bulk density and low porosity of the soil. As a result, there is a reduction in water holding capacity and an impediment to water infiltration (Downes and Stokes 1977).

The acidity of the soil on bare patches was significantly higher (pH = 4.7) than that of the soil in pasture on the no topsoil area (pH = 5.6). Pulford et al. (1988) noted that pyrite oxidation of spoil material lower down a dump profile resulted in acidic patches which appeared three years after rehabilitation. This may be the case on the Area 4 dump. Large bare patches have been appearing on the adjacent Chicken Creek Area 5 pasture rehabilitation. These have been prone to erosion due to the lack of vegetation cover. Measurements of the pH indicated that they were significantly more acidic (pH = 3.9) than the surrounding pasture (pH = 4.8) (Doronila and Fox 1990). The increase in acidity may be occurring at a faster rate than on the Chicken Creek Area 4 pasture. The increased acidity solubilises ions such as aluminium and manganese, which are toxic to plants in high concentration (Bradshaw and Chadwick 1980). The lower pH also reduces nitrogen availability as it is connected to the nitrate form which is easily leached through the soil. Phosphorus can be less available due to the higher acidity (Bradshaw and Chadwick op. cit.). This may explain the low nutrient levels on the bare patches. Compounded by the high acidity, electrical conductivity and sodium content were also much higher on the bare patches. Early work on the spoil materials from the mine, indicated that these were saline and there was a potential for the development of sodic conditions (Dames and Moore 1983). The high salinity (5.25 mS cm^{-1}) further compounds the problem of water availability of the spoil material. Field observations on the few clover seedlings present on the bare patches showed that these were generally stunted and the leaves were of a dark blue/green coloration, symptoms associated with high soil salt content (Russell 1988).

CONCLUSION

The more acidic and saline nature of bare patches suggest that a net migration of toxic ions occurred from the lower substrata of the dump. In order to contain such sectors, reliming may be necessary. To predict the appropriate rate of lime required it is necessary to identify the potential acidity. Doubleday (1974) noted that inadequate liming over a two year period caused acidity to increase and led to the subsequent failure of crop establishment.

The conductivity levels exceeded 4 m S cm^{-1}, a level considered to be restrictive to the growth of many crops (Richards 1954). The most used method to help displace exchangeable ions is to use gypsum ($Ca\ SO_4 . 2H_2O$). It is more efficient than calcium carbonate because it can displace more sodium. In addition, gypsum is much more economical (Russell 1988).

Amelioration of toxic conditions could be initiated by liming, combined with cultivation and reseeding to effectively cover the bare areas. In time the biomass produced by pasture growth should be adequate to improve the structure of the soil surface.

ACKNOWLEDGEMENTS

Mr Ashley Knight, final year biology student, is thanked for practical work undertaken.

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THE SENSITIVITY OF SOME WESTERN AUSTRALIAN CATERPILLARS TO FLUOROACETATE

L E Twigg, School of Environmental and Life Sciences, Murdoch University, Perth, Western Australia

Present Address: New South Wales Department of Agriculture and Fisheries, Vertebrate Pest Research Unit, A.R. and V.C., Forest Road, Orange, New South Wales.

ABSTRACT

The sensitivity of fluoroacetate (1080) of three species of lepidopteran and one species of hymenopteran larvae, which coexist with fluoroacetate-bearing vegetation in Western Australia, was determined. Larvae of **Perga dorsalis** (hymenopteran) and **Mnesamplea privata** feed mainly on eucalypts and were very sensitive to the toxin with LD_{50} values of 1.05 and 3.88 mg 1080 kg^{-1} respectively. **Spilosoma** sp. has a cosmopolitan diet and was moderately tolerant to fluoroacetate (LD_{50} 42.73 mg 1080 kg^{-1}). However, larvae of **Ochrogaster lunifer**, which when collected were feeding on fluoroacetate-bearing **Gastrolobium microcarpum**, were extremely tolerant to the toxin (LD_{50} c. 150 mg 1080 kg^{-1}). This suggests that co-evolution has occurred between the tolerant insects and the toxic plants.

INTRODUCTION

It has been recently demonstrated that several species of dasyurids which coexist with fluoroacetate-bearing vegetation are less sensitive to fluoroacetate intoxication than are dasyurids not exposed to these plants (King et al. 1988; Twigg 1986). Thirty-four of the 34 plant species in Australia known to produce fluoroacetate belong to the genera **Gastrolobium** and **Oxylobium** (leguminosae), and 33 of these species are confined to the south west of Western Australia (Aplin 1971). Depending upon the degree of exposure to the toxic vegetation, indigenous seed eating birds (Twigg and King 1988; Twigg 1986) and herbivorous mammals (King et al. 1978, 1981; Oliver et al. 1977, 1979; Mead et al. 1985) in this region have also developed tolerances to fluoroacetate.

The toxicity of fluoroacetate arises from its conversion within the mitochondria to fluorocitrate which competitively inhibits the tricarboxylic acid cycle enzyme, aconitate hydratase (Morrison and Peters 1954). This results in elevated citrate concentration in tissues and plasma (Buffa and Peters 1949; Mead et al. 1985), energy deprivation (Buffa et al. 1973) and death.

Insects are the major component of the diet of most dasyurids (Blackhall 1980; Kitchener 1981; Strahan 1983). In areas where fluoroacetate-bearing vegetation occurs, the ingestion of insects which feed on the toxic plants could account for the enhanced tolerance to fluoroacetate seen in those dasyurids which coexist with this vegetation. Consequently, to determine whether those insects which have the potential to consume the toxic plants have indeed developed a tolerance to fluoroacetate, the sensitivity to the toxin of several species of caterpillars which coexist with fluoroacetate-bearing vegetation in Western Australia was investigated.

METHODS

Origin and Housing of Experimental Animals

Caterpillars were hand collected from various localities in the south west of Western Australia: **Perga dorsalis** Leach (Hymenoptera: Pergidae) and **Spilosoma** sp. (Lepidoptera: Arctiidae) were from Perth (31° 57' S, 115° 51' E); **Mnesamplea privata** (Guenee) (Lepidoptera: Geometridae) were from Kulikup (33° 50' S, 116° 40' E); and the **Ochrogaster lunifer** Herrich-Schaeffer (Lepidoptera: Notodontidae) were from Dryandra (32° 29' S, 117° 01' E). Larvae were identified by staff of the CSIRO Department of Entomology, Canberra, and Dr B. Dell, Murdoch University, using both larvae fixed with Carnoy's reagent, and adults raised from caterpillars.

Larvae were kept in translucent plastic containers fitted with plastic fly-wire lids and were held at 23 ± 1°C with 70% relative humidity and a 12:12 h photoperiod. A paper towel liner was added to the bottom of each container. Caterpillars were supplied daily with fresh leaves of the plant species of which they were originally collected.

Mortality Data

Caterpillars were held for 1 week before commencing the experiments to allow them to become accustomed to captivity. Each caterpillar was individually weighed (±0.001 g) and then administered either aqueous commercial grade 1080 (Rentokil Laboratories, Perth; 94% sodium fluoroacetate by HPLC) or deionized water by injection into the haemocoel in the last 2-3 abdominal segments using a 28 gauge 10 µl Hamilton syringe. Dosed caterpillars rarely received greater than 10 ml per injection and control animals received equivalent amounts of deionized water. None of the caterpillars were re-used during the trials.

After dosing, caterpillars were inspected at frequent intervals for the first 24 hours and then three times daily for a further 4 days and the number of deaths recorded. The 5 day inspection period was similar in length to the time required for fluoroacetate to induce 100 percent mortality in the cabbage moth, **Pieris brassicae** (David and Gardiner 1953) and the aphid **Aphis fabae** (David and Gardiner 1951).

LD_{50} values and the 95% confidence limits were calculated according to the moving average method of Thompson (1947).

Determination of Whole Body Citrate Concentrations

Citrate concentration was determined in both dosed and undosed caterpillars. After decapitation, larvae were macerated with a glass rod in a 10 ml conical plastic centrifuge tube containing 2.0 ml of deionized water. To ensure the release of all cellular citrate, the cuticle was carefully removed and ground in a further 2.0 ml deionized water with a mortar and pestle. Homogenates were then combined in the centrifuge tubes using 1.0 ml deionized water for quantitative transfer.

Homogenates were deproteinized (2.5 ml 25 percent (w/v) trichloroacetic acid) and centrifuged at 4000 g for 15 minutes to remove precipitated protein. After filtration (Whatman No. 1 filter paper), a 0.5 ml aliquot of each

supernatant was diluted with 0.5 ml deionized water and the citrate concentration in the diluted supernatant was determined using the colorimetric method of Camp and Farmer (1967) according to King et al. (1981) and Twigg (1986). The recovery of added citrate (264 uM) from spiked supernatants was 82 percent (n=9), SE 3.9 percent), however, the citrate concentrations determined for the larvae were not corrected for this recovery.

RESULTS

The range (mean) in the body weights of the caterpillars used was; P. **dorsalis** 1.021-2.304 g (1.386 g), M. **privata** 0.261-0.538 g (0.421g), **Spilosoma** sp. 0.361-1.240 g (0.888 g) and O. **lunifer** 0.460-1.351 (0.989 g).

Larvae of P. **dorsalis** were collected from several species of eucalypts and were found to be very sensitive to fluoroacetate (LD_{50} 1.05 mg 1080 kg^{-1}); Table 1). The majority of deaths for the poisoned caterpillars of all species occurred around 14-18 h (range 2-48 h) after administration of 1080. To illustrate the fluoroacetate was disrupting the tricarboxlyic acid cycle of the caterpillars, the total body citrate concentrations of poisoned P. **dorsalis** larvae were determined 12 h after dosing. In larvae administered the equivalent of the LD_{50}, citrate concentration increased 1.6-fold and was significantly greater ($P<0.005$; $t=-4.81$, $n=8$) than that of the caterpillars which had not received 1080 (Table 2).

The larvae of M. **privata** were collected while they were feeding on young eucalypts, and were also very sensitive to fluoroacetate (LD_{50} 3.88 mg 1080 kg^{-1}; Table 3). However, larvae of an unidentified species of **Spilosoma**, collected from a wide variety of garden plants around Perth, were moderately tolerant to fluoroacetate (LD_{50} 42.73 mg 1080 kg^{-1}; Table 4). The total body citrate concentration in larvae of **Spilosoma** given 10 or 45 mg 1080 kg^{-1} was approximately 2- and 4-fold greater than that of the unpoisoned conspecifics (Table 5) and this increase was significantly greater ($P<0.001$; $t=-8.03$, $n=8$) at the 45 mg dose level.

Larvae of O. **lunifer** were collected while they were feeding on toxic **Gastrolobium microcarpum** (Sandplain poison). While the limited number of individuals collected prevented the determination of an accurate LD_{50} value for this species, these caterpillars appear to be very tolerant to fluoroacetate. No deaths occurred when larvae were given 50 or 100 mg 1080 kg^{-1} but larvae given 200 mg 1080 kg^{-1} or greater, succumbed (Table 6).

DISCUSSION

Unlike the availability of laboratory-bred animals, it is often difficult to obtain large numbers of animals which occur naturally. For this reason, the LD_{50} values described for the caterpillars in this paper were based upon a limited number of individuals and consequently, may not necessarily represent the absolute value for each species. They do, however, represent the only known data on the sensitivity to fluoroacetate of invertebrates which coexist with fluoroacetate-bearing vegetation. They also enable comparison of the trend in sensitivities to fluoroacetate between the species of caterpillars which may or may not include plants containing fluoroacetate in their diet.

Larvae of P. **dorsalis** and M. **privata** were very sensitive to fluoroacetate (Tables 1 and 3). The diets of these two species consists almost exclusively of Eucalyptus species (CSIRO 1970). Thus, even though these species coexist with fluoroacetate-bearing vegetation, their specific feeding habits are such that fluoroacetate-bearing plants are excluded from their diet. Consequently these species have not developed a tolerance to the toxin.

The diet of **Spilosoma** larvae is uncertain but they appear to feed on herbaceous plants and woody ornamentals (CSIRO 1970). Larvae of other members of Arctiidae feed on species of the Boraginaceae and may also attack seed pods of Crotolaria (CSIRO 1970). The **Spilosoma** sp. larvae were collected in relatively close proximity to the toxic plants. Because of the more cosmopolitan feeding habits of members of Arctiidae, **Spilosoma** larvae could potentially include the toxic plants in their diet which may account for the moderate level of tolerance seen in this species (Table 4).

The larvae of O. **lunifer** feed predominantly on species of **Acacia** and to a lesser degree on other legumes and trees (CSIRO 1970). O. **lunifer** larvae were collected while they were feeding on G. **microcarpum** and they are unlikely to be able to consume the toxic plants unless they could tolerate fluoroacetate. **Gastrolobium microcarpum**, which dominates the understorey at Dryandra, often constitutes 80 percent of the shrub layer. It can also contain up to 600 mg fluoroacetate kg kg^{-1} air dried sample (Aplin 1971). The relatively high fluoroacetate tolerance (estimated LD_{50} approximately 150 mg 1080 kg^{-1}; Table 6) of O. **lunifer** larvae compared to that of the other species investigated, may reflect the extent to which O. **lunifer** in Western Australia has had to adapt to the inclusion of fluoroacetate in its diet.

The elevated whole body citrate concentration in poisoned P. **dorsalis** and **Spilosoma** sp. larvae (Tables 2 and 5) indicates that, as in vertebrates, fluoroacetate intoxification results in disruption of the tricarboxylic acid cycle in these caterpillars. However, citrate accumulation in poisoned larvae appears to be maximal when larvae are given the equivalent to their respective LD_{50} values.

It appears that other Western Australian insects are also able to tolerate some fluoroacetate. When offered a choice of seed from three species of legume (G. **microcarpum**, **Bossiaea eriocarpa** and **Acacia pulchella**), seed-harvesting ants coexisting with fluoroacetate-bearing vegetation selected seed on the basis of size rather than the presence or absence of fluoroacetate (Twigg et al. 1983). Furthermore, seed weevils (Bruchidae) often lay their eggs in the seed-pods of toxic species of **Gastrolobium** and **Oxylobium** and the larvae complete their development and pupate in the pod resulting in the consumation of the entire seed (L. Twigg unpub. data). Seed-harvesting ants and seed weevils could not utilize fluoroacetate-bearing vegetation unless they were able to overcome the toxic effects of fluoroacetate. These observations support the suggestion that the enhanced tolerance to fluoroacetate displayed by the larvae of O. **lunifer**, and to a lesser degree those of **Spilosoma** sp., may in fact reflect the evolutionary selection pressure for the development of tolerance to fluoroacetate resulting from the need to ingest plants or plant parts which contain fluoroacetate.

It is difficult to directly compare the susceptibilities to fluoroacetate of the caterpillars investigated here with other published data on sensitivity of insects to fluoroacetate (see Matsumara and O'Brien 1963). The latter studies have been largely carried out using a lethal concentration technique and the exact amount of toxin ingested is therefore unknown. Moreover, the ambient temperature at which mortality was determined is often not reported. However, many insects appear to be very sensitive to fluoroacetate poisoning, and although its use is not recommended, 1080 has been patented as an insecticide (Tietze et al. 1930). Cabbage leaves dipped in a 1 mg ml^{-1} sodium fluoroacetate solution and supplied to larvae of the white cabbage moth were sufficient to kill all the larvae within 3-5 days (David and Gardiner 1953). While an artificially induced concentration of 1 mg sodium fluoroacetate kg^{-1} fresh weight in bean plants induced 100 percent mortality in aphids (A. fabae) 1-2 days after infestation.

The enhanced fluoroacetate-tolerance exhibited by those dasyurid mammals which coexist with fluoroacetate-bearing vegetation (King et al. 1988; Twigg 1986), allied with the high levels of tolerance to fluoroacetate of the caterpillars collected feeding on or near the toxic plants, suggest that those insects which consume these plants as food have evolved a degree of tolerance to the toxin. The results reported here also provide further support for the suggestion that the incorporation of insects which feed on fluoroacetate-bearing vegetation in the diet of dasyurids has resulted in the development of tolerance to fluoroacetate by these marsupials (King et al. 1988; Twigg 1986).

ACKNOWLEDGEMENTS

This work was carried out as part of my Doctor of Philosophy degree at Murdoch University, W.A., which was supervised by Dr R.J. Mead. Drs Dennis King and Jonathan Majer reviewed an earlier draft of the manuscript and Jonathan Majer, Stephen Sawyer, Des and Esma Twigg assisted with the collection of caterpillars.

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TABLE 1: Mortality data (mortality/number dosed) and LD 50 with 95 percent confidence limits for larvae of **Perga dorsalis** from Perth.

	Dose (mg 1080 kg^{-1})								
	0	0.57	0.69	0.84	1.02	1.23	1.49	1.80	2.18
Deaths	0/9	0/5	2/5	3/5	3/5	3/5	3/6	3/6	4/4
		95%LCL			LD_{50}			95%UCL	
		0.86			1.05			1.28	

TABLE 2: Citrate accumulation in **Perga dorsalis** larvae 12 hours after administration of 1080.

Dose (mg 1080 kg^{-1})	µmol citrate (g fresh weight)$^{-1}$ Mean	SEM	N
control	3.43	0.24	4
1.05 (LD_{50})	5.60	0.35	4
10	6.33	0.73	3

TABLE 3: Mortality data (mortality/number dosed) and LD_{50} with 95% confidence limits for larvae of **Mnesamplea privata** from Kulikup, Western Australia.

	Dose (mg 1080 kg^{-1})						
	0	1.98	2.90	4.24	6.21	9.09	13.30
Deaths	0/5	1/3	2/5	5/6	3/6	4/6	3/3
		95%LCL		LD_{50}		95%UCL	
		2.70		3.88		5.56	

TABLE 4: Mortality data (mortality/number dosed) and LD_{50} with 95% confidence limits for **Spilosoma** sp. larvae from Perth.

	Dose (mg 1080 kg^{-1})						
	0	10.00	23.13	30.59	40.46	53.50	70.76
Deaths	0/6	0/3	0/4	1/5	1/4	6/7	4/4

TABLE 5: Citrate accumulation in **Spilosoma** sp. larvae 12h after administration of 1080.

Dose (mg 1080 kg^{-1})	µmol citrate (g fresh weight)$^{-1}$		
	Mean	SEM	N
control	2.32	0.91	4
10	4.37	1.02	4
45	10.01	0.29	4
100	10.49	0.39	4

TABLE 6: Mortality data (mortality/number dosed) for larvae of **Ochrogaster lunifer** collected which when collected were feeding on **Gastrolobium microcarpum** at Dryandra, Western Australia.

	Dose (mg 1080 kg^{-1})					
	0	<50	50	100	200	>200
Deaths	0/5	1/16	0/3	0/3	2/2	6/6

THE SMALL VERTEBRATE GROUND FAUNA OF MULGA HABITATS NEAR WILUNA, WESTERN AUSTRALIA

J N Dunlop, School of Biology, Curtin University of Technology, Kent Street, Bentley, Western Australia, 6102.

INTRODUCTION

In the eastern Murchison all non-saline plant habitats are dominated, or at least co-dominated, by Mulga **Acacia aneura**. Vegetation formations include open scrub, scrub, low woodland and thicket or groves (Muir 1977). This study is based on the live-trapping of small ground living and scansorial vertebrate animals, in three apparently distinctive mulga habitats, near Mt Lawrence Wells (26° 48'S, 120° 12'E). The primary objectives were to survey the vertebrate ground fauna and to investigate the relationships between the various species and certain, broadly defined, habitat components. It was also considered worthwhile to analyse the data for relationships between trapping rates, as a measure to animal activity, and environmental variables. Davies (1973) working mainly with the flowering and fruiting cycles of shrubs, and with birds, concluded that reproductive activity in organisms adapted to the mulga lands was seasonal rather than opportunistic. The availability of moisture, and its ensuing productivity, although highly variable was nevertheless predictable in recurrence. Seasonal activity would be expected to be most evident in the small ground fauna which is not capable of rapid or widespread movement.

METHODS

The small vertebrate (<50g) ground fauna of the study area was sampled using pitfall and drift traplines in three representative mulga dominated habitats. A number of other habitats in the area were sampled by opportunistic hand-collection. One trapline was established in each of the main study habitats. These consisted of 10 or 11 lines pits (160mm diameter, 700mm depth) spaced along, and on alternate sides of, a 30-35m length of 300mm high flywire fence. The trapping efforts and habitat details for each sampling site are summarized below. (Vegetation physiognomy is described using the height/density classes of Muir 1977).

Trapline	Trap days	Habitat description
1	1628	Open mulga **Acacia aneura, A. linophylla** and **A. pruinocarpa** low woodland over **Triodia basedowii** mid-dense hummock grassland on red-brown clay loam. Subterranean termitaria over much of the area.
2	1467	Groved mulga woodland over clumped **Eremophila spectabilis** low scrub C on red-brown loam. Considerable dead-wood in groves.
3	1430	Open mulga woodland over open **Grevillea sarissa** and **Eremophila forrestii** scrub A over open **Plectrachne schinzii** hummock grassland on red loam sand. The area had been logged in the distant past.

