File 14

CONTROL OF *PHYTOPHTHORA* AND *DIPLODINA* CANKER IN WESTERN AUSTRALIA

ANNUAL REPORT TO THE

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THE CONTROL OF *PHYTOPHTHORA* IN NATIVE PLANT COMMUNITIES

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Annual report to the Australian Nature Conservation Agency (March 1995)

Summary

Aerial spraying of the chemical phosphonate is a promising method of application. In the South Sister trial phosphonate applied aerially at 8.5 % at 26 l/ha reduced plant death in the treated area for up to 18 months. Low levels of phosphite ion are still being detected in the shoots and roots 18 months after spraying.

In the Gull Rock trial, 10 % phosphonate applied at 60 l/ha controlled the disease in all treated plots in all reps in the first twelve months. After one year the number of deaths in treated plots started to increase in two reps. Phosphonate is still controlling the disease in the two other reps, despite the phosphite concentration in the shoots and roots being below 1 $\mu g\ g^{-1}$.

The initial concentration of phosphonate residues in plant tissue after spraying with 10 % phosphonate was generally too low to achieve long term protection against *Phytophthora cinnamomi* infection.

Higher concentration of the fungicide applied in new trials (20 to 40 % at 60 l/ha applied twice) resulted in substantially higher than in Albany trials initial concentration of the active ingredient in the plant tissue, which is expected to ensure longer term infection control.
Phosphonate is distributed in the shoots and roots of the treated plants and the first harvest of *Banksia telmetia* demonstrated that the chemical was equally distributed in the shoots and roots.

Droplet size of the spray is one of the most important factors determining the success of fungicide application. Rate per hectare should be set depending on the canopy structure and the amount of leaf area per unit area.
40 % phosphonate applied in our trials is currently the highest concentration commercially available and has not been used to treat plants before.

Introduction

Phytophthora cinnamomi is a vigorous pathogen that kills a wide range of plant species by attacking their root system. The fungus is widespread in the south-west of Western Australia and it is estimated that 1500 to 2000 species of the estimated over 8000 species of vascular plants in the South-West may be susceptible to infection. (Shea, 1991). Many of them are highly endemic and have been brought to the brink of extinction.
Apart from impacting on the structure and genetic diversity of heath and shrublands, animal communities would also be affected because of severely altered habitat. Therefore, dieback disease currently poses the greatest conservation threat facing Western Australia.

In the past, the diseases caused by *Phytophthora* were usually controlled using specific cultural practices and soil fumigation (Coffey, 1991). However, these methods were difficult to apply in field conditions. The discovery of systemic fungicides characterised by symplastic mobility open new possibilities of *Phytophthora* disease control.

Phosphonate is an aqueous solution of di-potassium phosphonate, held at pH 5.7 to 6.0. The active component of the fungicide is the phosphite ion (PO_3^-).
The chemical has been used to control *P. cinnamomi* in economically significant crops for several years. Phosphonate was also found to be very effective against the infection in jarrah and some species of *Banksia* (Shearer, *pers. comm.*). It is cheap (the price dropped by about 40 % in the last two years), biodegradable and non-toxic to people, animals and soil microflora. Foliar application of phosphonate did not affect microbial numbers in the rhizosphere of avocado seedlings (Wongwathanarat and Sivasithamparam, 1991). Also, growth of the mycorrhizal fungus was unaffected by application of the fungicide to maize (Wellings *et al.*, 1990).
Phosphonate is the only chemical that can be safely applied to extensive areas of native flora.

The fungicide has been field trialed by CALM in various areas in the south-west on plant communities already infested with *Phytophthora cinnamomi*. The trials have shown that one application of phosphonate gives excellent control of the fungus over several years in some *Banksia* species (Shearer, *pers. comm.*).

The chemical can be injected into the trunk using a hydraulic injector. This technique is successful with phosphonate because the PO_3^- ion is transported in the conductive tissues of the tree to the leaves and from leaves to roots.
Phosphonate can also be sprayed onto the foliage using a backpack sprayer.
Phytophthora cinnamomi has had a high impact on the population of rare and endangered *Banksia brownii* at Millbrook Reserve 30 km north of Albany. A foliar application field trial carried by CALM demonstrated that phosphonate protected the plants for up to three years. The infection front moved three metres past the experimental plots leaving the sprayed plants healthy.