Trapping was conducted over 14 (7-12 day periods) between June 1988 and May 1989. During each trapping period maximum and minimum temperature was recorded at each site using a thermometer placed in deep shade beneath mulga canopy and 1.0-1.5m above the ground. Each site also had a rain gauge mounted on a post in the open. At the end of each period a soil moisture sample was taken from each study site. Soil was taken from between 5 and 10 cm depth and always from beneath mulga canopy and leaf litter. The soil samples which were from 700-1200g in size, were weighed shortly after collection, dried in an oven at 100°c for 2 hours and then reweighed. Moisture was expressed as a percentage of dry soil weight.

All small vertebrates captured were weighed to 0.1g on a laboratory top-pan balance. Snout-vent length (SVL) was measured in reptiles, foot length and tail base diameter in mammals. All animals were examined for evidence of reproduction. Most captures were marked by toe-clipping and released close to the trapping point. A voucher specimen for most taxa was lodged with the Western Australian Museum.

RESULTS AND DISCUSSION

The small vertebrate ground fauna of the study area was found to consist of 4 frogs (Hylidae, Leptodactylidae), 8 geckos (Gekkonidae), 5 legless lizards (Pygopidae), 7 dragons (Agamidae), 9 skinks (Scincidae), 2 blind snakes (Typhlopidae), 6 venomous snakes (Elapidae), a python (Boidae), 3 marsupial mice (Dasyuridae) and 2 rodents (Muridae).

A full systematic list is presented in Table 1 below including species captured on these three traplines and collected by hand in other habitats in the area. For each trapline the capture rates are shown as the total number of individuals trapped and in parenthesis, the number caught standardised for effort as captures/1000 trap days.

TABLE 1: Species captured on the three traplines or collected by hand in other habitats of the area.

TAXA	Pitfall Trapline 1	2	3	*Other Habitats R	SMW	BR	RG	SA
Neobatrachus sp			10(7.0)					
Neobatrachus wilsmorei			20(14.0)					
Litoria rubella			3(2.1)					
Diplodactylus conspicillatus		13(8.0)						
Diplodactylus elderi		14(8.6)	5(3.5)					
Diplodactylus pulcher		1(0.6)	3(2.0)					
Diplodactylus stophurus			3(2.1)					
Diplodactylus granariensis							+	
Gehyra varegata		4(2.4)				+		
Nephrurus vertebralis			12(8.4)					
Rhynchoedura ornata		4(2.6)	1(0.7)			+		
Delma butleri		14(8.6)	8(5.6)					
Delma nasuta		1(0.6)						
Lialis burtonis		4(2.6)	4(2.3)					
Pygopus lepidopodus		6(3.7)	1(0.7)					
Pygopus nigriceps		2(1.2)	2(1.4)					
Ctenophorus caudicinctus				+		+		
Ctenophorus inermis			+		+			
Ctenophorus isolepis			1(0.7)					
Ctenophorus reticulatus		1(0.6)	3(2.0)					
Ctenophorus salinarum		1(0.6)	1(0.7)					+
Ctenophorus scutulatus		1(0.6)						
Pogona minor		1(0.6)	2(1.4)					+
Tymanocryptus cephala		1(0.6)						
Varanus brevicauda		3(1.8)						
Varanus caudolineatus		1(0.6)	4(2.7)					
Varanus gouldii			1(0.7)					
Varanus panoptes			1(0.7)		+	+		
Ctenotus leonhardii		16(9.8)	1(0.7)	2(1.4)				
Ctenotus pantherinus		71(43.6)	1(0.7)					
Ctenotus schombourgkii		1(0.6)						
Egernia depressa			2(1.4)					
Eremiascincus richardsonii					+	+		
Lerista desertorum				1(0.7)				
Lerista muelleri		1(0.	(10.7)					
Rhamptyphlops hamatus		3(1.8)						
Rhamphotyphlops waittii				1(0.7)				
Demansia psammophis					+			
Denisonia fasciata					+	+		
Pseudonaja modesta						+		
Pseudonaja nuchalis					+	+		
Rhynoplocephalus monachus				2(1.4)				
Morelia perthensis					+	+		
Ningaui ridei		8(4.9)	2(1.4)					
Sminthopsis macroura		1(0.6)						
Sminthopsis ooldea		5(3.1)						
Mus musculus		3(1.8)				+		
Pseudomys hermannsburgensis		4(2.4)	2(1.4)					
		185(113.6)	25(17.0)	78(54.5)				

*Other Habitats - R, ridges; SMW, stony mulga woodland; BR, breakaways; RG, gallery woodland; SA, samphire, saltbush, bluebush scrub.

Small vertebrate densities in the area were low in comparison to mulga areas elsewhere, such as the eastern Pilbara. This may result in part from climatic factors (eg a slightly lower rainfall at Wiluna) but is more likely to reflect the impact of grazing, by sheep and rabbits, on near ground habitats. The effect is especially marked in the small dasyurid marsupials which were present at very low densities indeed. For example Ealey's Marsupial Mouse **Ningaui timealey** can be caught at a rate of approximately 68 per 1000 trap days in its preferred habitat in the eastern Hamersley Ranges. Ride's Marsupial Mouse **N. ridei**, the sibling species at Mt Lawrence Wells, is captured in similar habitat at only 5 per 1000 trap days.

The highest densities of small vertebrates in the Mt Lawrence Wells area occur where mulga codominates with a mid-dense understratum of hummock grass (**Triodia**).

Much lower densities are found where the hummock grass stratum is sparse or absent (Table 1). The importance of hummock grassland to Australian arid zone small vertebrates is well established (Cogger 1984) and has been suggested as a factor promoting the great speciation of our desert lizards.

A number of fauna habitats can be identified within the mulga dominated vegetation of the area. The dendrogram Figure 1 uses the pitfall trap data to group species occurring together at high frequency and separate those with little or no association (species/species similarity analysis). The resulting classification often reflects the key habitat factors influencing the distribution of species. At the 50-60% level three broad clusters reflect the habitats selected for sampling, supporting the view that they do actually represent distinctive environments to the fauna. The uppermost group inhabits dense mulga woodland without an understratum of hummock grass. The central group occupies mulga over a well developed (mid-dense) hummock grass stratum. Both these faunal communities occur on red-brown loam. The lower group inhabits mulga over open hummock grassland on sand.

At and above the 70% similarity level more subtle associations with habitat become evident. **Pseudomys hermannsburgensis, Lerista muelleri, Pygopus nigriceps** and **Egernia depressa** are probably associated with the litter and deadwood in the dense mulga groves. **Varanus caudolineatus** and **Diplodactylus pulcher** are semi-arboreal and would make use of the living trees as well as the deadwood. Dense hummock-grass with some emergent shrubs and trees, especially mulga, is probably the preferred micro-habitat of **Lialis burtonis, Ningaui ridei, Ctenotus leonhardii, Sminthopsis ooldea** and **Pygopus lepidopodus.** Subterranean termitaria (abundant at trapline 1) may be an important habitat component for **Rhamphotyphlops hamatus, Varanus brevicauda, Gehyra variegata** and **Rhynchoedura ornata.** One group occurs only where there is hummock grass and all other factors may be relatively unimportant; these spinifex specialists are **Delma butleri, Diplodactylus elderi** and **Diplodactylus conspicillatus.**

Within the lower cluster two sub-groups are probably significant. One is clearly associated with the mulga trees (three are semi-aboreal) and includes **Pogona minor, Rhinoplocephalus monachus, Dipladactylus stophurus** and **Litoria rubella.** The second sub-group are burrowers (the two frogs **Neobatrachus** for aestivation) are clearly associated with the sandy substrate. Burrowing behaviour was observed in the Knob-tailed Gecko **Nephrurus vertebralis.**

FIGURE 1: Species/species similarity dendogram based on pitfall data from three mulga dominated habitats at Mt Wilkinson, Western Australia.

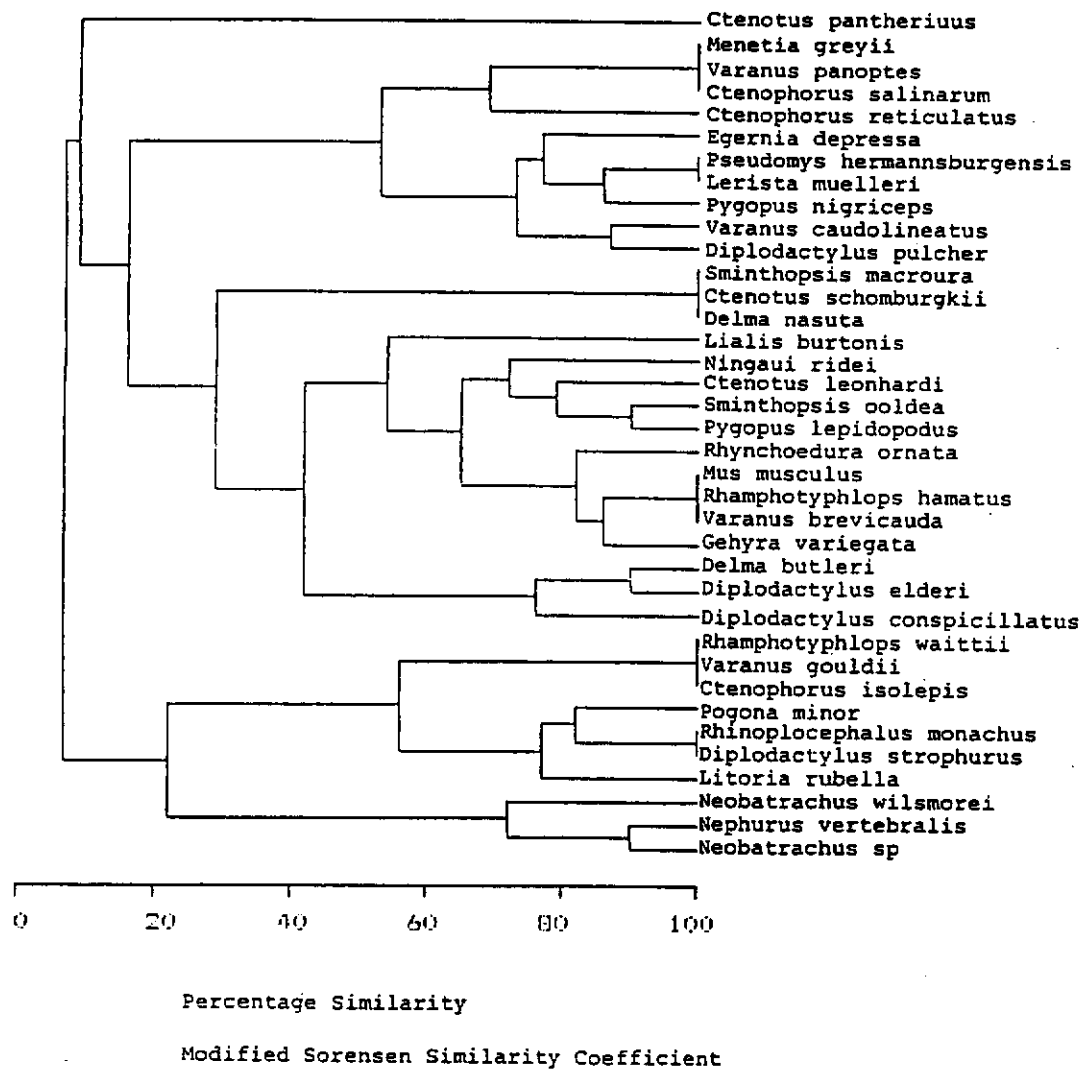
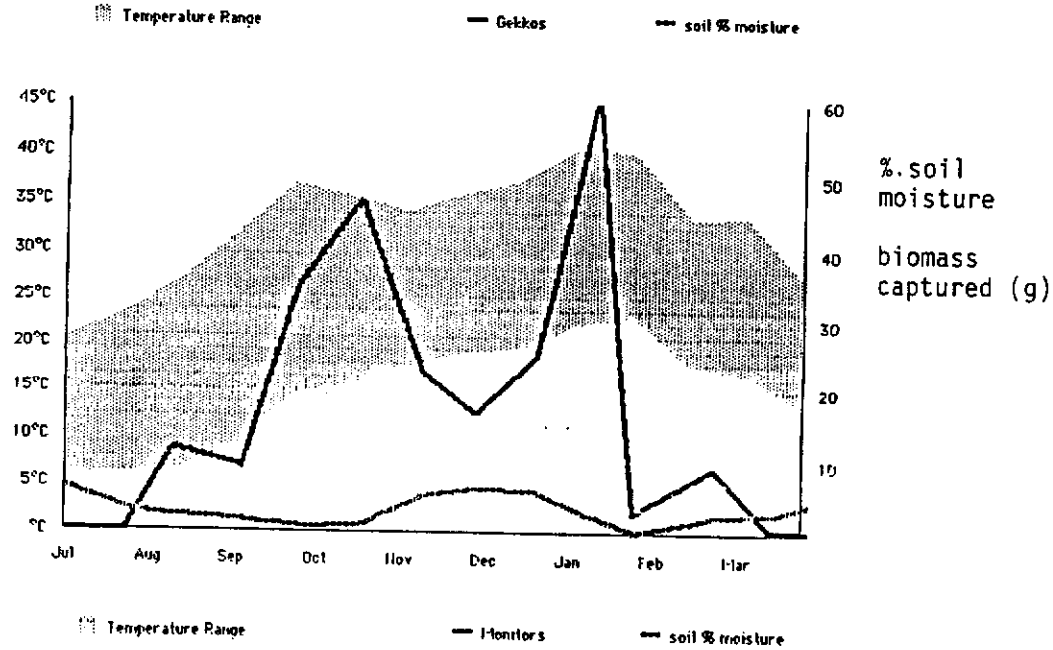
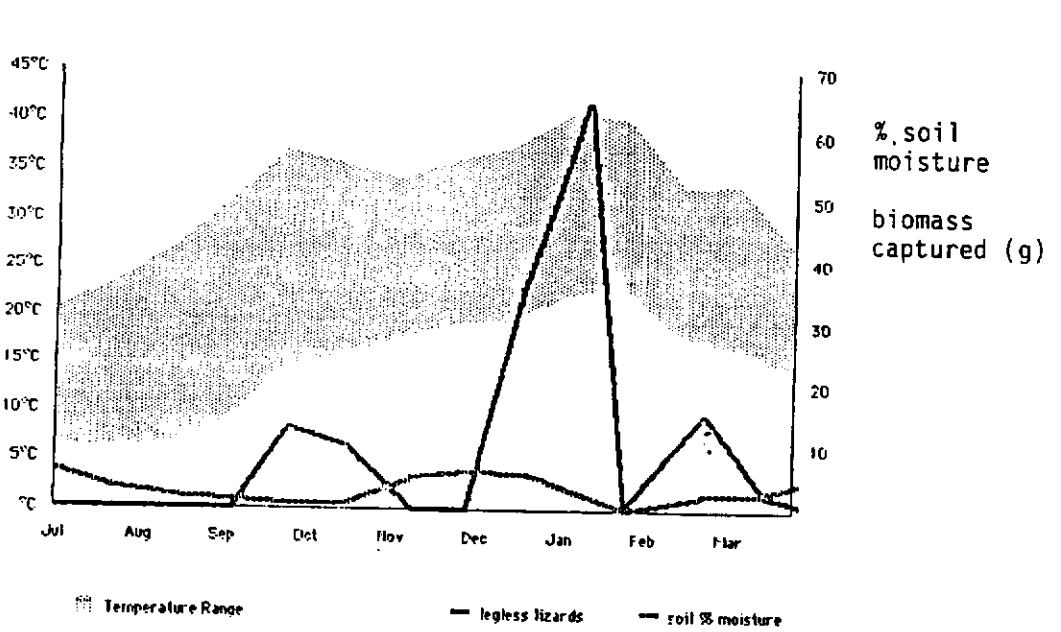


FIGURE 2: The seasonal activity of Gekkos, Monitors and Legless Lizards measured as the biomass captured per sampling period shown against the mean temperature range per period and the percentage soil moisture.

(a)



(b)



(c)

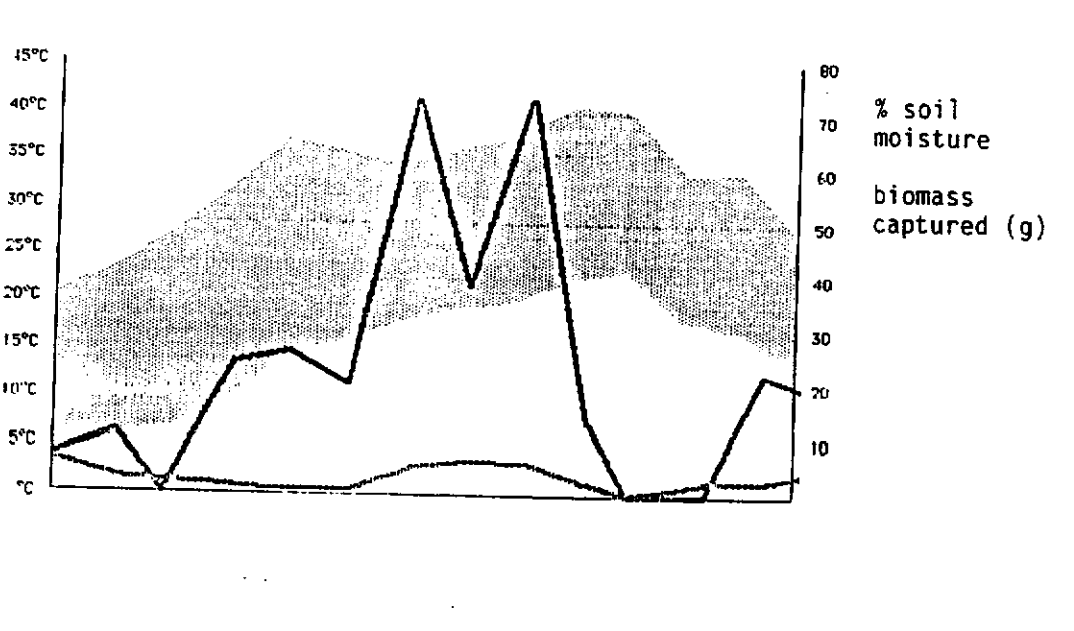


FIGURE 3: The seasonal activity of dragons measured as the biomass captured per sampling period shown against the mean temperature range per period and soil moisture.

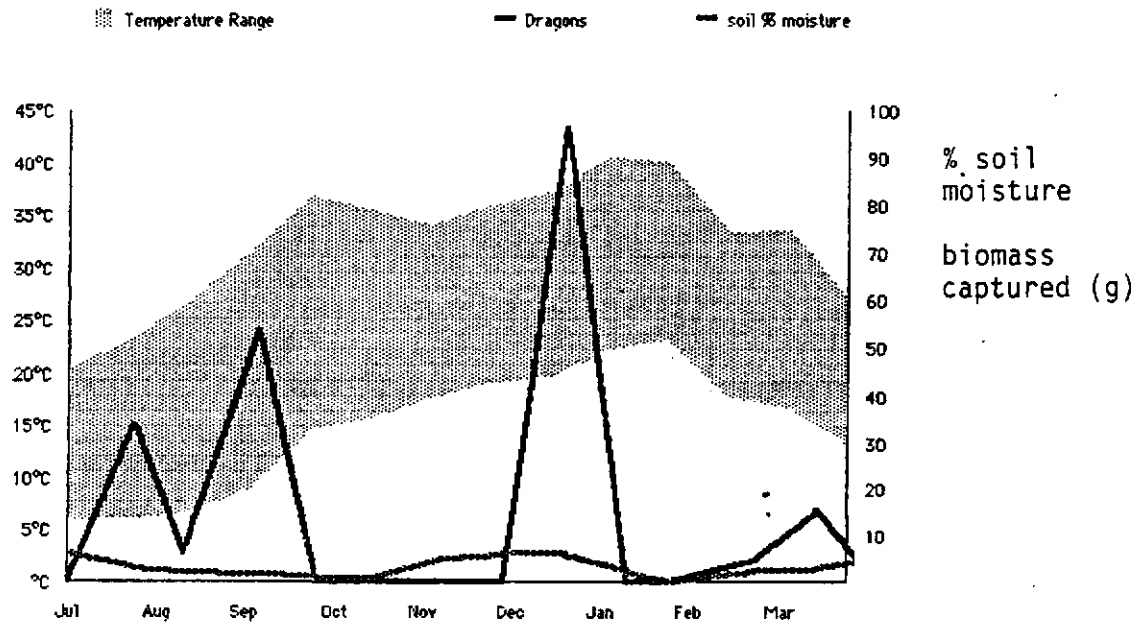
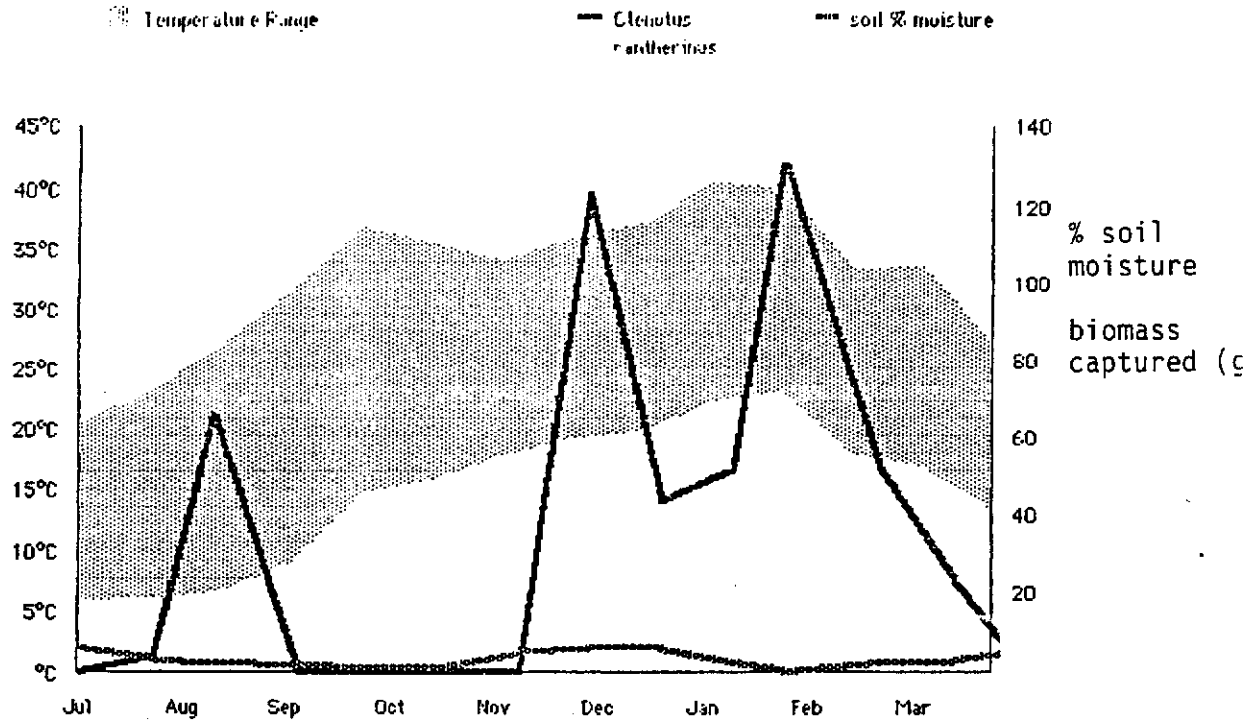


FIGURE 4: The seasonal activity of **Ctenotus pantherinus** and **C. leonhardii** measured as the biomass captured per sampling period shown against the mean temperature range per period and the percentage soil moisture.

(a)



(b)

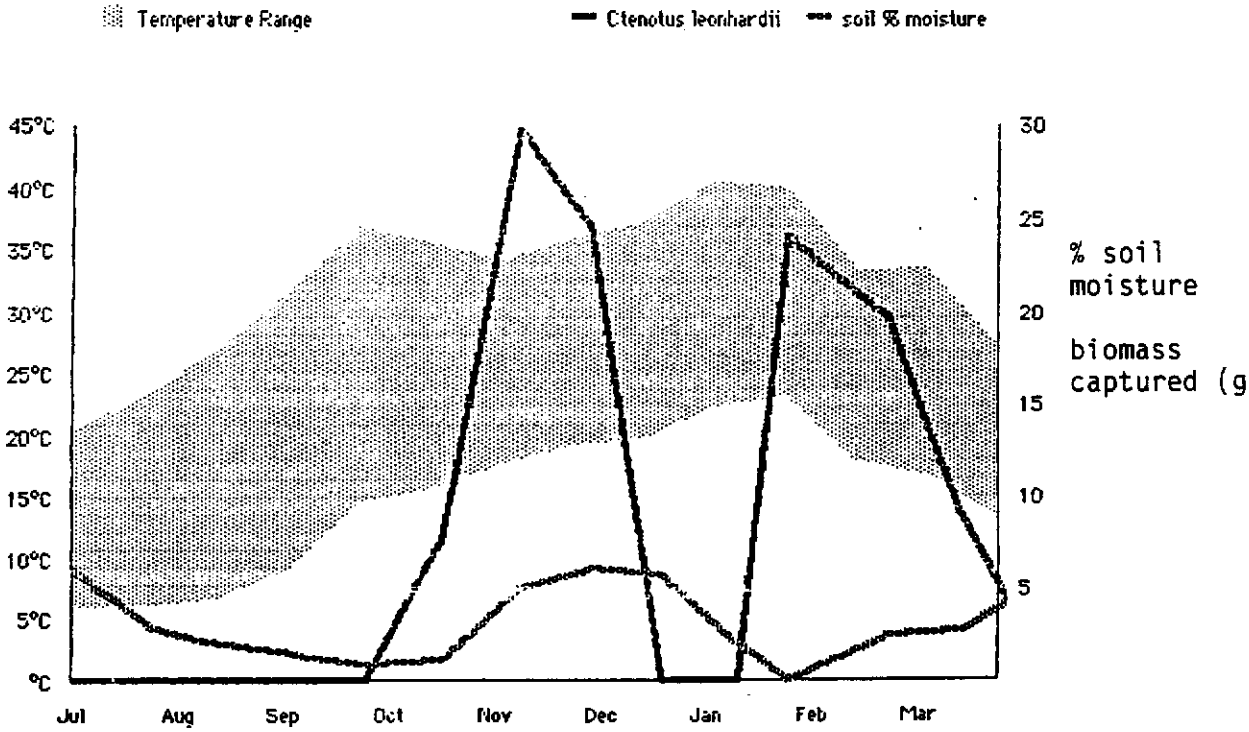


FIGURE 5: The seasonal activity of frogs and blind snakes measured as the biomass captured per sampling period shown against the mean temperature range per period and the percentage soil moisture.

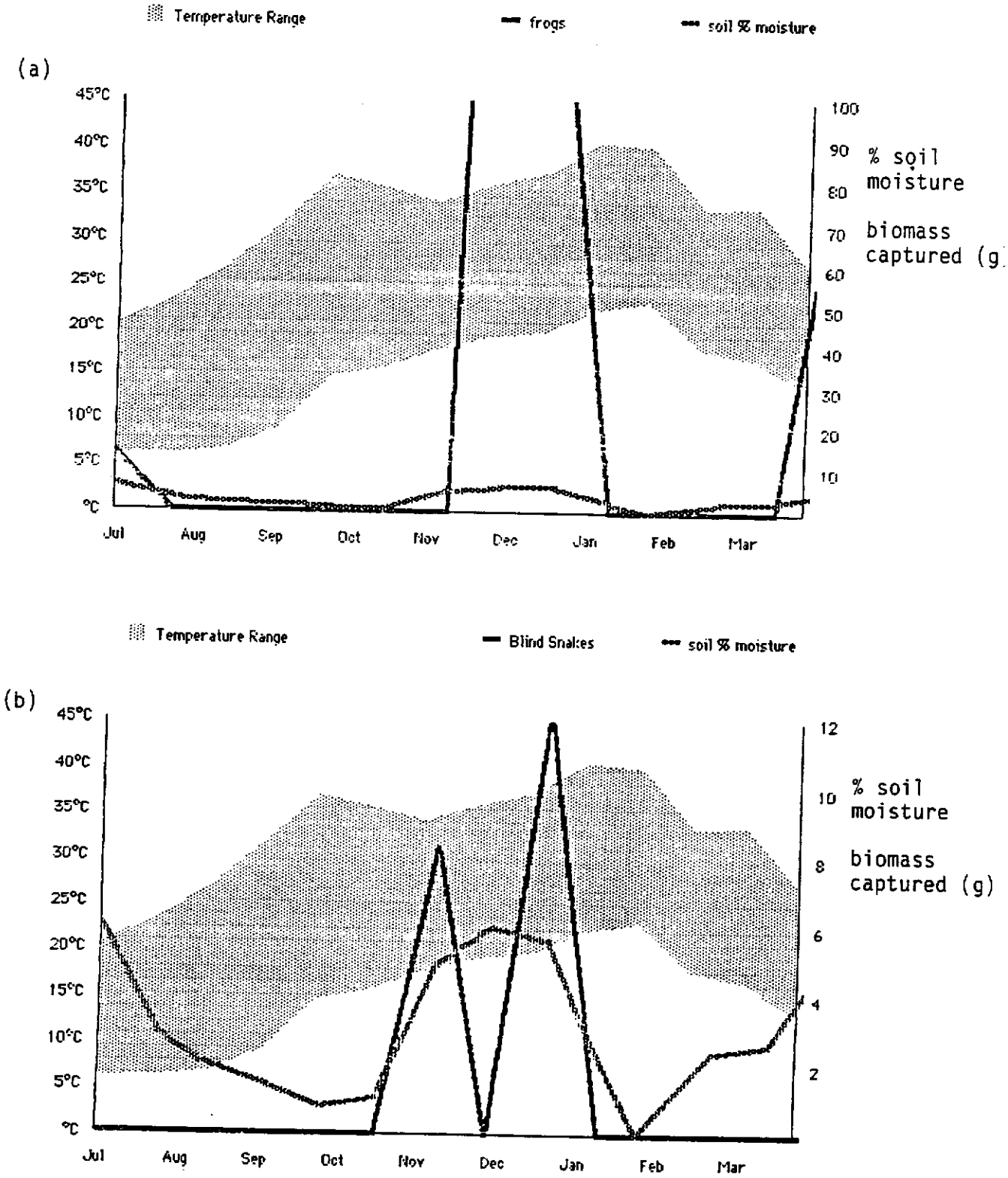
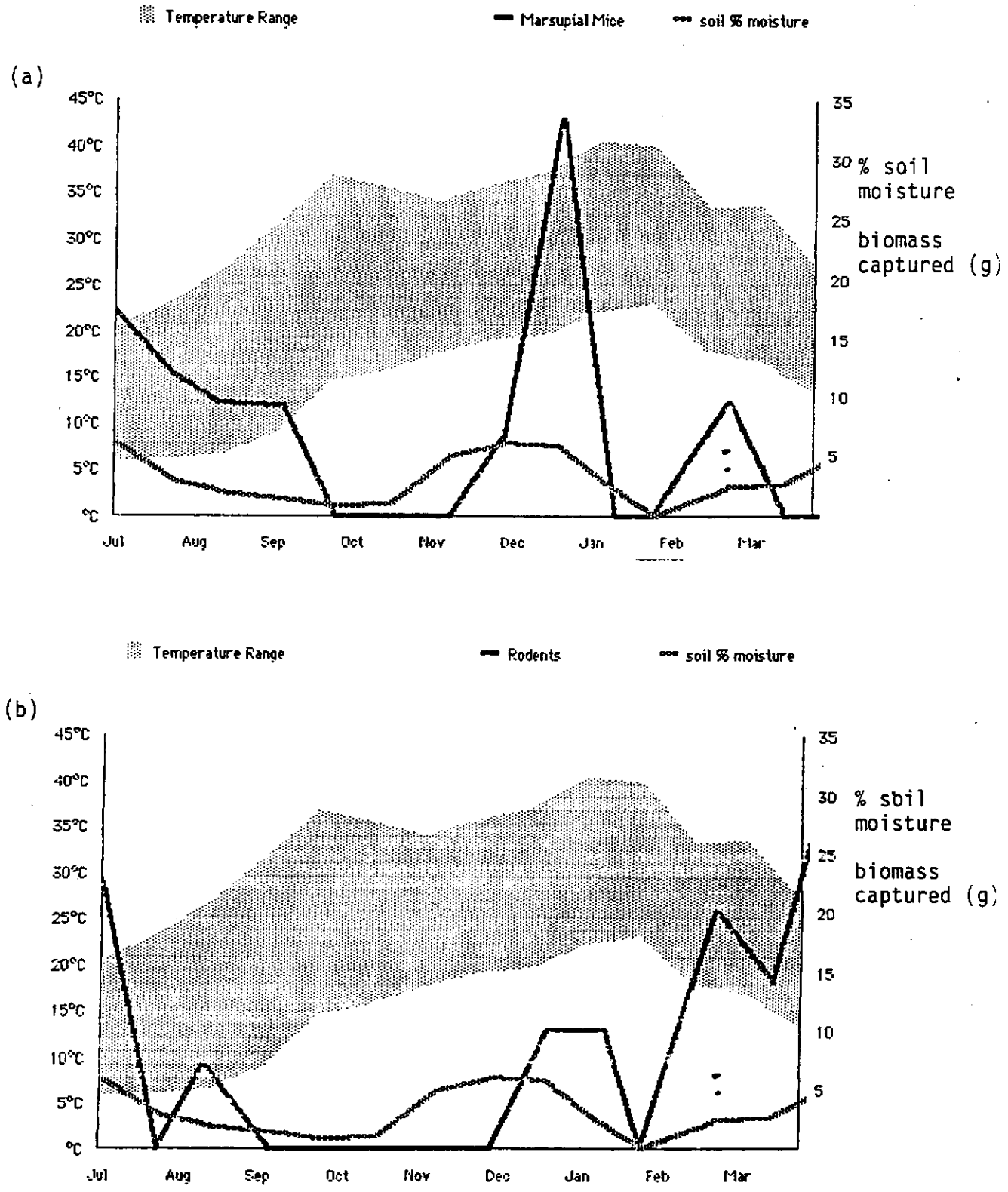


FIGURE 6: The seasonal activity of Marsupial Mice and Rodents measured as the biomass captured per sampling period shown against the mean temperature range per period and the percentage soil moisture.



Small vertebrate activity has been expressed in terms of captured 'biomass' (weight g) per sampling period rather than as the number of individuals. This was to avoid over-emphasizing the importance of juveniles in the seasonal activity patterns.

In some families the individual species showed similar peaks and troughs in capture rates. In these cases the data for all species was lumped. The gekkos (Gekkohidae), monitors (Varanidae) and the legless lizards (Pygopodidae) showed clear activity responses to ambient temperature (Figures 2a, b and c). Gekkos appeared to be most active during those parts of the year with the highest diurnal maxima, although the temperatures of the early darkness hours may be the actual operating factor. The monitors also showed definite activity peaks corresponding to the annual peaks in diurnal temperatures. By contrast, the legless lizards although active during the warmer parts of the year, were more active during the slightly cooler, often cloudy and humid, periods.

The dragons (Agamidae) showed an early peak in activity with respect to other reptiles (Figure 3) due no doubt to their ability to raise body temperature by basking and orientation. Egg laying appears to begin early in spring, much sooner than in other herptile groups.

The activity patterns of the skinks (Scincidae) showed more variation between species. For example **Ctenotus pantherinus** has an early activity peak (Figure 4a) which is not present in **Ctenotus leonhardii** (Figure 4b).

As expected frogs showed peaks of surface activity during periods of higher soil moisture (Figure 5a). The same trend was evident in the blind snakes (Typhlopidae - Figure 5b).

The activity of small mammals was probably least controlled by short term environmental variables and more by intrinsic 'seasonality'. Marsupial mice were most active during spring and again in mid to late summer (Figure 6a). The early peak, due to the movements of adult animals, was probably associated with mating whilst the latter peaks coincided with the independence of the cohorts of young (Dunlop and Sawle 1982, Muir 1983). Rodents in the arid zone frequently show peaks of activity in winter and this was the case in the study area (Figure 6b). These peaks may be associated with the availability of seed during the winter months or be the consequence of rapid reproduction during the autumn months.

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TREE AND SHRUB ESTABLISHMENT ON COAL MINE INTERBURDEN

A I Doronila and J E D Fox, School of Biology, Curtin University of Technology, Kent Street, Bentley, Western Australia, 6102.

SUMMARY

Results are presented after 2 years for a coal dump restoration trial contrasting overburden types and topsoil addition. Seed of a suite of 16 native woody trees and shrubs was sown into the trial in June 1987. The sowing rate was 4.8 kg ha^{-1}.

Acacia species survived best on Class I material with topsoil. The Myrtaceae (**Eucalyptus, Melaleuca**) were more numerous on Class I with no topsoil. **Acacia lateriticola** was the most abundant **Acacia**, whereas **Eucalyptus wandoo** was the most abundant **Eucalyptus** species present.

Acacia saligna and **A. extensa** were tallest plants at 2 years. The tallest eucalypts were **E. gomphocephala** and **E. wandoo**. Both **E. gomphocephala** and **A. myrtifolia** showed significant height responses to high phosphorus application on Class I material with no topsoil.

INTRODUCTION

A major limitation to revegetation of coal mine dumps is the acidic nature of the interburden materials combined with low fertility (Bartle and Riches 1978). Potential understorey species such as **Acacia pulchella, A. extensa**, and **A. saligna** have shown some tolerance to the hostile conditions of these substrates if lime and fertiliser treatments are applied (Koch and Bell 1985; Fox et al. 1987). Other legume species such as **Kennedia coccinea** and **Paraserianthes lophantha** have performed well on undifferentiated spoil materials treated with lime (Fox and Mathie 1982; Fox, Patroni and O'Dea 1985). Candidate overstorey species of **Eucalyptus** have also been grown in pot trials at Curtin University. For example, both **E. camaldulensis** and **E. gomphocephala** grew poorly on undifferentiated spoil material (Fox, Patroni and O'Dea op. cit.) whereas addition of fertiliser enhanced their growth on two classes of interburden material (Fox and Colquhoun 1987).

Two contrasting interburden materials were tested with combinations of topsoil, lime and phosphorus application in a field trial on the Marron Pool Dump at the Muja Open Cut coal mine, Collie. The objectives of this trial were to confirm the value of placing less acidic interburden on dump surfaces and to contrast the other variables. A suite of 16 species was sown onto the prepared surface. This paper describes the status of the sown species after 2 years.

MATERIALS AND METHODS

The experimental site was established on a relatively flat, upper surface of the Maroon Pool Dump, Muja Open Cut coal mine, Collie, Western Australia, 150 km S.E. of Perth on June 18, 1987 (Owens and Fox 1988).

Two experimental blocks (24 m x 20 m) were created using contrasting interburden materials, (Ceres-Diana Class I and Ate-Bellona Class III). The Class I material (mean pH = 4.75) was of uniform texture, grey-white in colour, whereas the Class III material (mean pH = 3.59) was a dark grey to black shaley material. Topsoil which had been stockpiled for 2 years was layered 20 cm deep on half of each interburden block to create four main blocks. Each of these main blocks (12 m x 20 m) was further subdivided into 4 smaller subplots of 6 m x 10 m, with different combinations of lime and phosphate fertiliser.

Lime in the form of crushed limestone was applied at different rates depending on interburden material. Topsoil amended interburdens and Class III only substrate received lime at rates of nil or 10 t ha^{-1} while Class I only substrate received lime at rates of nil or 3 t ha^{-1}. Prior field experiments indicated that the topsoil used was of an acidic nature and thus required a higher lime rate than the less acidic (Class I) interburden material (Fox and Colquhoun 1987).

Prior to seed sowing one of two rates of superphosphate and molybdenum fertiliser was applied to subplots (6 m x 10 m) at 136.9 kg ha^{-1} (12.2 kg P ha^{-1}) or 400 kg ha^{-1} (35.6 kg P ha^{-1}). At sowing all blocks received 200 kg ha^{-1} of super/potash 3-2, this provided a further 11 kg ha^{-1} P and 36 kg ha^{-1}. The layout of the experimental site is illustrated in Figure 1.

A list of the seed used is given in Table 1. Fourteen native species were obtained from the Department of Conservation and Land Management. These had been collected from various locations near Collie. Seed of two other leguminous species (**Viminaria juncea, Paraserianthes lophantha**) from Curtin University Field Trial Area were also used. All **Acacia** spp. and the **Paraserianthes lophantha** seed were pretreated with boiled water then cooled and air dried prior to sowing. Seed was sown on 19 June 1987. Seed was planted as mixtures, the rates of sowing (g ha^{-1}) and total number of seeds per plot are also given in Table 1.

The trial site has been assessed on five occasions, twice in 1987, September 4-5 and October 14-15; twice in 1988, June 1 and October 14; and July 5 1989. The treatments were initially assessed using 1m x 1m quadrats located randomly. At each assessment the heights and number of sown species were recorded for each quadrat (Owens and Fox 1988). The final assessment involved the measurement of all plants found on each treatment plot. Most of the species were difficult to identify at the earlier visits, and consequently assessments were general. The results presented below describe the status of sown species at July 1989, at two years from sowing.

RESULTS

The number of plants per species present on the 4 main blocks after two years are summarised in Table 2. The least number of survivors was on Class III material in the absence of topsoil (CIII). On Class I (CI) there were more seedlings in the absence of topsoil. All eucalypts and five of the seven *Acacia* species shared significant c^2 values between blocks.