In order to control spread of infection effectively over large areas, the chemical must be applied by aircraft. The aerial application field trials in the Albany region are being conducted with the aim to provide information on the appropriate rates of application, the duration of protection of phosphonate achieved by this method of application and to determine when re-spraying is required. The effectiveness of follow-up application is also being assessed.

Aerial spraying is the next step in the fight against *Phytophthora* dieback. Although phosphonate does not eliminate disease, it is a powerful prophylactic fungicide and it offers the only available option in the prevention of further losses of rare and endangered plant species from dieback through effective protection of healthy plant communities, in areas where the pathogen is present, in the short to medium term.

1. Assessment of the efficacy of aerial application of phosphonate

The fungicide has been field trialed in the past five years in several areas in the south-west on plant communities already infected with *Phytophthora cinnamomi*. Foliar application and trunk injection trials have shown that one application of phosphonate gives excellent control of the fungus over several years (Shearer, *pers. comm.*). However, in order to control spread of infection effectively, the chemical must be applied aerially using aircraft. This method of application would be most suitable if long fronts of infection were to be treated. It would also permit treatment of most remote areas cost-effectively, without disturbance to the treated and neighbouring areas, therefore eliminating any possibility of accidental spread of the disease. There are some fundamental differences between the two methods of application. Aerially applied systemic fungicides are applied in low volumes, usually 20-50 l/ha (Jacobsen, 1986). Also, because of the aircraft's speed and the dynamics of the physics involved, various factors must be considered. These should include the size of the target and the need for adequate coverage (number of droplets deposited per unit area at various levels of the plant canopy), droplet size, effective application swath width and uniformity of application. It is critical that before any of these factors can be considered, proper calibration of the aircraft is achieved.

The aim of the aerial trials is to provide information on the appropriate rates of application and the duration of protection of phosphonate achieved by this particular method of application. The results from these experiments will be used to formulate a management strategy and to determine when re-spraying is required.

1.1 Aerial application trial at South Sister Nature Reserve (30 km east of Albany)

In the beginning of May 1993 we commenced aerial application trial at South Sister Nature Reserve where there is a population of rare and endangered *Banksia brownii*

(feather-leaved banksia). This species of *Banksia* is very susceptible to infection from *Phytophthora*, occurs only in a few locations and all populations are infected with the pathogen.

The South Sister site (8.8 ha) was sprayed with 8.5% concentration of potassium phosphonate, using Synertrol (0.5%) as a wetting agent. The chemical was applied from aeroplane, in a low volume of 26 l/ha. A local contractor was selected to do the job (Giles Aviation). A follow-up spray was carried out six weeks later.
Twenty *B. brownii* trees were randomly selected and marked. Their health was assessed. In order to determine the coverage of the spray within the plant canopy and droplet size, water-sensitive papers were attached to the upper leaves and also put directly onto the ground below the trees. The use of water-sensitive papers is not directly quantitative but does give a quick visual estimate of droplet size and density in various points of the plant canopy. The relationship between the volume of liquid carrier used and the density of spray deposition on the plant is important because the biological efficacy of a fungicide is determined not only by rate of application but also by spray droplet density on the plant.
In our trial, droplet size (VMD-volume mean diameter) ranged from 100 to 500 μm with 70 % of droplets being in the range of 100 to 300 μm and droplet density was 50-60 drops/cm^2.
It is generally recommended that droplet VMD should be between 300 and 500 μm as it prevents excessive drift. However in our situation smaller droplets are preferred as they penetrate the dense canopy much better due to good horizontal movement of the spray. The wind speed was only 3 km/hr so spray drift was minimal. Droplet penetration through the canopy was good as the water-sensitive papers laid under the trees had only 20 % fewer drops than the papers attached to the upper leaves.
Leaf samples were taken for chemical analysis to determine the concentration of phosphite ion in the plant tissue. The marked trees were assessed for any visible signs of phytotoxicity, but none were observed.

The concentration of the active ingredient was boosted by the second application of phosphonate but it decreased to a low level of 0.6 ppm six months after spraying. Despite the low concentration of the phosphite ion in plant tissues the plants remained healthy.
One Year and a half after spraying the concentration of phosphonate residues decreased to 0.17 μg g^{-1} and two marked trees died.