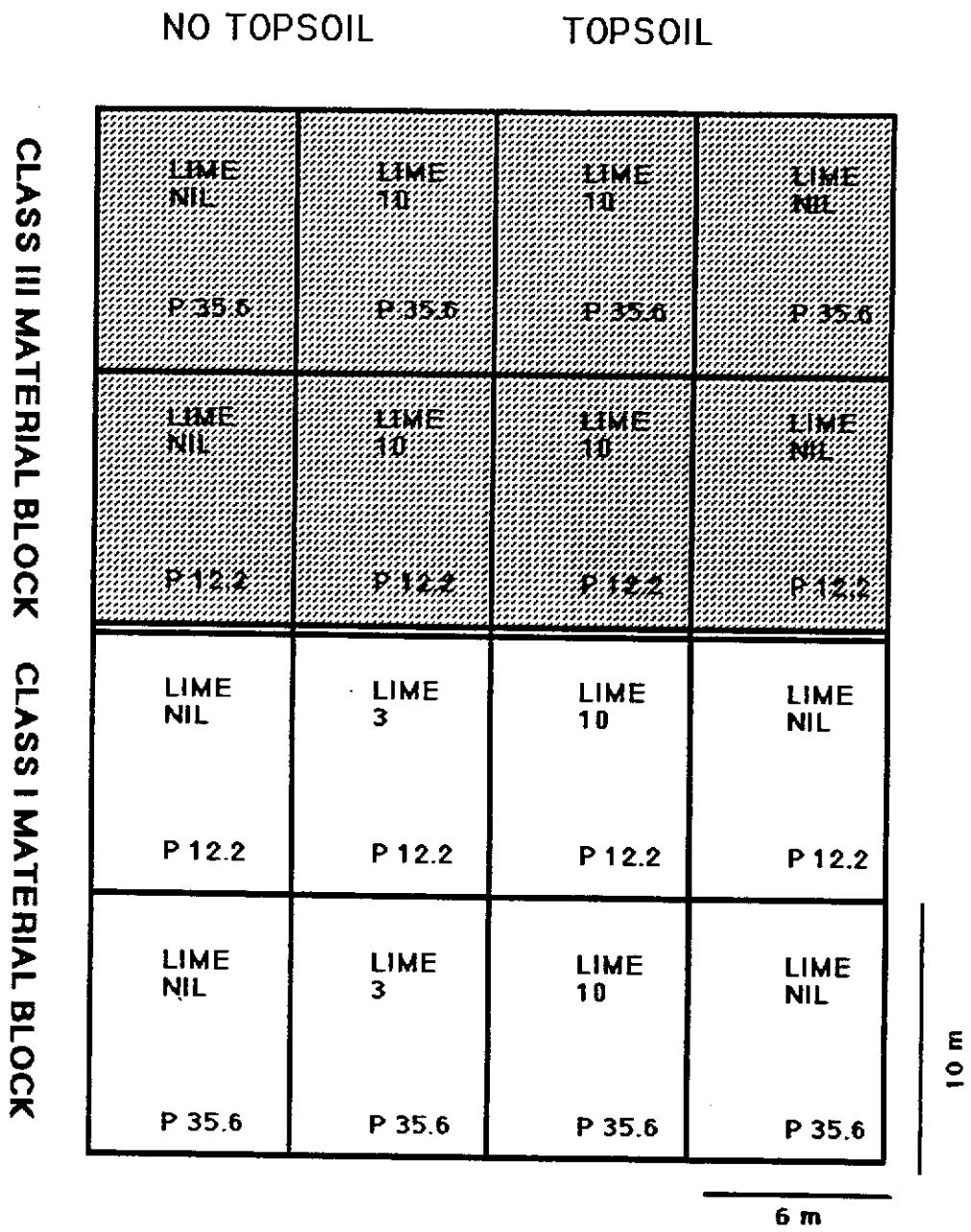

FIGURE 1: Design of factorial experiment on Marron Pool Dump.

TABLE 1: Species list and sowing rate for the Marron Pool Field Trial.

Species	Seed sown g ha^{-1}	No of seed per g	Wt sown on 6 x 10m (g)	No of seed sown on 6 x 10m
Acacia drummondii	400	230	2.4	552
A. extensa	500	31	3.0	93
A. lateriticola	500	170	3.0	510
A. myrtifolia	500	84	3.0	252
A. pulchella	150	165	0.9	148
A. saligna	150	65	0.9	58
A. urophylla	200	186	1.2	223
Eucalyptus accedens	150	110	0.9	99
E. camaldulensis	150	416	0.9	374
E. gomphocephala	250	240	1.5	360
E. patens	250	212	1.5	318
E. wandoo	150	350	0.9	315
Kennedia coccinea	500	38	2.0	11
Melaleuca preissiana	500	n.a.	3.0	n.a.
Paraserianthes lophantha	150	15	0.9	13
Viminaria juncea	300	106	1.8	190
Total	4800		28.8	

4.8 kg ha^{-1} sown of 16 species.

All but two of the seven **Acacia** species were most abundant on topsoil amended Class I material (CITS). **A. saligna** and **A. urophylla** had their greatest abundance on Class III material with topsoil (CIIITS), although both **A. myrtifolia** and **A. extensa** were better represented on this material. The most abundant **Acacia** species overall was *A.* **lateriticola** (N =164) and the least was **A. pulchella** (n = 30).

Three of the five eucalypts had greatest numbers on Class III with topsoil (**E. camaldulensis, E. accedens,** E. patens). The total number of **Eucalyptus** seedlings was highest on Class I, largely due to the high representation of **E. wandoo** on this substrate; **E. gomphocephala** was also most abundant on Class I. As for **Acacia** species Class III without topsoil had poorest representation of eucalypts. Whereas topsoil addition resulted in more eucalypts on Class III material, numbers on Class I with topsoil were lower than without topsoil. **Melaleuca preissiana** was most abundant on Class I without topsoil and poorly represented on the other three substrate blocks.

Figure 2 illustrates the mean heights of the five best plants for each species on three of the four main blocks and Table 3 summarises analysis of variance of these. Class III without topsoil and species with less than 2 plants on CI (**Paraserianthes lophantha** and **Viminaria juncea**) were excluded from this analysis as too few plants were present.

All **Acacia** species were significantly taller on Class I with topsoil. **A. extensa** and **A. saligna** were shortest on Class I, whereas the other five *Acacia* species were significantly smallest on Class III with topsoil.

All eucalypt species except for **E. camaldulensis** grew significantly taller on Class I without topsoil. **E. camaldulensis,** had tallest plants on Class III with topsoil but differences were not significant between treatment blocks. **Kennedia coccinea** (for which length rather than height was used) and **Melaleuca preissiana** did not show significant differences between treatments.

The number and heights of plant species in response to phosphorus fertiliser treatments are summarised in Table 4. Table 5 summarises the effect of lime applications on each of the four main blocks.

Each of **A. drummondii, A. extensa, A. lateriticola** and **A. myrtifolia** had their best representation on Class I topsoil without lime and the high phosphorus rate. They were also generally tallest on these treatments. **A. urophylla** reached its highest numbers of individuals on topsoiled Class III with lime and high phosphorus. It, however, grew tallest on the Class I topsoil at the higher phosphorus rate on both limed or unlimed substrates. **A. pulchella** had its highest number and tallest individuals on Class I topsoil with the highest application of lime and phosphorus. **A. saligna** had similar numbers on both topsoil treatments, but this species was tallest on the low phosphorus and high lime rate of Class I with topsoil.

Among the eucalypts **E. patens** was the only one surviving on Class III substrate without topsoil. It occurred on the limed plots. The most abundant eucalypt, **E. wandoo,** was the least well distributed across treatments and reached its highest abundance on Class I without topsoil. Its numbers and heights were similar across lime and phosphorus treatments for this substrate. **E. accedens** had its greatest abundance on Class III with topsoil, which had low phosphorus and high lime but it grew taller on Class I with lime and high phosphorus. The greatest number of **E. camaldulensis** was on Class III with topsoil in the absence of lime and the lowest phosphorus rate. **E. gomphocephala** numbers were similar on the Class I material on either of the lime or phosphorus treatments, however, it grew much taller with the addition of lime at the high phosphorus rate.

Kennedia coccinea grew much better on the Class I blocks with and without topsoil and had its longest plants on Class I only substrate with lime and low phosphorus. **Melaleuca preissiana** plants were much more abundant on Class I without topsoil plots compared to the other treatments. This species had its highest numbers on low phosphorus and lime treatments, but it grew taller on Class I with topsoil plus a high phosphorus rate.

Paraserianthes lophantha was characterised by its general absence but grew taller on Class I with topsoil plus lime and high phosphorus. **Viminaria juncea** also had its greatest number on this substrate but grew taller on the unlimed plus high phosphorus treatment.

TABLE 2: Numbers of plants recorded after two years, with c2 significant levels (*p> 0.05; ** p> 0.01; *** p>0.001) on the 4 main blocks (12 x 20 m).

SPECIES	NUMBERS								c^2	Signi-ficance
Acacia drummondii	CITS	>	CI	>	CIITS	>	CIII		5.95	*
	67		28		21		1			
A. extensa	CITS	>	CIITS	>	CI	>	CIII		14.2	***
	53		49		21		1			
A. latericola	CITS	>	CI	>	CIITS	>	CIII		9.68	**
	96		47		21		0			
A. myrtifolia	CITS	>	CIITS	>	CI	>	CIII		7.08	**
	55		49		24		6			
A. pulchella	CITS	>	CIITS	>	CI	>	CIII			NS
	15		11		4		0			
A. saligna	CIITS	>	CITS	>	CI	>	CIII			NS
	12		11		8		0			
A. urophylla	CIITS	>	CITS	>	CI	>	CIII		6.81	**
	35		18		13		0			
Eucalyptus accedens	CIITS	>	CI	>	CITS	>	CIII		47.42	***
	66		26		21		0			
E. camaldulensis	CIITS	>	CITS	>	CI	>	CIII		49.28	***
	79		26		26		0			
E. gomphocephala	CI	>	CITS	>	CIITS	>	CIII		44.09	***
	74		52		42		0			
E. patens	CIITS	>	CI	>	CITS	>	CIII		23.14	***
	38		17		13		4			
E. wandoo	CI	>	CITS	>	CIITS	>	CIII		51.73	***
	221		102		28		0			
Kennedia coccinea	CITS	>	CI	>	CIITS	>	CIII			NS
	16		7		2		1			
Melaleuca preissiana	CI	>	CITS	>	CIITS	>	CIII		28.94	***
	111		6		4		2			
Paraserianthes lophantha	CITS	≥	CITS	>	CI	>	CIII			NS
	2		2		1		0			
Viminaria juncea	CITS	>	CIITS	>	CI	≥	CIII			NS
	13		11		0		0			
Non sown natives										
Bossiaea eriocarpa	CIITS	>	CITS	>	CI	≥	CIII			NS
	26		21		0		0			
Gompholobium spp.	CITS	≥	CIITS	>	CI	≥	CIII			NS
	4		4		0		0			
All Acacias	CITS	>	CIITS	>	CI	>	CIII		61.42	***
	315		198		145		8			
All Eucalyptus	CI	>	CIITS	>	CITS	>	CIII		288.83	***
	364		253		214		4			
Other species (sown/unsown)	CI	>	CITS	>	CIITS	>	CIII		58.25	***
	119		67		44		3			
Total Numbers	CI	>	CITS	>	CIITS	>	CIII		366.59	***
	628		596		495		15			

TABLE 3: Mean heights of 2 year old plants (cm) with F values (ANOVAR) and significance levels (*p> 0.05; ** p> 0.01; *** p>0.001) on 3 main blocks.

Species	Tallest	Height	Shortest	FValue	Significance
Acacia drummondii	CITS 80.7[a]	CI 66.3[ab]	CIIITS 49.5[b]	8.4	**
A. extensa	CITS 147.2[a]	CIIITS 122.5[a]	CI 68.4[b]	36.3	***
A. latericola	CITS 78.6[a]	CI 44.7[b]	CIIITS 39.0[b]	63.7	***
A. myrtifolia	CITS 112.3[a]	CI 67.6[b]	CIITS 66.2[b]	22.0	***
A. pulchella	CITS 45.9[a]	CI 28.3[ab]	CIIITS 26.1[b]	2.9	*
A. saligna	CITS 147.2[a]	CIIITS 89.4[a]	CI 43.6[b]	16.0	***
A. urophylla	CITS 70.0[a]	CI 58.5[a]	CIIITS 34.3[b]	10.8	***
Eucalyptus accedens	CI 41.0[a]	CITS 30.0[ab]	CIIITS 21.5[b]	5.1	**
E. camaldulensis	CIIITS 34.8	CI 33.7	CITS 29.9	0.7	NS
E. gomphocephala	CI 74.7[a]	CITS 63.3[a]	CIIITS 33.1[b]	14.4	***
E. patens	CI 33.0[a]	CITS 28.2[ab]	CIIITS 19.7[b]	4.5	*
E. wandoo	CI 73.3[a]	CITS 63.8[ab]	CIIITS 39.6[b]	4.9	*
Kennedia coccinea (length)	CI 120.1	CITS 98.0	CIIITS 72.0	0.9	NS
Melaleuca preissiana	CITS 30.3	CI 29.3	CIIITS 19.3	2.6	NS

TABLE 4: The numbers and mean heights of sown plant species in response to phosphorus applications.

SPECIES	SUBSTRATE							
	CI		CITS		CIII		CIITS	
Phosphorus rate (kg/ha)	122	356	122	356	122	356	122	356
	[Number Mean height (cm)]							
Acacia drummondii	20	8	23	44	0	1	10	11
	62.1	72.4	70.9	90.6	-	10.0	50.2	48.5
A. extensa	9	12	22	31	0	1	18	31
	54.7	80.6	149.5	144.9	-	8	133.2	111.9
A. lateriticola	24	23	34	62	0	0	9	12
	44.6	44.8	78.1	79.1	-	-	38.3	39.7
A. myrtifolia	11	13	18	37	1	5	22	27
	48.9	91.6*	111.0	113.5	5	4.8	69.2	63.2
A. pulchella	1	3	7	8	0	0	7	4
	20.0	31.0	35.0	55.5	-	-	26.6	25.3
A. saligna	5	3	2	9	0	0	2	10
	54.2	26.0	205.0	130.7	-	-	54.0	101.2
A. urophylla	9	4	8	10	0	0	7	28
	57.0	60.7	61.3	78.7	-	-	32.9	36.7
Eucalyptus accedens	1	25	12	9	0	0	43	23
	33.7	48.3	33.1	-	-	23.8	19.3	
E. camaldulensis	16	10	18	8	0	0	40	39
	42.3	24.0	29.1	31.3	-	-	36.2	33.4
E. gomphocephala	35	39	30	0	0	20	22	
	55.8	93.7*	56.0	70.7	-	-	34.3	31.2
E. patens	7	10	5	8	2	2	25	13
	43.4	27.2	16.6	37.8	6.0	6.0	20.0	19.5
E. wandoo	113	108	53	49	0	0	10	18
	71.1	75.4	55.4	70.2	-	-	57.4	14.6
Kennedia coccinea	4	3	6	10	1	0	1	1
(length)	145.3	86.7	104.7	94.0	20	-	-	-
Melaleuca preissiana	67	44	3	3	2	0	4	0
	34.0	24.6	28.0	50.7	17.0	-	19.3	-
Paraserianthes lophantha	1	0	0	2	0	0	2	0
	90	-	-	135.0	-	-	99.0	-
Viminaria juncea	0	0	2	11	0	0	4	7
	-	-	16.2	156.5	-	-	38.3	34.0

*High P significant at 5% level on Class I material.

TABLE 5: The numbers and mean heights of sown plant species in response to lime rate.

SPECIES	SUBSTRATE							
	CI		CITS		CIII		CIITS	
lime rate (t/ha)	0	3	0	10	0	10	0	10
	[Number Mean height (cm)]							
Acacia drummondii	8	20	37	30	0	1	5	16
	48.1	79.1	92.2	69.3	-	10.0	47.8	50.4
A. extensa	10	11	34	19	0	2	26	23
	62.8	74.4	154.0	140.0	0	8.0	74.4	139.5
A. latericola	11	36	58	38	0	0	7	14
	36.4	52.1	76.4	80.8	-	-	38.8	39.1
A. myrtifolia	9	15	37	18	1	5	33	16
	67.4	67.7	117.5	107.0	5.0	4.8	72.5	59.1
A. pulchella	1	3	6	9	0	0	4	7
	20.0	31.0	42.3	48.3	-	-	30.0	23.9
A. saligna	5	3	8	3	0	0	6	6
	30.8	65.0	144.2	155.3	-	-	76.4	84.4
A. urophylla	2	11	10	8	0	0	1	34
	69.0	55.9	69.4	70.6	-	-	19.8	43.0
Eucalyptus accedens	12	14	14	7	0	0	25	41
	31.0	51.0	18.3	24.8	-	-	30.8	28.9
E. camaldulensis	7	19	14	12	0	0	47	32
	29.6	36.4	27.6	33.8	-	-	32.1	37.5
E. gomphocephala	37	37	33	19	0	0	31	11
	64.7	84.8	68.1	58.6	-	-	29.6	39.0
E. patens	6	11	9	4	0	4	24	14
	28.0	35.8	34.1	18.7	-	6.0	19.4	20.1
E. wandoo	112	109	62	40	0	0	20	8
	66.3	80.2	84.1	41.5	-	-	47.6	28.4
Kennedia coccinea	1	6	8	8	1	0	0	2
(length)	110.0	121.8	107.5	88.5	20.0	-	-	72.0
Melaleuca preissiana	37	74	6	0	2	0	0	4
	33.8	24.8	39.3	0	17.0	-	-	19.3
Paraserianthes lophantha	1	0	0	2	0	0	2	0
	90.0	-	-	135.0	-	-	99.0	-
Viminaria juncea	0	0	8	5	0	0	6	5
	-	-	103.0	69.7	-	-	46.2	69.1

DISCUSSION

Each species is considered and general comments are presented in the conclusion.

Acacia drummondii

These species grew best on the least acidic substrate and its survival and height growth followed the pattern:

CITS > CI > CIIITS > CIII

On CITS, though not significant, a higher phosphorus rate appeared to enhance survival of **Acacia drummondii** as well as produce the tallest plants. No liming on this substrate gave better plants and more survivors. In contrast on CI and CIIITS liming increased survival and produced taller plants. The response observed could have been due mainly to substrate effects as the less acidic conditions presumably were less detrimental to plant growth.

Acacia extensa

Both survival and growth of this species was determined by the addition of topsoil to the soil material. Both numbers and heights of **Acacia extensa** were one and a half to twice as great on topsoil blocks as on CI only. Lime and phosphorus produced no significant effects but the trends seen were for increased height with high P on CI and no difference on the CITS or CIIITS in terms of growth and survival. Liming of CITS depressed numbers and heights. However on CI and CIIITS liming gave the opposite effect of encouraging both survival and growth.

Fox, Patroni and O'Dea 1985 reported that liming of spoil material did not enhance early growth of this species. On more acidic material it appears that later stages of development must be sustained by a less acidic substrate.

Acacia lateriticola

Growth responses followed the pattern established with **A. drummondii** of best growth and survival on CITS and nil on CIII. This species has a similar habit to **A. drummondii** but its better response in this trial suggests that it could persist better over the longer term.

Acacia myrtifolia

Of the larger growing **Acacia** species this survived well on the more acidic substrate CIIITS. It also had the most survivors on CIII substrate, but growth response as indicated by height was significantly less on CIIITS compared with CITS. On the CI substrate there was a significant effect due to the higher P application, while on CITS and CIIITS no such response was observed. The addition of lime did not appear to increase survival of **Acacia myrtifolia** on any of the substrates.

In earlier glasshouse experiments (Fox, Patroni and O'Dea 1985) this species appeared to perform well on toxic materials amended with lime. The current study suggests that with its ability to establish initially on the more acidic substrates and despite its slower growth on these materials it may persist well on the dumps.

Acacia pulchella

This was the least frequently encountered of the **Acacia** species. This may be due to the low seeding rate used for this species (Table 1). As with other **Acacia** species the best response was on CITS for both numbers and heights. An increase in phosphorus application increased height on CITS but not on the other substrates while liming did not give any advantage. This species is known to tolerate acidic conditions (pH < 4.0) but liming and fertiliser application enhances growth (Fox, Gazey and Barrett 1987; Koch and Bell 1985).

Acacia saligna

A. saligna was also infrequently encountered on the treatment blocks. It has a comparatively large seed and was sown at the lowest rate, some 1/3 to 1/9 of the other **Acacia** species. Most survivors were on the unlimed CITS plots with higher phosphorus rate. The best plants were found on unlimed CITS with low P while CIIITS with no lime and high P gave plant heights comparable to the least toxic substrate (CITS).

Though not appearing to do well in early pot trials (Fox, Gazey and Barrett 1987) once established **Acacia saligna** may persist for a number of years provided that it does not succumb to the rust fungus **Uromycladium** which tends to shorten the life of this **Acacia**.

Acacia urophylla

Of all the **Acacia** species this survived best on CIIITS but it only grew half as tall as on CITS. A high phosphorus application enhanced its growth on the above substrate but not on the others. Liming produced more survivors on CIITS as well as enhancing plant growth.

There were more survivors on CIIITS than on the other substrates combined however these were only half as tall as those on the best substrate CITS. High phosphorus rates enhanced growth on CITS and increased survival on CIIITS while liming appeared to improve survival on CI and CIIITS. This species appeared to be the most acid tolerant of the **Acacia** species.

Eucalyptus accedens

Individuals counted and measured on CIIITS may have been classed as **E. wandoo**. It is likely that survival may be similar on CI and CITS. Liming and high phosphorus application on CI gave highest growth. Growth responses may be due to the less acidic nature of the CI interburden.

Eucalyptus camaldulensis

This species had more survivors on CIIITS than on other combinations. No significant differences were observed for **Eucalyptus camaldulensis** in height comparisons for main blocks. Liming of CI increased numbers as well as giving taller plants but on CIIITS liming was associated with fewer survivors.

Early growth of this species has been reported to respond to liming of acidic spoil material (Fox, O'Dea and Patroni 1985) as well as to fertiliser treatments of Class III materials (Fox and Colquhoun 1987). These effects were not noticeable in the present field trial but it is possible that this species is tolerant of the more acidic conditions

of CIIITS and although appearing to grow slowly, it may be able to persist in the long term.

Eucalyptus gomphocephala

Survival and growth of this species were significantly greater on the least acidic substrates versus the most acidic. The response pattern was CI > CITS > CIIITS. On CI substrates it showed a significant height response to the high P application. On the other blocks more individuals were found at higher P application but only CITS were these taller. Survival gave mixed results with lime/no lime. CI had most individuals with numbers the same for lime and no lime, the limed plants were taller.

CITS on limed plots had more survivors and plants were taller than with lime while on CIIITS without lime there was a comparable number of individuals but they were only one third as tall as those on the best treatment: CI with lime. This species had the tallest individuals of all the eucalypts despite the fact that it is naturally found on the limestone areas of the western coastal plain. Its abundance and growth on the different substrates may be indicative of its natural preference for the less acidic substrates.

It is likely that eventual long term persistence of **E. gomphocephala** will largely be determined by its ability to cope with acidity at depth as its roots penetrate deeper into the more acidic layers of the dump.

Eucalyptus patens

This species was most numerous in CIIITS however individuals growing on CI and CITS were significantly taller. High phosphorus applications did not produce taller plants on CI or CIIITS, in fact the best ones were found growing on CI with low phosphorus. On CITS high phosphorus gave taller plants. There were more plants on unlimed CIIITS but these were shorter than on limed CI and unlimed CITS.

This was the only eucalypt found growing on CIII substrate with lime. This confirms that E. patens is very tolerant of acidic conditions. Several reports have cited it as a species which is acid tolerant. Bartle and Riches (1978) found it growing on old abandoned coal spoil dumps while in long term pot trials it has been shown to persist for 20 months on various interburden materials (Fox, Colquhoun and Owens 1988).

Eucalyptus wandoo

This was the most abundant eucalypt species found on CI. There were no significant differences in height but it grew taller on the limed substrate. No difference was obtained with phosphorus rates though on CITS the high P application was associated with taller individuals. On CIIITS despite a high P application plants were 80% shorter than on the best treatment. The response of this species appears to be mainly determined by the suitability of CI as a substrate and given appropriate amounts of P fertiliser and lime application it may persist in the long term.

Kennedia coccinea

Nearly all individuals of this species were found in CITS and CI. There were more plants on CI with lime and those were also the longest. On CITS there were similar numbers but the addition of lime did not produce larger plants. On CIIITS **Kennedia coccinea** was found only in limed plots. Though no significance differences were observed for this species in either number or length of plants in the main blocks it appears to grow best on the less acidic topsoil treated spoil material. On CI it had nearly all survivors on the lime treated plots. No apparent differences in growth could be detected for phosphorus applications. The response observed may be similar to other plants with nitrogen fixing symbiosis (such as the **Acacias**) where topsoil treatments with less acidity appear to enhance the relationship.

Melaleuca preissiana

Nearly all individuals of this species were found on the CI substrates. Liming appeared to enhance survival on CI as there were more on this treatment than all the others combined. It appears that this species has very poor acidity tolerance and the possible reason for its success on CI could be due to the drought tolerance mechanism. Other species of **Melaleuca** have been shown to have biochemical adaptations to drought resistance (Naidu et at 1987).

Paraserianthes lophantha

Due to the low seed rate used for this species no inferences could be made on its performance in the trials other than that it will survive on topsoil treatments.

Viminaria juncea

This native legume grew only on topsoil treatments and CITS was associated with significantly taller individuals than CIIITS. Unlimed with high P CITS was associated with more survivors and taller plants than any of the other treatments. This indicates that it will grow on the less acidic interburden with a high P application.

Competition for essential nutrients, sunlight and water was not apparent. The treatment blocks with the most individuals (CI and CITS) also produced the tallest plants for most of the species. The differential responses of each species are explained sufficiently by the main treatment effects of spoil quality and topsoil treatment. It will be of interest to see whether these trends persist into the subsequent year.

CONCLUSION

In all **Acacia** species the general performance was superior in the less acidic topsoil amended Class I material. Some species eg **A. extensa** and **A. urophylla** appeared to tolerate the more acidic substrate CIIITS but they did not grow there as well as on CITS. This response may be related to possible functional N_2-fixation which is more likely to occur in less acidic conditions. The N_2-fixing bacteria in the symbiosis are generally sensitive to low pH (Habish 1978). It appears that a high rate of phosphorus may also assist in the growth of Acacia species. Nitrogen fixing nodulation in **Acacia** species has been shown to be a distinctly seasonal event most effective during the wet season (Langkamp **et al.** 1981; Monk **et al.** 1981). The trial has been exposed to only two winter seasons at Collie so that the full effectiveness of nodulation in influencing growth may not have been realised. Effective nodulation is more likely to occur in the less acidic conditions which CITS provides, early height growth may reflect this.

Two year old **A. pulchella** in natural ecosystems appears to derive over two-thirds of its nitrogen from nodule activity (Monk **et al.** 1981). It can be assumed that for effective growth of Acacia species an increase in liming may enhance plant growth due to enhancement of nodulation. Loneragan and Dowling (1958) have shown that increase in Ca^{2+} may offset the effects of acidity in nodulation of **Rhizobium** with **Trifolium subteranneum**.

Other leguminous species such as **Paraserianthes lophantha, Kennedia coccinea** and **Viminaria juncea** known to have symbiotic N_2 fixing associations, may also have their long term survival enhanced on topsoil treatments on the less acidic CI interburden.

Eucalypt species may be divided into two groups. Firstly those that are less tolerant of acidic conditions such as **E. accedens, E. gomphocephala** and **E. wandoo** and secondly species which appear to survive just as well on the more acidic conditions such as **E. camaldulensis, E. patens.** Amelioration by liming of the initial acidic conditions may allow the first group to persist in the early years of growth but over the longer term conditions may be much more detrimental to these species as their roots invade the deeper layers of the dump. The second group may show better growth in future assessments.

Melaleuca preissiana may also be less tolerant of the more acidic conditions and appears to behave similarly to the first group of eucalypts.

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NURSERY POTTING MIXTURE FOR *SANTALUM ALBUM* L. IN TIMOR

J E D Fox[A], I K Surata[B] and S Suriamidhardja[B]

A: School of Biology, Curtin University of Technology, Kent Street, Bentley, Western Australia, 6102.

B: Forest Research Institute, Kupang, Indonesia

SUMMARY

Seedling growth of **Santalum album** at Kupang is considerably enhanced when sand is added to local soil materials. The best mixture in terms of dry matter production, height of seedlings and collar diameter was 3 parts sand to 5 parts of local soil. Soil from Sikumana (lithosol) was superior to that from Oilsonbai (grumosol) as the preferred local soil. The type of clay in the potting medium influences plant growth considerably, whereas the calculated percentages of sand achieved in mixtures were similar for both basic media.

A linear relation occurs between seedling height and dry weight over the range of potting mixtures used. Despite this useful correlation, seedling height may not indicate the best condition as it is desirable for root growth and consequent haustorial development with pot host, to be strong in relation to top growth.

INTRODUCTION

Considerable variation exists in preferred potting mixtures for growing tree seedlings (Wijesuriya and Fox 1985). Availability and costs of materials are prime considerations. If local soils are of suitable texture and give good growth of seedlings then refinements may include fertilizer trials, sterilization techniques or the introduction of suitable microbial symbionts. In cases where the local materials are problematical then mixing different sources is the first option for improvement. Heavy clay soils with an abundance of fine particles may be difficult to work with and allow little aeration for optimal root growth. On the other hand coarse sandy materials tend to have low water holding capacities, necessitating frequent watering. When mixing materials the resultant medium itself should be homogenous otherwise plant growth will be uneven.

Members of the parasitic genus **Santalum** pose the added complication of an early host requirement. Although early seedling growth can flourish for 6 months or so in the absence of a host (e.g. Wijesuriya and Fox 1985, Widiarti 1989) early growth must largely rest on cotyledonary/endosperm nutrition. At Kupang, N.T.T., Indonesia, early height growth of **S. album** with **Breynia cernua** as a pot host exceeded that of several more conventional pot hosts (**Lycopersicon esculentum, Capsicum annuum, C. frutescens**) (Anon 1987). After 9 months in a 1:1 soil/sand mixture height growth of **Santalum album** with **Breynia cernua** was only exceeded by the host **Calotropis gigantea** (Kharisma and Sutarjo 1988). The latter is very vigorous and tends to grow rapidly in pots, thus providing considerable competition to sandalwood. For this reason, the use of **Breynia cernua** was preferred in the present trial.

Few reports are available regarding suitable potting mixes for **Santalum**. Hirano (1977) achieved some success with manufactured materials in artificial mixes. These would be expensive and difficult to arrange for field trials at remote locations. At the Sandal Research Centre (Bangalore, India) a commonly used potting mixture is sand/red earth/manure in a 3:3:1 ratio. A mixture of 1 part sawdust, 1 part pine bark and 1 part sand produced greater growth over 6-7 months from germination in **Santalum spicatum** than a 1:1 mixture of sawdust and sand (Wijesuriya and Fox 1985). In that experiment each potting mixture (except control) had added nutrients. In a recent experiment conducted at Bogor, Java, much greater effects due to potting media on growth of **Santalum album** than to the type of host plant used were reported by Widiarti (1989).

In the trial described in the present paper two local soils of the Kupang area were used with a coarse grained sand in admixture. The objectives were firstly to confirm that the addition of sand to local soil improves growth of **Santalum album**, secondly to contrast the two local soils, and thirdly to determine the optimum admixture of sand conducive to enhanced growth at the nursery stage.

METHODS

Seed of **Santalum album** and **Breynia cernua** was sown on June 1st 1988 into trays in a shade house at Oilsonbai nursery, Kupang. One seedling of each species was transplanted into sets of pots containing sand/soil mixtures on July 30th 1988. Plants were harvested on December 2nd 1988.

Fresh river sand was mixed with soil from Oilsonbai (grumosol) and Sikumana (lithosol) to provide sets of pots in 9 percentage sand treatments. Mixtures ranged from 0 to 100 percent sand with increments of sand of $^1/_8$ (12.5 percent). Fifteen replicate pots for each of Oilsonbai and Sikumana soils were established. At harvest 5 randomly selected replicate pots from each of the 18 treatments were harvested, the remainder were planted out in the field at Oilsonbai on December 3rd 1988.

Above ground height of the stem, diameter of the stem at the base and plant dry weight were obtained for each **S. album** harvested. Dry weights were taken to two decimals of a g after drying in an oven for 24 hr at 60°C. The soil materials were analysed at the Soil Research Institute at Bogor.

RESULTS

Soil Characteristics

The source of sand (Tarus) used in the mixture may be described on its textural structure (Table 1) as a sandy loam, whereas the Oilsonbai grumosol was a clay. The Sikumana material was on the border between silty clay loam and sandy clay loam (Mott 1988). Table 1 summarises the results of analyses undertaken at Bogor. In addition to nutrient elements shown, attention is drawn to iron: present in quantity in the sand, as a trace only in the grumosol and absent from the lithosol. For most nutrient elements the Oilsonbai grumosol was better endowed than the Sikumana lithosol, although the latter had much the highest phosphorus content.

The potting mixtures differed in their final constitutions in proportion to the admixture. Figure 1 indicates that the Oilsonbai mix always had considerably more clay materials as a percentage than did the Sikumana material.

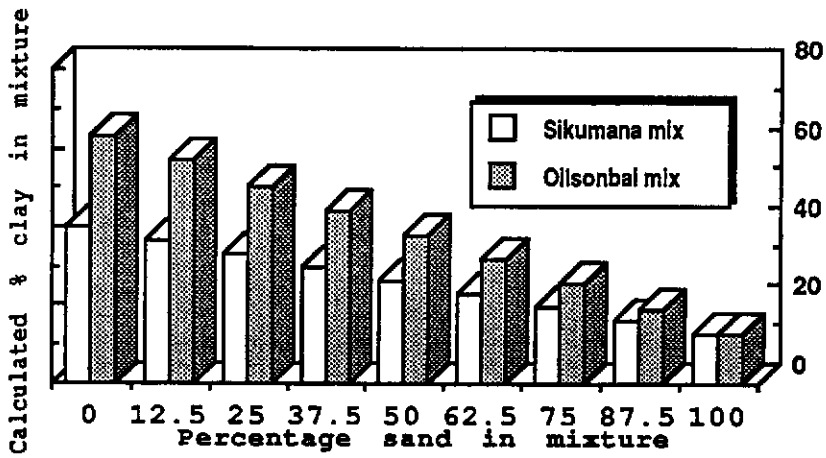

FIGURE 1: Calculated percentage clay in the mixed materials by percentage sand treatment.

TABLE 1: Characteristics of soil materials used

		MATERIAL		
		Tarus	Oilsonbai	Sikumana
		Sand	Grumosol	Lithosol
Physical attributes				
Percent	sand	78.2	18.2	17.2
	silt	8.8	18.8	42.8
	clay	13.0	63.0	40.0
Conductivity (m shos/cm)		47	40	45
pH, H_2O 1:1		8.23	7.21	7.73
KCl 1:1		7.12	6.35	7.03
Chemical attributes				
Bases meq/100g				
	K	21.36	18.64	14.16
	Na	93.83	23.77	17.55
	Ca	65.55	151.33	112.69
	Mg	33.22	63.07	9.90
	Sum	213.96	256.81	154.30
Cation exchange capacity		2.17	10.26	3.08
Percent	organic C	0.38	1.24	1.50
	total N	nil	0.16	0.08
mg/kg	Fe	60.901	0.941	nil
	total P	1.582	2.980	7.699
	Cu	0.211	2.085	2.085
	Zn	0.442	0.863	1.218

TABLE 2: Mean harvest values for height (cm) stem diameter (cm) and dry weight (g) (n=10).

Percent sand in mixture	Height	Stem Diameter	Dry Weight
0	20.63 e	0.362 c	1.779 e
12.5	21.36 e	0.335 c	1.967 de
25.0	31.27 b	0.471 a	3.794 b
37.5	40.41 a	0.507 a	5.542 a
50.0	26.47 c	0.415 b	3.058 bc
62.5	25.68 cd	0.369 bc	2.785 cd
75.0	22.29 de	0.341 c	2.103 de
87.5	15.19 f	0.266 d	0.898 f
100	10.47 g	0.189 e	0.257 f
LSD p 0.05	4.06	0.050	0.857

Harvest values with the same letter do not differ significantly using the least significant difference test (Sokal and Rohlf 1981).

Harvest Values

For each of the 3 harvest parameters significantly better growth was attained with 37.5 percent sand added to the soil (Table 2).

Growth in 100 percent sand was considerably poorer than in the unamended local materials, with all sand giving only one seventh of the yield of all soil with no added sand, and about half the height. The second best treatment was addition of 25 percent sand to the local soils, which did not have a significantly smaller stem diameter than the best treatment.

Comparison of Oilsonbai and Sikumana Materials

Table 3 gives mean values (n = 5) and significance levels for a comparison of dry weights as between the two local soils used in the mixtures.

TABLE 3: Mean dry weights at harvest for plants grown in Sikumana and Oilsonbai soils mixed with sand.

Percent sand in mixture	SOURCE Sikumana	Oilsonbai
0	1.576 de	1.982 cd
12.5	2.252 bcd	1.682 de
25.0	4.552 a	3.036 bc
37.5	5.666 a	5.418 a
50.0	2.884 bc	3.232 b
62.5	3.238 b	2.332 bcd
75.0	1.892 cd	2.314 bcd
87.5	1.094 de	0.702 ef
100	0.286 e	0.228 f
LSD p 0.05	1.290	1.169

Harvest values with the same letter do not differ significantly using the least significant difference test.

The Sikumana based soil mixture generally produced better yield (dry weight), although this was not true for the base material with no sand added or with 50 and 75 percent sand material added (Figure 2). Additions of sand to the Sikumana material had a more rapid effect on dry weight yield, with a significant response to 25 percent addition over weight at zero sand. It is possible that this was due to the better phosphorus status of the Sikumana soil.

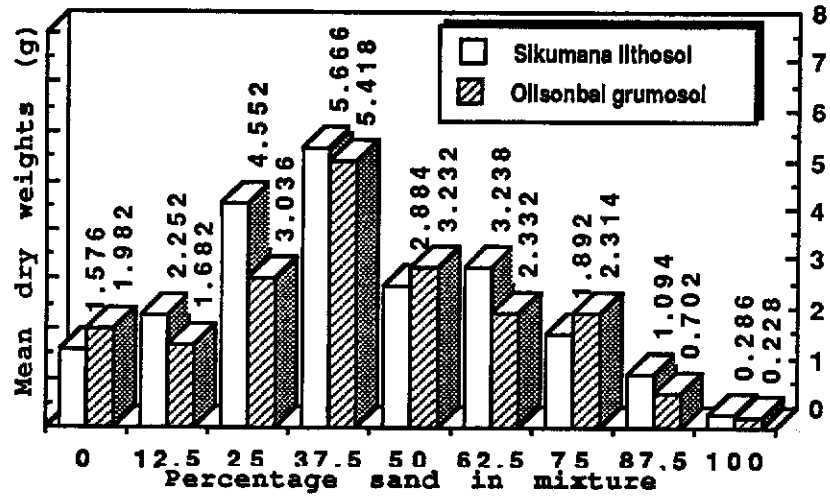

FIGURE 2: Mean dry weights for plants raised in mixtures based on two local soils.

The response in Oilsonbai grumosol was smaller and came with larger increments of sand added, so that a significant response was not achieved until 3 parts sand, 5 parts soil was reached.

There was a significant correlation between mean dry weights and seedling heights overall, and for both local soil bases (Figure 3).

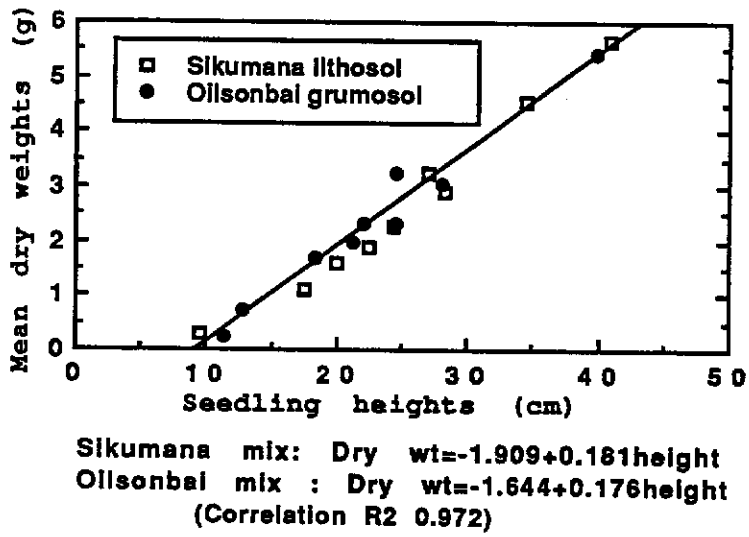

FIGURE 3: Dry weight/height relationship at harvest.

This suggests that for a number of purposes, mean height calculations should be quite adequate to characterise performance in relation to treatment in **Santalum album.**

As the Sikumana and Oilsonbai soils differed in initial proportions of sand and clay, so the addition of equal quantities of the sand mixture brought differing effects in relation to growth. If total proportions of sand in mixtures are plotted against yield (Figure 4), then a similar pattern is shown with maximum yields at about 40 percent sand. Silt varied also (Table 1), with Sikumana having much more. Yield against percentage of clay estimated for mixtures indicate that Sikumana yields peaked at about 30 percent clay but that the Oilsonbai material peaked at 45 percent clay (Figure 5). It may be assumed that effects of clay were more important on yield than effects of sand, reflecting the low levels of nitrogen and phosphorus present in the sand.

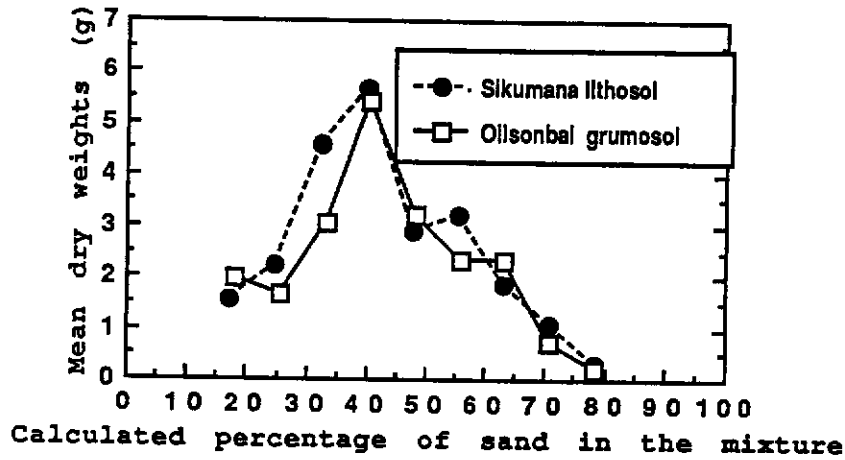

FIGURE 4: Mean dry weights related to estimated sand in mixtures.

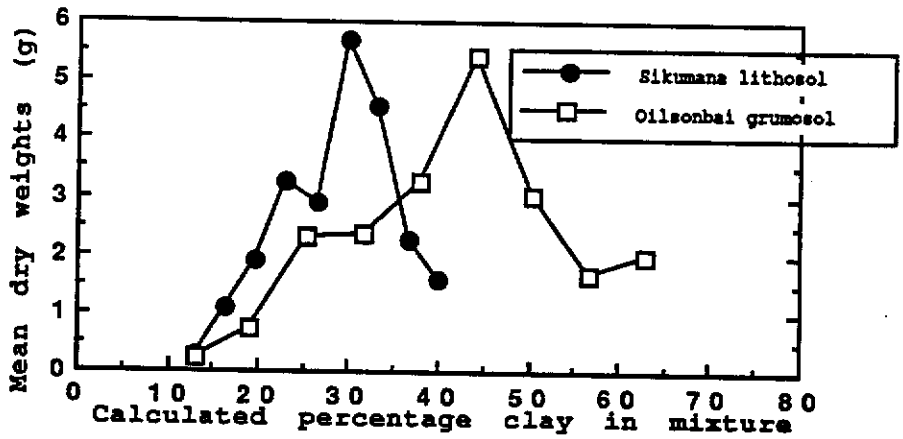

FIGURE 5: Mean dry weights related to estimated clay in mixtures.

Similar patterns were seen in relation to height growth between the two materials (Figures 6 and 7) with the calculated percentage of clay clearly more important. In both mixtures height growth peaked at about 40 cm with 40 percent of total sand from all sources, but there was a considerable difference associated with the proportion of clay.

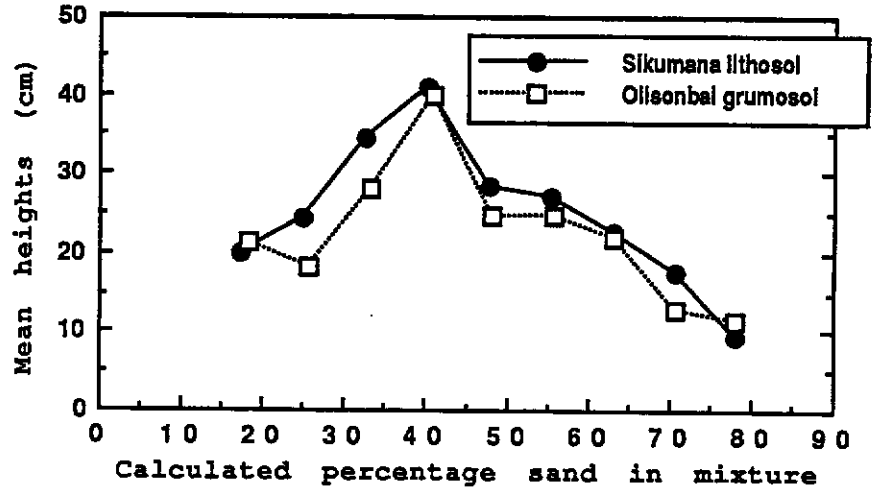

FIGURE 6: Mean heights related to estimated sand in mixtures.

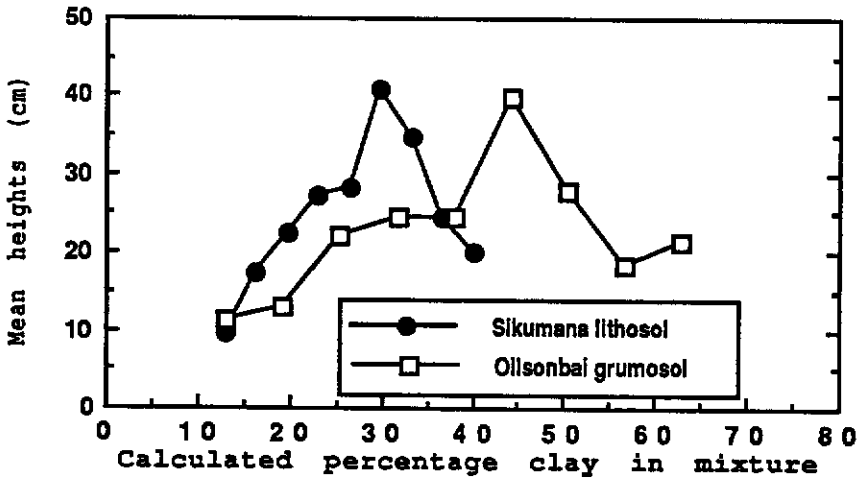

FIGURE 7: Mean heights related to estimated clay in mixtures.

DISCUSSION

Uniformity of the trial was ensured by the use of a single species (**Breynia cernua**) of pot host. This was reflected in the constant linearity of the dry weight/height relationship for harvested plants and a general pattern of dry weight response to proportions of sand added to the local soils. Each local soil may, by implication, be assumed to have an ideal admixture of sand which leads to improved early growth of **Santalum album** in the nursery stage. As a general rule, perhaps heavier clays should be mixed at 1:1 to 1:2 with sands, and soils less clayey at about 1:2 to 1:3. For the two soils tested, the results are clear cut. This is not however an endorsement of the value of **Breynia cernua** as a preferred pot host.

For satisfactory plantation establishment the investment in potting must achieve a useful balance between root and shoot growth. The aim of good nursery practice is for the production of planting stock most likely to survive in the field. To this end it is likely that higher root than shoot weights, preferably coupled with satisfactory 'in pot' haustorial attachment to a primary host, which itself can survive transplanting would be the most desirable feature to aim for. At 6-7 months from germination shoot/root ratios of 1.4 to 2.9 have been observed in **Santalum spicatum** grown in a range of potting mixtures (Wijesuriya and Fox 1985). It remains to be shown whether field survivals can be related directly to good pot performance.

Widiarti (1989) grew **Santalum album** seedlings at Bogor for 6 months in 5 different growth media (Table 4). It was suggested that seedlings grew best in burned chaff and that a nursery potting mix could be a soil/sawdust mixture of equal parts, with burned rice chaff. Inspection of Widiarti's (1989) data indicate that the main ground for concluding best growth in burned rice chaff was that this produced the lowest shoot/root ratio, of 2.05. However this material produced little height growth, and was poorest in both root and total dry weight. Plants grown in the sawdust only treatment appear to have given greatest values for height, stem diameter, top weight and total dry weight. This treatment had unbalanced shoot/root weights with a ratio of 3.65. The mixture recommended by Widiarti (1989), that of equal parts soil/sawdust, produced heaviest root weight but this was not significantly greater than in three of the other four treatments.

Root and shoot weights were not obtained in the trial reported here, so no direct comparison with the Bogar study can be made. It is likely however that balanced natural soil materials will provide at least as good early seedling growth as admixtures with organic media. The use of these types of materials will inevitably tend to introduce variability in seedling performance dependant on the degree of rotting attained and effects on water holding capacity of the potting mixtures. The use of sand may be mainly beneficial through effects on pot drainage, particularly when heavy clay soils are the main local resource.

TABLE 4: Mean harvest values of a growth medium trial at Bogor, for height (cm), stem diameter(cm) and dry weight (g).

Growth medium	Height	Stem diameter	Dry weight
Soil/sawdust 1:1	15.07 bc	0.302 ab	0.333 a
Soil/sawdust 1:3	13.09 d	0.286 b	0.271 a
Sawdust	20.87 a	0.335 a	0.360 a
Soil	16.21 b	0.279 b	0.312 a
Burned rice chaff	13.73 cd	0.287 b	0.193 b

After Widiarti (1989). Harvest values with the same letter do not differ significantly using Duncan's multiple range test. Dry weights estimated from published root weights and shoot/root ratios.

Analysis of the contribution to mixture of the proportional representation of particle sizes indicates that the Oilsonbai grumosol mixtures remained texturally a clay until 50 percent admixture with sand.

The necessity for the availability of iron to young **Santalum** seedlings has been demonstrated by Hirano (1977). The sand source used in the trial reported here is rich in iron and the potential beneficial effects due to this component remain to be evaluated.

CONCLUSIONS

A planting stage seedling height growth of 25 cm or more was obtained with 25 to 62.5 percent sand added to natural soils in the Kupang area. This is accepted as the target size for 6 month seedlings ready for field planting. Seedlings must also have good root development and haustorial connections to the pot host.

Analysis of shoot/root weights is desirable to confirm that the primary aim of good nursery practice is achievable using preferred treatments.

It is recommended that Sikumana soil be preferred to Oilsonbai in preparing nursery potting mixtures at the Kupang area. The Sikumana material is best prepared by mixing it in the ratio of 3 parts local Tarus sand to 5 parts Sikumana soil. It is often easier to alter this to 1 part sand, 3 parts soil. Growth should not be unduly affected.

If the Sikumana material is not available and Oilsonbai grumosol has to be used, then the same basic ratio also applies, but in practice perhaps 1 part sand to 1 part soil may be just as good.

As a general principle it should be noted that clays require a higher admixture of coarse materials than do clay loams.

ACKNOWLEDGEMENTS

The Soil Research Institute, Bogor analysed the soils for which data are presented in Table 1. The Department of MIPA at Widyamandira University, Kupang allowed the use of laboratory facilities to enable dry weight determinations to be made. The Australian Centre for International Agricultural Research provided support for the Sandalwood research programme.

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GROWTH OF THREE SUBTERRANEAN CLOVER (*TRIFOLIUM SUBTERRANEUM*) CULTIVARS ON COAL MINE INTERBURDEN MATERIALS AMENDED WITH FLY ASH.

J E D Fox, A I Doronila and B K Owens, School of Biology, Curtin University of Technology, Kent Street, Bentley, Western Australia, 6102.

INTRODUCTION

Revegetation of coal mine dumps in Collie, W.A. is limited by the highly acidic nature of the spoil material and its poor nutritional status. Lime is routinely applied to neutralize acidity in both the waste dumps and in applied topsoil, prior to cultivation and sowing at the Muja open cut dumps (Stedman 1988).

Acid neutralizing materials are absent in the Collie coal basin (Bartle and Slesser 1989). The costs of obtaining and transporting lime are therefore high. Fly ash, the major solid waste product from coal fired power stations, may be used as an ameliorating agent. It is readily available in large quantities from the adjoining Muja power station. Aside from its acid neutralizing capability, fly ash may also improve the water holding capacity of soils (Campbell **et al.** 1983). Fly ash may also be a source of nutrients **e.g.** magnesium (Hill and Lamp 1980).

Subterranean clover (**Trifolium subterraneum**) is a pasture species used in the initial stages of dump revegetation. It is considered to be tolerant of acidic soil conditions and the related nutrient status (Fox and Turner 1984).

The aim of the study reported here was to evaluate the growth response of three cultivars of **T. subterraneum** (cvs. **Esperance, Northam** and **Trikkala**) grown in three coal spoil materials with various amendments of fly ash.

MATERIALS AND METHODS

The three spoil materials used were suggested as suitable for coal dump surface rehabilitation by Dames and Moore (1983). Ate-Overburden and Galatea-Hebe are greyish sandy clay loams with mean pH levels of 5.4 and 5.3 respectively. The third material, Ceres-Diana, is a whitish loamy sand with mean pH of 5.6. All three materials contain only traces of the essential minerals nitrogen, phosphorus and potassium (less than 1 p.p.m.).

Fly ash from Fremantle power station was incorporated into these three spoil materials at concentrations of 0, 2, 5, 10 and 20 percent by weight. Sets of 10 replicate 1.5 litre capacity plastic pots were filled with the fly ash/spoil mixtures, giving a total of 450 pots (3 spoil materials x 5 fly ash concentrations x 3 subclover cultivars x 10 replicates). Seed of **Trifolium subterraneum** cvs. **Esperance, Northam** and **Trikkala** were inoculated with **Rhizobium**. Several seeds were sown into each pot. Seedlings were thinned to two per pot after 2 weeks.

Five plants per treatment were harvested at 12 weeks and the rest at 20 weeks. Dry weights were obtained at each harvest. The first harvest weights were used to calculate relative growth rates (R.G.R.) between 12 and 20 weeks. Nodules were counted on **Esperance** and **Trikkala** plants harvested at 20 weeks.

Analysis of variance was used to compare dryweights between treatments after 20 weeks growth. Soil pH was recorded from 3 pots at each harvest.

RESULTS

The mean dry weights of subclovers after twenty weeks are summarised in Table 1.

TABLE 1: Mean dry weights (g) of **Trifolium subterraneum** after 20 weeks in relation to fly ash concentration and interburden.

Subclover cultivar	Percentage fly ash 0	2	5	10	20	F value	Sig.
Esperance	0.073[a]	0.594[c]	0.512[c]	0.315[b]	0.149[ab]	27.04	***
Northam	0.111[ab]	0.751[c]	0.304[b]	0.141[ab]	0.050a	25.34	***
Trikkala	0.110[a]	0.513[b]	0.235[a]	0.135[a]	0.109[a]	13.92	***

	Interburden Material: Ate Overburden	Ceres Diana	Galatea Hebe	F value	Sig
Esperance	0.254	0.380	0.352	1.65	NS
Northam	0.278	0.258	0.278	0.03	NS
Trikkala	0.149	0.288	0.226	2.19	NS

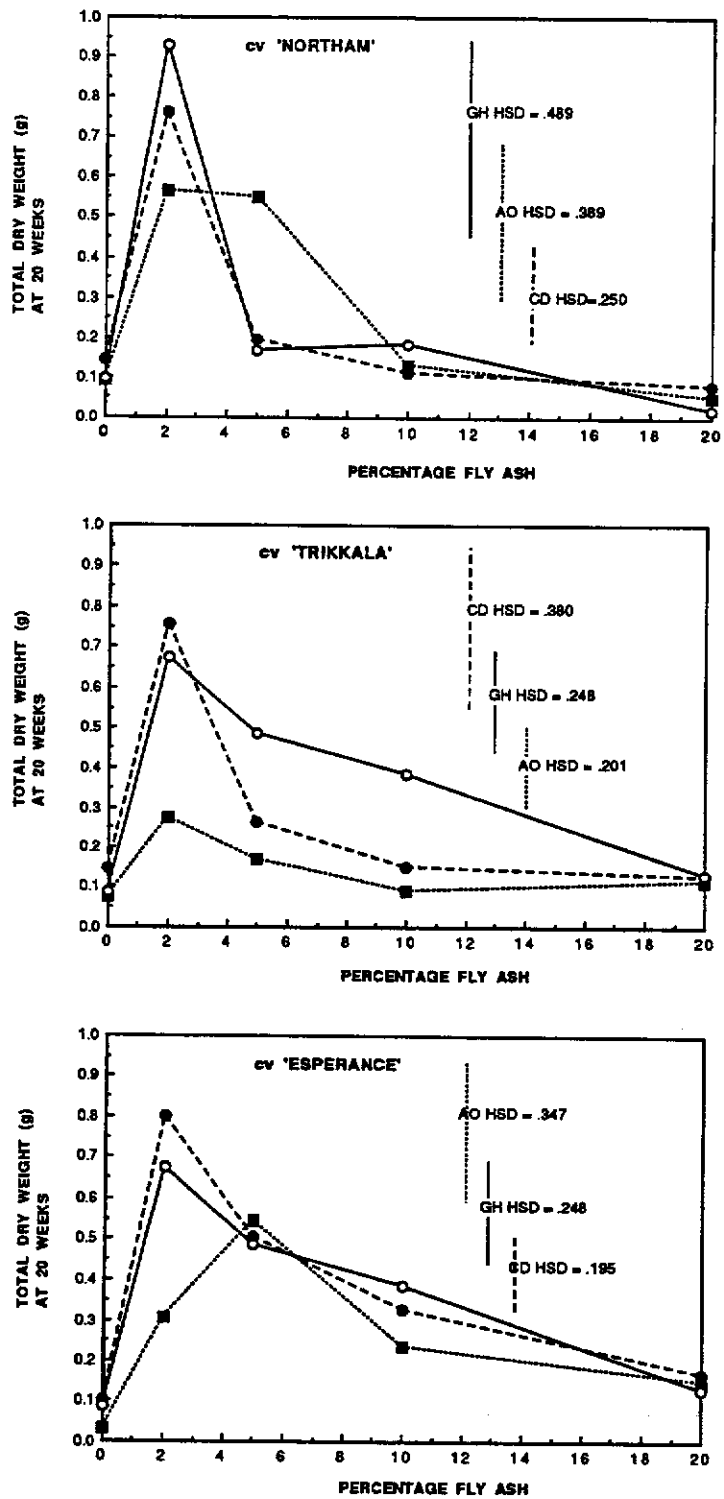
* Note: Mean values with the same letter are not significantly different, using Tukey's highest significant difference test.

All three cultivars gave significantly highest yields with 2 percent fly ash. Cv. **Esperance** harvest was not significantly lower at 5 percent fly ash. Cvs. **Northam** and **Trikkala** also gave second highest yields at 5 percent fly ash concentration, however these were significantly larger than the 2 percent yield.

There was no significant difference in overall mean yield between cultivars grown in each of the three spoil materials (Table 1). There were interesting differences

between cultivars in yields across the treatments (Figure 1). Whereas most yields were greatest at 2 percent fly ash, both cvs. **Northam** and **Esperance** yielded well with 5 percent fly ash in the Ate-Overburden material. **Trikkala** was poor in this material. Yields were generally greatest in Galatea-Hebe at 2 percent fly ash. Both **Esperance** and **Trikkala** had yields significantly higher than the controls at levels up to 10 percent fly ash added to Galatea-Hebe.

FIGURE 1: Mean yields of **Trifolium subterraneum** cultivars grown in fly ash amended spoil materials. (Bars indicate ranges of significant differences using Tukey's highest significant difference test.)



The root nodulation response of subclover cvs. **Esperance** and **Northam** is summarised in Table 2.

TABLE 2: Mean number of nodules present in each subclover grown in fly ash amended spoil material after 20 weeks.

Subclover cultivar	Percentage fly ash 0	2	5	10	20	F value	Sig.
cv. **Esperance**							
Spoil material							
Ate-Overburden	1.30^{a}	11.45^{b}	10.30^{b}	8.60^{b}	11.70^{b}	8.85	***
Ceres-Diana	5.90^{a}	19.23^{b}	14.30^{ab}	16.40^{ab}	9.70^{ab}	3.75	*
Galatea-Hebe	6.80^{a}	19.60^{b}	11.90^{ab}	17.20^{ab}	6.80^{a}	13.36	***
cv. Northam							
Ate-Overburden	6.20^{a}	17.80^{b}	12.10^{b}	4.00^{a}	0.00^{a}	10.98	***
Ceres-Diana	6.60^{ab}	13.80^{b}	6.40^{ab}	6.00^{ab}	4.20^{a}	3.90	*
Galatea Hebe	7.20^{b}	21.00^{c}	3.80^{ab}	7.40^{b}	0.00^{a}	14.35	***

Note: Mean values with the same letter are not significantly different, using Tukey's highest significant difference test.

Unamended spoil materials gave the least number of nodules. Nodule formation in **Esperance** was significantly enhanced by fly ash addition. In cv. **Northam** nodulation was significantly improved at 2-5 percent in Ate-Overburden and at 2 percent in Ceres-Diana and Galatea-Hebe. Nodulation in **Northam** was depressed at 20 percent fly ash in all 3 materials.

TABLE 3: Relative growth rates (g g^{-1} day^{-1}) between the two harvests (12-20 weeks.

	Relative Growth Rates Percentage fly ash 0	2	5	10	20	F value	P
cv. Esperance							
Spoil Material							
Ate-Overburden	-0.014^{a}	0.011^{ab}	0.019^{b}	0.017^{b}	0.021^{b}	4.797	**
Ceres-Diana	0.017	0.019	0.027	0.026	0.023	1.429	NS
Galatea-Hebe	0.003^{a}	0.024^{b}	0.019^{b}	0.028^{b}	0.007^{a}	6.709	***
cv.Trikkala							
Ate-Overburden	0.010	-0.0001	0.007	-0.003	0.012	0.468	NS
Ceres-Diana	0.010	0.014	0.010	0.014	0.005	0.275	NS
Galatea-Hebe	0.001	0.006	0.024	0.015	0.011	0.964	NS
cv. **Northam**							
Ate-Overburden	0.005	0.020	0.005	0.017	0.006	0.794	NS
Ceres-Diana	0.007	0.024	0.009	0.016	0.002	2.389	NS
Galatea-Hebe	0.						
	0.016^{b}	-0.007^{ab}	0.008^{ab}	-0.015^{a}	3.070	*	

There was no significant difference in the relative growth rate (R.G.R.) of cv. **Trikkala** grown in any of the treatments (Table 3). The R.G.R. of cv. **Esperance** was significantly higher between 2-10 percent fly ash amended Galatea-Hebe while a negative R.G.R. was noted in unamended Ate-Overburden. Cv. **Northam** grown in 2 percent fly ash amended Galatea-Hebe was only significantly greater than in 20 percent fly ash concentration. All other treatments did not differ.

Acidity increased slightly over the first 12 weeks in the Ceres-Diana and Ate-Overburden treatments. There was a small upward drift in pH in Galatea-Hebe (Table 4). Comparing the 12 and 20 week recordings it is noted that fly ash amendments increased pH levels significantly after 20 weeks in Ate-Overburden for all concentrations except the 10 percent level. Ceres-Diana acidity was also ameliorated in all fly ash amendments. Galatea-Hebe did not show any significant change in pH for any fly ash additions, but a trend towards neutrality was observed.

TABLE 4: The pH levels of fly ash amended spoil material at 12 weeks and 20 weeks (n=3).

Spoil material	Fly ash concentration	pH level 12 weeks	pH level 20 weeks	Sig.
Ate-Overburden (initially 5.4)	0	5.0	5.4	NS
	2	4.9	6.0	*
	5	4.9	6.0	*
	10	4.9	5.8	NS
	20	4.9	6.1	*
Ceres-Diana (initially 5.6)	0	5.4	5.5	NS
	2	5.5	6.1	*
	5	5.5	6.3	*
	10	5.6	6.4	*
	20	5.6	6.4	*
Galatea-Hebe (initially 5.3)	0	5.5	5.3	NS
	2	5.5	5.8	NS
	5	5.5	5.5	NS
	10	5.6	6.2	NS
	20	5.8	6.3	NS

DISCUSSION

Harvest yields obtained at 2-5 percent fly ash amendments appear to be comparable with fertilizer (NPK) responses in the three spoil materials (Fox and Turner 1985). Cv. **Esperance** was most responsive and **Northam** least responsive to fly ash amendment. Galatae-Hebe material appears to be better able to provide enhanced growing conditions with fly ash amendment. Fly ash may act as a source of nutrients thus enhancing dry matter production. Phosphorus levels (32 mg g^{-1}; $NaHCO_3$ extract) from East Perth power station fly ash are reported to be among the highest in Australian fly ashes. Other plant nutrients present in medium to high concentrations are Ca, Mg, S, Cu, Zn, Mn and Fe (Aitken et al. 1984).

Subterranean clover is grown in mixed swards with pasture grasses. Comparatively high levels of fly ash materials have been reported is enhancing yield of the pastures species **Agropyron smithii** (Howard et al. 1977). Where a mixed legume/grass pasture is favoured then initial attention needs to be paid to the lower statured legume. Subterranean clover has value in its nitrogen enhancing capacity. This is dependent on adequate nodulation and the **Rhizobium** symbiont is affected by soil acidity level.

Amelioration of spoil acidity was shown after 20 weeks by the incorporation of fly ash. This may have enhanced phosphorus uptake.

The poor growth of the subclover cultivars in the higher levels of fly ash (10 and 20 percent) may have been due to toxicity effects. Unweathered fly ash contain boron and aluminium at quantities sufficient to damage plant tissue (Jones and Lewis 1960). There may have been sufficient of these elements to depress growth at higher concentrations of fly ash. Depression of nodulation is associated with nitrogen levels (Harper and Gibson 1984) but nitrogen, though not tested for here, is believed to be relatively low in fly ash.

Another factor which may have depressed growth in the higher levels of fly ash could have been the increased moisture retention effect. Moisture retention has been shown in fly ash amended sand (Campbell et al. 1983). This is advantageous in dry conditions. The trial was conducted during the wet winter period when waterlogging of pots occurred for several days. Rhizobial nodules are sensitive to a reduction in available soil oxygen (Dakora and Atkins 1989). Plants stressed through waterlogging may have reduced root uptake of nutrients, oxygen and enhanced uptake of toxic ions (Clucas and Ladiges 1979).

CONCLUSION

This study indicates that fly ash from Western Australian coal has the capacity to ameliorate acid spoil dump materials. It may provide a source of inexpensive acid neutralizing material. Fly ash may also provide a source of nutrients essential for plant growth. Concentrations of 2 to 5 percent appear most beneficial to **Trifolium subterraneum** growth. The response of this species at these fly ash levels is considered to be comparable to that of dump material amended with c. 200 kg ha^{-1} of 3:2 superphosphate/potash fertiliser. Differences in response to fly ash occur between both cultivars and substrate materials.

ACKNOWLEDGEMENTS

The following are thanked for their technical assistance; G. Joyce, P. Mioduszewski, I. Velentik, R. Stevens and T. Supeni. The Griffin Coal Mining Company Limited and the State Energy Commission provided the interburden and fly ash materials respectively. This work has been supported financially by The Griffin Coal Mining Company Limited.

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ENVIRONMENTAL ASSESSMENT FOLLOWING SAND MINING: A CASE STUDY FROM ENEABBA, WESTERN AUSTRALIA.

J M Osborne, School of Biology, Curtin University of Technology, Kent Street, Bentley, Western Australia, 6102.

Abstract

Extraction of heavy mineral sands from the deposits of the Western Australian northern coastal plain is achieved through open pit mining. Following mining, progressive restoration procedures are undertaken. Mining operations extend into a reserve set aside for conservation purposes. In this area the long term rehabilitation objective is the re-establishment of the range of plant associations which were present prior to mining. Regular assessment is required to determine whether the desired plant community is revegetating successfully through the slow process of ecosystem recovery. A State Government interdepartmental Committee oversees the rehabilitation work carried out by the mining company.

This paper reviews the problem of establishing an assessment regime for the rehabilitation such that:

i. the assessment regime evaluates revegetation success, as established by the Government Committee,

ii. the assessment regime is repeatable and accurate, and

iii. the assessment regime is cost, labour and time effective.

The development of an appropriate sample size for broad acre rehabilitation assessments at Eneabba, is also addressed. A stratified random sampling covering an area of 180 m^2 provided estimates of species richness and plant density comparable with a random sample regime of 400 m^2, for a one hectare rehabilitation. On considerably larger rehabilitation blocks, a figure of seven or eight, 20 m line transects, provided an accurate estimate of plant parameters with 95 percent statistical confidence.

Introduction

Australia has become established as the major supplier of the world's mineral sand requirements. Production was originally based on the east coast of the continent, but has, over the past ten years, become more concentrated in Western Australia. This State is now the world's major producer of mineral sands.

The term "heavy mineral sands" includes a wide variety of minerals. Ilmenite dominates most Western Australian deposits with associated, variable amounts of rutile, leucoxene, zircon, monazite, kyanite and xenotime present.

This paper specifically refers to one mineral sand operation, located at Eneabba, 300 km north of Perth, in Western Australia. The heavy mineral deposits are associated with a past beach and coastal dune system. Mining operations commenced in 1973.

The undisturbed native vegetation of the region consists of sclerophyllous low shrubland heath. The most numerous elements are species of Myrtaceae, Proteaceae and Leguminosae with a ground cover of sedges (Cyperaceae) and the rush-like Restionaceae. Although the soils are naturally impoverished in respect of nutrients, the region is very rich in plant species. A vegetation survey prior to sand mining recorded over 429 species in an area of 20 sq km (Hnatiuk and Hopkins 1981: Hopkins and Hnatiuk 1981). The region experiences a dry mediterranean climate, with hot, dry summers, cool, wet winters, and rainfall averaging 530 mm.

At Eneabba, open cut mining procedures are currently in operation. Initially, the low shrub heath vegetation on the area is cut and mulched, and the topsoil is stripped for later use on rehabilitated areas. Removed overburden materials either fill previous pits or become walls of tailings dams. The mined mineral bearing sands are transported to wet separation plants where gravitational processes extract the heavy minerals. The remaining clays, silts and siliceous sands form a slurry of slimes and sand tailings, which are piped to worked out pits to dry. Overburden materials, sand tailings and slimes are mixed to some extent, reconstructed and then rehabilitated.

Part of the current mining lease of the Company operating at Eneabba extends into an A-class nature reserve, where the rehabilitation requirement is the re-establishment of a functioning ecosystem, with the range of plant densities, and the species richness exhibited prior to mining.

Rehabilitation techniques applied by the Company to ensure appropriate revegetation include:

i. application of fresh topsoil containing viable seed, vegetative propagules and organic matter;

ii. growth of a light cover crop to stabilise topsoil;

iii. addition of mulched vegetation, which contains seed and stabilises soil;

iv. direct-seeding onto the topsoil of locally collected seed; and

v. planting of nursery stock.

Regular quantitative assessment of the rehabilitation is required to determine whether the desired plant community is revegetating successfully through the slow process of ecosystem recovery. Currently over 70 hectares of rehabilitation requires assessment, prior to the Company being relieved of further rehabilitation responsibilities. A State Government interdepartmental Committee, in consultation with the mining company, oversees the rehabilitation work and assessments carried out by the Company.

Sampling Strategies for Eneabba Rehabilitation

When choosing a particular sampling strategy for the revegetated areas the stated objectives of the rehabilitation work need to be considered. A sampling strategy which reflects the aims of the rehabilitation will ensure that the information collected is appropriate for an evaluation of rehabilitation success. Other aspects for consideration

include the attributes of the vegetation to be measured, and the practicability of implementing the sampling.

For the rehabilitated mine areas at Eneabba, the sampling strategy chosen must allow the investigator to obtain measures of the plant community characteristics, species richness, plant density and canopy cover. These are the characteristics selected by the State Government Interdepartmental Committee for evaluating revegetation success. Secondly, the sampling strategy utilised must provide a sample that is representative of the regenerating plant community (i.e. the sampling is repeatable and accurate). Finally, it is considered important that the sampling strategy is cost, labour and time effective.

Recognised and commonly used strategies for obtaining a vegetation sample employ random sampling, systematic sampling or a combination of both. At Eneabba a quantitative trial in a one hectare rehabilitation area was established to compare the relative merits of three such sampling strategies designated random, subsampling and stratified random (see Table 1). Two one-hectare revegetated areas were trialled by each of two botanical surveyors.

TABLE 1: Quantitative trial to compare the relative merits of three sampling strategies (from Osborne, Brooks and Carey 1985).

- two rehabilitation areas (2 years old; 3 years old)
- two surveys in each (each with 2 botanists) for the following:

	Sampling Strategy		
	Random	Subsampling	Stratified random
Quadrat size	10 m x 10 m	10 1 m x 1 m within the 10 m x 10 m	transect lines of 20, 1 m x 1 m contiguous quadrats
per hectare replicates	4	4	9
Area samples per hectare	400 m^2	40 m^2	180 m^2

Random sampling was instigated at a rate of four, 10 m by 10 m quadrats per hectare, as proposed by the Government Committee. The Committee also suggested subsampling of the 10 m by 10 m quadrats might be appropriate, and specified this be done using ten, 1 m by 1 m quadrats randomly located within each of the four main quadrats. The stratified random strategy was a combination of systematic sampling (Mueller-Dombois and Ellenberg 1974) and random sampling. The sampling required each rehabilitation area to be arbitrarily divided into similar sized blocks, with a line transect of 20 contiguous one metre square quadrats randomly placed within each block. The one hectare trial area was divided into 9 blocks, with a randomly located 20 metre line transect in each.

As would be expected with replicate sampling procedures, in both rehabilitation sites estimates of density (per m^2) were the same for the three sampling strategies trialled in this Eneabba minesite study ($p > 0.25$; Table 2, from Osborne et al. 1985). The standard 1 m by 1 m quadrat size enabled comparison of the mean number of species per unit area (m^2) for the stratified random and subsampling procedures. In both rehabilitation sites, the estimates of number of species per square metre did not differ ($p > 0.25$; Table 2, from Osborne et al. 1985).

Estimates of the species richness of the rehabilitated one hectare areas were generally similar for the stratified random and random sampling strategies. Stratified random sampling covering an area of 180 m^2 provided information about species richness reasonably comparable with a sampling of 400 m^2. A considerably lower species richness was recorded in both areas for the subsampling regime, indicating that the total area of 40 m^2 sampled by this regime was of insufficient size for an accurate estimation of species richness. Approximately half the species sampled by the random and stratified random strategies were not included in the sample (Table 2).

TABLE 2: Plant densities (m^2), number of species (m^2) and species richness for two, one hectare rehabilitation sites (from Osborne et al. 1985).

		Area 1 (2 yr old)			Area 2 (3 yr old)		
		Random n=4	Sub-sampling n=4	Strati-fied n=9	Random n=4	Sub-sampling n=4	Strati-fied n=4
Density/m^2							
Survey 1	x	2.78	2.83	3.12	3.65	4.52	4.96
	s	1.20	0.91	1.45	0.95	1.72	1.22
Survey 2	x	2.29	3.83	2.84	2.82	4.48	4.88
	s	0.92	2.11	1.18	0.91	1.53	1.85
No. species/m^2							
Survey 1	x	-	1.98	2.08	-	3.85	4.09
	s	-	2.08	0.85	-	1.27	1.33
Survey 2	x	-	2.30	1.99	-	3.75	3.95
	s	-	1.99	0.71	-	1.14	0.96
Richness							
Survey 1		62	25	61	105	51	96
Survey 2		60	23	49	97	46	97

Division of a one hectare rehabilitation area into nine blocks with random location of a 20 m line transect of 1 m by 1 m quadrats in each, provided plant community estimates of species richness, density, and number of species per unit area, comparable with a sampling regime of four 10 m by 10 m quadrats per hectare. The proposed line transect sampling is practicable. It is both more manageable and more economical than the four 10 m by 10 m quadrat technique. It can also lead to a more statistically acceptable randomisation of data capture in that any fertility/topography/moisture gradients may be sampled. With the random 10 m by 10 m quadrat system such gradients may be inadvertently missed.

Sample Size for Eneabba Rehabilitation

There is now a requirement at Eneabba for annual broad area rehabilitation assessments, now in excess of 270 hectares. A small sample size may lead to an inaccurate plant parameter estimation. On the other hand, a sample that is too large provides estimates that are more accurate than is required. The sampling then becomes unduly costly and time consuming.

Data from three rehabilitation areas of 8, 11 and 14 hectares were processed. On each rehabilitation area 18 line transects of 20 m by 1 m provided numbers of species and densities for each square metre. Recommended sample size for the three rehabilitations based on procedures modified from Cochran (1963) and Snedecor and Cochran (1982) are given in Table 3 (for details see Appendix 1, Tables 4 and 5).

FIGURE 1: The basis for statistical procedures followed in processing the rehabilitation data.

The sample size depends on the following:

- degree of precision required
  - use of estimate
  - consequences of large error
    - confidence levels, e.g. 95%
    - accuracy, e.g. 10% of the mean x_{s_t}
- variability or spread of the data
  e.g. $s_{x_{s_t}}$
  s_t
  s
- sample method
  i.e. stratified random
- type of estimating procedure
  i.e. interval estimation is the appropriate procedure

Figure 1 outlines the basis for the statistical procedures followed in this data processing. The required interval estimation, and the stratified random sampling method employed were assumed for the data processing. The use of the plant parameter estimate, and the consequences of a sizeable error in the estimate, determined the degree of precision required for an estimate of the sample size. Initial sampling (see Osborne et al. 1985) provided estimates of the variability or data spread, thus helping select the sample sizes for data collection from the larger rehabilitation areas.

Ninety-five percent confidence levels were set for the plant parameter estimates of density (per m^2) and number of species (per m^2). For the suggested sample sizes it can be assumed with 95 percent confidence that estimates of the mean plant parameters lie within 10 percent of the mean.

For the relatively young rehabilitation areas at Eneabba of approximately 8 to 14 hectares, generally seven, 20 m by 1 m transects estimated plant parameters within 10 percent of the mean, with a 95 percent statistical confidence (Table 3).

Conclusions

A stratified random sampling covering an area of 180 m^2 provided estimates of species richness and plant density comparable with a random sample regime of 400 m^2, for a one hectare rehabilitation. On considerably large rehabilitations of 8 to 14 hectares, a figure of seven or eight, 20 m line transects, provided an accurate estimate of plant parameters with 95 percent statistical confidence. Seven or eight transects provide an initial assessment of sample size, which should be reasonably generalistic to other Eneabba rehabilitation blocks of similar, and larger areas. With increased or decreased variability, the figure may need modification.

TABLE 3: Required quadrat and transect numbers to provide mean plant parameter estimates within 10 percent of the mean, with 95 percent statistical confidence.

	Data Set 1	Data Set 2	Data Set 3
No. species per m^2	142; 7 transects	111; 6 transects	104; 5 transects
No. plants per m^2	(a) 365; 18 transects	139; 7 transects	124; 6 transects
Rehabilitated	1983	1983	1982
Assessed	1986	1985	1985
Vegetation age	3 years	2 years	3 years
Area	7.62 ha	13.6 ha	10.5 ha

(a) Two quadrats recorded respectively 49 and 16 **Beaufortia elegans** and **Beyeria brevifolia** plants, contributing considerably to variability.

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Appendix 1

TABLE 4: Number of species per m^2 quadrat, 1983 eight hectare rehabilitation, data collected 1986. x_t = mean number of species per m^2 for each transect, with s_t, standard deviation.

Transect	1	2	3	4	5	6	7	8	9	0	11	12	13	14	15	16	17	18	19	20	Total species	x_t	s_t
1	4	2	1	4	1	2	2	1	0	3	2	4	1	2	4	2	5	0	1	0	18	2.05	1.504
2	10	5	6	3	4	1	3	2	3	7	4	1	4	5	4	1	4	2	2	3	31	3.70	2.203
3.	1	0	10	2	2	1	3	2	2	4	6	1	6	4	0	3	3	0	2	2	26	2.70	3.430
4	6	4	4	5	5	3	4	4	2	2	4	4	3	1	3	1	3	2	2	0	23	3.10	1.518
5	2	3	2	3	5	1	1	1	3	4	5	2	1	2	4	2	1	3	1	1	23	2.35	1.349
6	0	2	4	0	2	1	6	1	2	1	1	0	0	2	3	3	2	1	1	2	17	1.70	1.491
7	3	1	2	2	2	3	2	7	2	2	3	0	2	0	3	1	2	3	1	3	25	2.20	1.473
8	2	3	2	0	3	1	3	1	5	4	2	6	2	3	5	1	1	4	1	1	25	2.50	1.638
9	7	7	6	4	0	4	6	2	4	4	5	4	7	4	6	2	2	1	3	0	31	3.90	2.222
10	6	4	3	3	4	5	2	3	0	3	4	5	1	1	1	1	1	2	2	1	24	2.60	1.667
11	3	6	6	1	3	5	2	4	2	4	5	2	4	5	1	2	1	1	4	6	36	3.35	1.785
12	5	4	10	7	6	2	6	5	7	5	4	5	6	4	4	5	5	3	5	1	42	4.95	1.905
13	11	4	8	7	4	5	3	4	4	7	5	4	2	3	3	3	3	4	4	1	36	4.45	2.282
14	8	3	5	3	4	4	4	4	4	4	0	3	1	3	6	3	0	2	3	2	30	3.30	1.867
15	10	6	5	3	3	2	2	3	3	2	4	8	6	6	4	4	6	6	4	2	40	4.45	2.164
16	2	7	3	0	7	3	5	4	2	4	3	5	2	6	1	4	2	1	0	3	26	3.20	2.067
17	3	3	0	2	5	4	3	7	1	5	2	4	9	2	5	3	2	4	2	5	36	3.55	2.089
18	4	4	2	1	1	1	1	1	1	2	2	2	2	1	1	3	3	6	4	9	28	2.55	2.064

The estimation of an appropriate sample size for the 1983 rehabilitation data number of species per m^2 data (Table 4), follows Snedecor and Cochran (1982) and Cochran (1962).

n = estimated number of 1 m x 1 m quadrats, with 20 quadrats per transect

s = standard deviation (calculated from a stratified sample, which Snedecor and Cochran (1982) note is a reasonable approximation, i.e. more than 10 sampling units per stratum and stratification is proportional).

$t_{0.05(2)[df]}$ df = n-1 (number of 1 m x 1 m quadrats from initial sample minus 1).

Noting approximations are often made to the z distribution or the value 2 ($z = 1.96$, $\alpha = 0.05$), e.g. Brewer and Woolly (1983), Snedecor and Cochran (1982). These values provide a 95 percent statistical confidence that estimates of the sample mean lie within a percent of the mean for the estimated sample size.

L = allowable error in the sample mean, this has been taken as 10 percent of the stratified sample mean x_{s_t}.

deff = design effect (see Cochran 1963, Kish 1965, Snedecor and Cochran 1982).

deff = $\frac{\text{variance of the estimate from stratified sampling}}{\text{variance of the estimate given by random sampling}}$

The sample size calculated for a simple random sample is multiplied by the design effect, for use with the stratified random plan.

$$n = \frac{t_{0.05(2)[df]} \text{ multiplied by } s}{L}^2$$

xst = 3.1722

L = 10 percent of x_{s_t}.

$\therefore L$ = 0.3172

t = 1.95

s = 2.0905 (obtained from Table 5), total mean square, (see Snedecor and Cochran 1982)

$\therefore$ n = 166.852

deff = design effect = $\frac{3.74 \text{ (from Table 5)}}{4.37}$

= 0.856

TABLE 5: ANOVA summary, species per m^2 data, 1983 rehabilitation assessed 1986 (Table 4). Total mean square and within strata mean square used for variability estimates.

Source of variation	df	SS	MS	F	P
Between strata	17	289.92	17.05	4.56	p<0.001
Within strata	342	1279.40	3.74		
Total	359	1569.32	4.37		

The estimated number of quadrats from the stratified sampling of the rehabilitation, required to provide an estimate of the mean number of species per square metre within 10 percent of the mean, with 95 percent statistical confidence, is:

n multiplied by deff

= 142.78 $\frac{143}{20}$ = 7.15 transects

= 143 quadrats

= 7 stratified random transects, of 20 contiguous one-metre square quadrats.

TECHNIQUES FOR ASSESSING ISOZYME ACTIVITY IN *SANTALUM ALBUM* L. AND PRELIMINARY RESULTS.

L M Egerton-Warburton, School of Biology, Curtin University of Technology, Kent St, Bentley, Western Australia, 6102.

Summary

An outline of methods suitable for assessing allozyme variation in **Santalum** species is given. Methods describe a suitable grinding buffer for seed or foliage material and soaking cum running buffers for cellulose acetate plates. Twelve enzyme systems were assessed and of these, eleven were observed to exhibit activity. Of these systems, those most suitable for assessing provenance variation within **Santalum** species were shikimate dehydrogenase, menadione reductase, glutamate oxalo-acetate transaminase and glucose-phosphate isomerase in juvenile leaf tissue and alcohol dehydrogenase in seed. Alcohol dehydrogenase was the only enzyme assayable in dry or moist seed.

Introduction

Species from the family Santalaceae are of commercial interest for their aromatic woods (**Santalum spicatum** and S. **album**) and as a cultivated fruit and nut crop (**S. acuminatum**, quandong) (Hewson and George 1984). The most useful species is considered to be **S. album**, which is widely grown throughout southern India, Indonesia and Hawaii, and is currently being established in Western Australia.

Parthasarathi **et al.** (1986) noted that considerable morphological variation existed both within and between populations of **S. album**. Although morphological characters are genetically determined, they can be subject to environmental influences. Hence, for accurate and reliable provenance determination, the detection of distinct genetic traits would be desirable.

Peroxidase activity in bark of **S. album** has been used as an indicator of oil producing capacity of this species (Parthasarathi **et al.** 1986). However, there is no literature describing isozyme techniques suitable for screening **S. album** provenances for variation or evolutionary changes. This paper describes electrophoretic techniques for isozymes suitable for screening **S. album** provenances.

Materials and Methods

Seed of **S. album** was made available from bulk seed collected at Bangalore, India in April 1989. The hard outer testa was removed immediately prior to grinding. Seeds were not soaked, as allozyme patterns were adequately resolved from dry seed. Juvenile foliage was collected from **S. album** trees which had been established in the Field Trial Area on the Bentley Campus of Curtin University of Technology for three to four years from Bangalore seedstock. Foliage was used for most allozyme assays as **Santalum** species generally show low germination levels (Barrett and Fox 1989) requiring large numbers of seed to be used in order to provide a representative sample size. In addition, only one enzyme (alcohol dehydrogenase, *Adh*), of four systems assayed (alcohol dehydrogenase, acid phosphatase, shikimate dehydrogenase and malate dehydrogenase) was active in seed. For this study, a minimum of 36 seed or 24 foliage samples were assessed for each of 12 enzyme systems (Table 1).

Individual seeds or first leaves were ground in 0.8-0.9 or 0.4 ml respectively of 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), 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 (Coates 1988).

Methods were based on the Helena Laboratories Titan III Zip Zone cellulose acetate electrophoresis system (Helena Laboratories, Melbourne). Ground seed or foliage samples were transferred to sample cells and directly loaded onto plates using the applicator.

Before loading, plates were soaked in running buffer (4 °C) for 10 minutes. An 80 mM Tris, 1 mM Na_2EDTA, 5.7 mM maleic acid, 1 mM $MgCl_2$ and 8 mM citric acid, pH 8.6 gave the best resolution for all enzyme systems assayed (after Coates 1988). After loading, the plated were placed face down in the tank with ends resting on blotting paper. Plates were electrophoresed for 45 minutes at 20 mA and 5°C.

Consistent and interpretable enzyme activity occurred in 12 enzyme systems; the staining procedures for these systems are given in Table 1. Four ml of just molten 3% 'Bacto-Agar' was added to four ml of stain buffer and reagents (the reaction mixture), mixed well and poured over plates. The addition of agar proved beneficial as maximum contact occurred between plate and stain reagent and diffusion of enzymatic products on the plate was prevented. Most enzymes stained readily within 15 to 20 minutes, after which the agar overlay was washed off, plates rinsed in 7 percent acetic acid and air dried. Each zone of enzyme activity was assumed to represent a single enzyme locus. Loci were designated numerically beginning with the fastest migrating zone.

Results and Discussion

Two enzymes of the twelve assayed were found to be either only weakly active or monomorphic at a single locus. The enzyme 6-Phosphogluconate dehydrogenase (locus *6Pgd-1*) was assayed but found to be weakly active and hence a phenogram of the locus was not included. Peroxidase (*Per*) activity was detected at only one locus and appeared to be monomorphic in all samples tested. This result disagrees strongly with Parthasarathi **et al.** (1986) whose data suggested that *Per* activity in **Santalum** species should be highly polymorphic.

Esterase

Esterase (*Est*) activity occurred at three loci, with *Est-1* and *Est-2* being monomorphic. Five patterns of alleles occurred in *Est-3* (Figure 1a), with patterns 1 and 4 the most common and pattern 2, the least common.

Shikimate dehydrogenase

Shikimate dehydrogenase (*Sdh*) was characterised by two loci (Figure 1b). Only two patterns, with one or two alleles, occurred at *Sdh-2* with most samples exhibiting

TABLE 1: Enzymes, buffers and stains used in cellulose acetate electrophoresis of **S. album.**

Enzyme (Locus, EC number)	Tissue ***	Buffer	Stain solution or substrate	Other reagents	Source#
Alcohol dehydrogenase (*Adh*, EC 1.1.1.1)	S	4 ml 0.1 M Tris pH 8.0	0.4 ml ethanol	0.2 ml NAD, 0.3 ml each MTT (methyl thiazolyl blue) and PMS (phenazine methosulphate)*	*a*
Esterase (*Est*, EC 3.1.1.1)	J	4 ml 0.1 M Tris-maleate pH 6.5	8 mg α-napthyl acetate	12 mg Fast Garnet GBC in 0.2 ml acetone	*a*
Glucose-6-phosphate dehydrogenase (*G6PD*, EC 1.1.1.49)	J	4 ml 0.1 M Tris pH 8.0	12 mg glucose-6-phosphate	0.2 ml each NADP, $MgCl_2$, MTT and PMS*	*a*
Glutamate dehydrogenase (*Gdh*, EC 1.4.1.3)	J	4 ml 0.1 M Tris pH 8.0	40 mg Na glutamate	0.2 ml each NAD, MTT and PMS	*a*
Glucose-phosphate isomerase (*Gpi*, 5.3.1.9)	J	4 ml 0.1 M Tris pH 8.0	10 mg fructose-6-phosphate	0.2 ml each NADP, $MgCl_2$, MTT and PMS 4 i.u. glucose-6-phosphate dehydrogenase	*a*
Glutamate-oxaloacetate transaminase (*Got*, EC 2.6.1.1)	J	4 ml 0.1 M Tris pH 8.0	10 mg pyridoxal-5-phosphate 0.4 ml α-ketoglutarate pH 8.0* 0.4 ml L-aspartate pH 8.0*	12 mg Fast Garnet GBC	*b*
Isocitrate dehydrogenase (*Idh*, EC 1.1.1.42)	J	4 ml 0.1 M Tris pH 8.0	20 mg DL-isocitric acid	0.2 ml each NADP, $MgCl_2$, MTT and PMS	*a*
Menadione reductase (*Mdr*,EC 1.6.99.22)	J	4 ml 0.1 M Tris pH 7.0	8 mg menadione	5 mg NADH 0.4 ml MTT	*b*
Peroxidase (*Per*, EC 1.11.1.7)	J	28 ml acetate buffer ** pH 5.0	2 ml 30% H_2O_2	100 mg dianisidine diHCl in 70 ml 50% ethanol	*c*
6- Phosphogluconate dehydrogenase (*6-Pgd*; EC 1.1.1.44)	J	4 ml 0.1 M Tris pH 8.0	10 mg 6-phosphogluconic acid	0.2 ml each NADP, $MgCl_2$, MTT and PMS	*a*
Phosphoglucomutase (*Pgm*, EC 2.7.5.1)	J	4 ml 0.1 M Tris pH 8.0	10 mg glucose-1-phosphate	0.2 ml each NADP, $MgCl_2$, MTT and PMS 4 i.u. glucose-6-phosphate dehydrogenase	*a*
Shikimate dehydrogenase (*Sdh*, EC 1.1.1.25)	J	4 ml 0.1 M Tris pH 8.0	5 mg shikimic acid	0.3 ml each NADP, $MgCl_2$, MTT and PMS	*b*

* Concentrations of stock solutions as follows: 40 mM NAD, 25 mM NADP, 14.5 mM MTT, 6.5 mM PMS, 1.0 mM $MgCl_2$, 50 mg ml^{-1} L-aspartic acid pH 8.0, 50 mg ml^{-1} α-ketoglutaric acid pH 8.0.

** Acetate buffer: 11.2 g sodium acetate and 4 ml glacial acetic acid per litre of deionised water (pH 5.0).

*** S, seed J, juvenile leaf tissue

Sources: *a* Richardson **et al.** (1986) *b* Moran and Hopper (1983) *c* Brewbaker **et al.** (1968)

pattern 2. Segregation occurred in *Sdh-1*, with four distinct patterns of alleles. Of these patterns, numbers 1 and 4 were the most common, and pattern 2 the least common.

Menadione reductase

Activity of menadione reductase (*Mdr*) was observed at both *Mdr-1* and *Mdr-2*, with *Mdr-1* being the more active (Figure 1c). Of the three patterns of alleles at *Mdr-2*, pattern 2 occurred the most often, with only 8 percent of samples showing pattern 3. At the *Mdr-1* locus, 50 percent of samples were monomorphic (pattern 4). Of the polymorphic forms, patterns 2 and 3 were equally represented.

Glucose-6- phosphate dehydrogenase

Enzyme activity of glucose-6-phosphate dehydrogenase (*G6pd*) resolved at two loci, *G6pd-1* and *G6pd-2* (Figure 1d). Polymorphic forms occurred at *G6pd-2*, where pattern 5 was commonest (54 percent of samples) and pattern 4, rarest (8 percent of samples). Only two patterns were observed at *G6pd-1*, with the majority of samples represented by pattern 1.

Glutamate oxalo-acetate transaminase

Four loci were detected for glutamate oxalo-acetate transaminase (*Got*), with both *Got-2* and *Got-4* being monomorphic (Figure 2a). For the *Got-3* locus, patterns 1 and 2 occurred in equal proportions. At *Got-1*, pattern three was found in 62 percent of samples, with only 4 percent of samples showing pattern 2.

Phosphoglucomutase

Three patterns of alleles were observed at *Pgm-2*, with most showing pattern 1 (three alleles at the locus) (Figure 2b). Activity at *Pgm-1* was much weaker, although interpretable, with most samples being monomorphic (pattern 2).

Glutamate dehydrogenase

Activity of glutamate dehydrogenase (*Gdh*) was resolved at three loci. Of these, *Gdh-2* and *Gdh-3* both showed patterns of either one or two alleles per locus (Figure 2c). *Gdh-2* was essentially monomorphic (pattern 2) and *Gdh-1* was monomorphic when present.

Glucose-phosphate isomerase

Glucose-phosphate isomerase (*Gpi*) activity occurred at two loci, *Gpi-1* and *Gpi-2*. *Gpi-2* was monomorphic and *Gpi-1* exhibited pattern 3 in 42 percent of samples tested (Figure 2d).

Isocitrate dehydrogenase

Only two allele patterns were observed for isocitrate dehydrogenase (*Idh*) at *Idh-1*, with most samples showing pattern 1 (Figure 2e). *Idh-2* was occasionally detected on plates, but monomorphic when present.

Alcohol dehydrogenase

Alcohol dehydrogenase (*Adh*) was the only active, interpretable enzyme in **S. album** seed. Most samples showed pattern (three alleles per locus), with pattern 1 the least common (2 percent of samples tested) (Figure 2f).

Acknowledgements

This study was supported by funding from the Australian Centre for International Agricultural Research, Project Number 2261. Dr J-P Orsini is thanked for his comments on the manuscript.

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Figure 1a

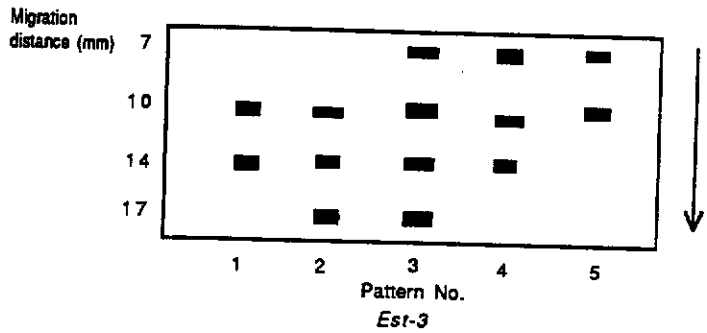


Figure 1b

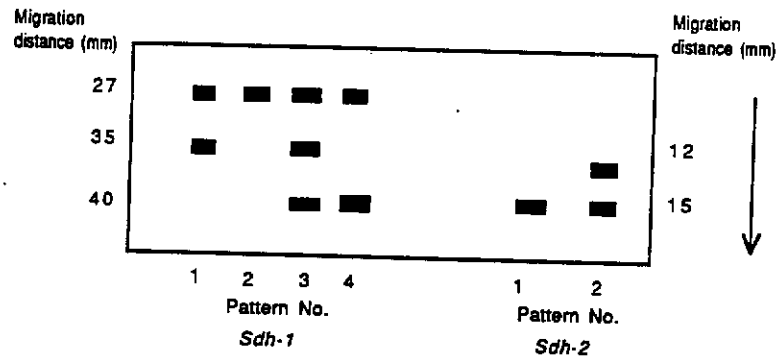


Figure 1c

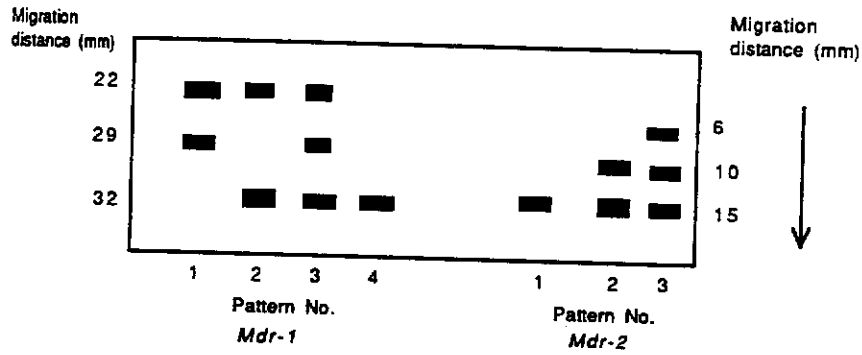
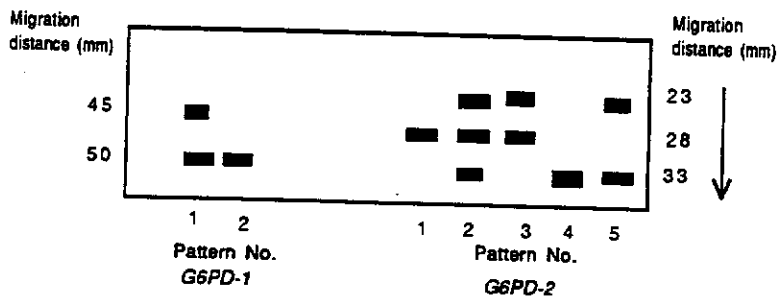
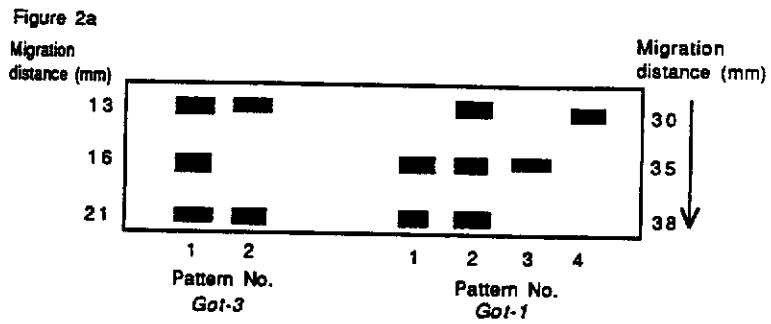
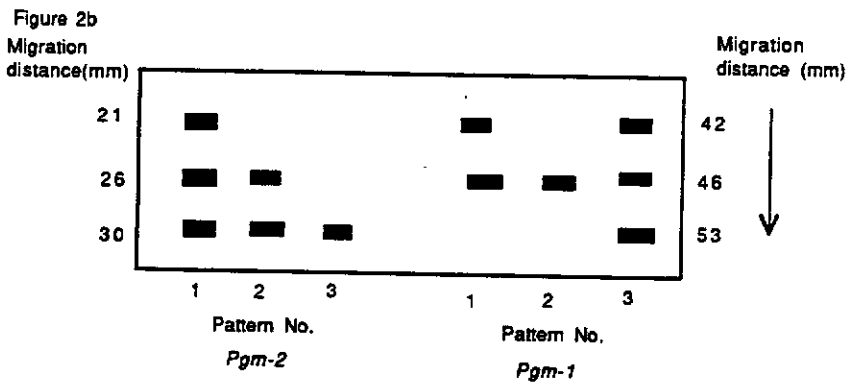


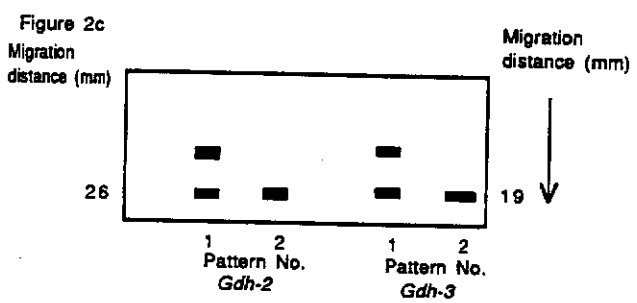
Figure 1d

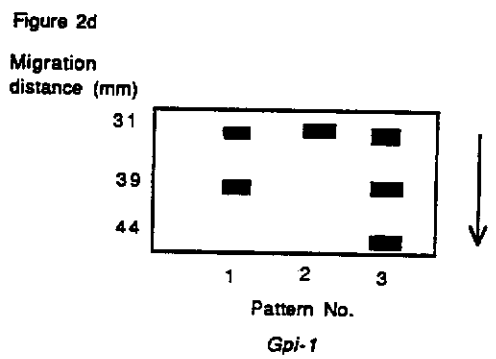


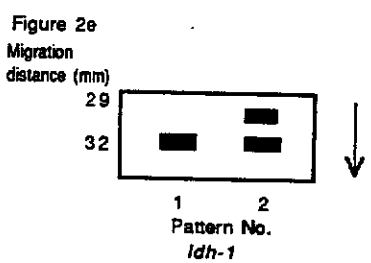
FIGURES 1a-d: Zymograms of **Santalum album**, with schematic representation of phenotypes for Esterase (*Est*), Shikimate dehydrogenase (*Sdh*), Menadione reductase (*Mdr*) and Glucose-6-phosphate dehydrogenase (*G6pd*) respectively.

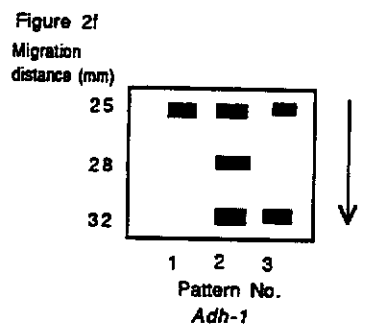












FIGURES 2a-e: Zymograms of **Santalum album**, with schematic representation of phenotypes for Glutamate oxaloacetate transaminase (*Got*), Phosphoglucomutase (*Pgm*), Glutamate dehydrogenase (*Gdh*), Glucose-phosphate isomerase (*Gpi*), Isocitrate dehydrogenase (*Idh*) and Alcohol dehydrogenase (*Adh*) respectively.