Table 1 Phosphite concentration (μg g^{-1}) in leaf tissue of *B. brownii*.

Sampling time	PO_3 (μg g^{-1})
before 1st spraying	0
after 1st spraying	1.3
before 2nd spraying (5 wks after 1st)	1.3
after 2nd spraying	6.2
six months after spraying	0.6
eighteen months after spraying	0.17

The concentration and the rate per hectare applied to this site was too low to assure long term protection of the treated plants. The plants were protected for up to eighteen months. In order to prevent further deaths in the area phosphonate will have to be applied at higher rate and concentration.
The results from the most recent trials indicate that higher concentration of phosphonate can be applied to plants without causing phytotoxicity, therefore after careful assessment of the most recent work it is planned that the area will be resprayed.

1.2 Aerial application trial in the Gull Rock National Park

A fully replicated field trial in the Gull Rock area near Albany was established in April 1993 to determine the effectiveness of phosphonate in the control of *Phytophthora cinnamomi* in *Banksia coccinea* (red-flowered banksia). The fungus has had high impact in that area and infection fronts are causing wide spread destruction.
B. coccinea is a keystone species as its flowers are an important food source for small marsupials and birds. It is also a commercially important species.
The trial consists of eight plots (4 pairs of sprayed and control) which have been set up on the infection front. The plots are 40 m long and 20 m wide. Within each sprayed plot an assessment sub-plot was marked (20 m long 10 m wide). Twenty plants were marked in each of the assessment sub-plot. Plant height was measured every six weeks before spraying and periodically after the treatment. Change in the rate of plant growth is a good indicator of phytotoxicity so the pre and post-spray measurements will allow to determine whether the chemical has any deleterious effects on the plants. The number of dead plants within each plot was also measured before spraying and has been recorded after spraying to establish the duration of the effectiveness of the fungicide. Plant samples are taken for chemical analysis to determine the concentration of the phosphite ion.
The plots were treated in early November and a follow-up spraying was carried out in the first week of December 1993.
The plots were sprayed twice with 10 % phosphonate (with 0.5% Synertrol as a wetting agent) in a low volume of 30 l/ha which gave the effective rate of 60 l/ha. The second spraying in December was done at the same rate.
In order to monitor the coverage of the spray and droplet size, water-sensitive papers were placed in the treated plots. After the first spraying we noticed that the coverage had not been satisfactory which was shown by a low number of droplets per unit area (20-30 /cm^2). Poor coverage was caused by excessive drift that occurred despite only slight to moderate wind (10-25 km/hr).
In the second spraying we increased the droplet size from 100-300 µm to 300-600µm which improved coverage significantly to a desired number of around 60 droplets/cm^2. Despite the increased droplet size the penetration of the plant canopy was excellent. It appears that application of bigger droplets is more beneficial in areas where windy conditions prevail such as the south-west. In addition, when only small area is to be sprayed, increased droplet size is preferred as it will contribute to improved precision of the application and minimise losses through drift.
The results from the first four harvests are presented in Table 2.
The concentration of the active ingredient was significantly increased by the second application of phosphonate. Improved converge due to increased droplet size in the

follow-up application resulted in significantly higher concentration of phosphite in plant tissue. The substantial differences in phosphite concentration between plots resulted from uneven application as the pilot had some difficulties in targeting the small experimental plots.
One year after of the fungicide application the concentration of phosphite ion decreased and ranged between 0.18 and 0.4 $\mu g\ g^{-1}$.

Table 2 Phosphite concentration in leaf tissue of treated *B. coccinea* plants.

	before 1st spr. ($\mu g\ g^{-1}$)	after 1st spr. ($\mu g\ g^{-1}$)	before 2nd spr.* ($\mu g\ g^{-1}$)	after 2nd spr. ($\mu g\ g^{-1}$)	after twelve months ($\mu g\ g^{-1}$)
rep **A**	0	0.9	0.15	4.15	0.4
rep **B**	0	4.3	5.1	13.2	0.3
rep **C**	0	1.4	1.2	6.2	0.18
rep **D**	0	2.5	2.4	34.2	0.32

* 2nd spraying was done three weeks after 1st

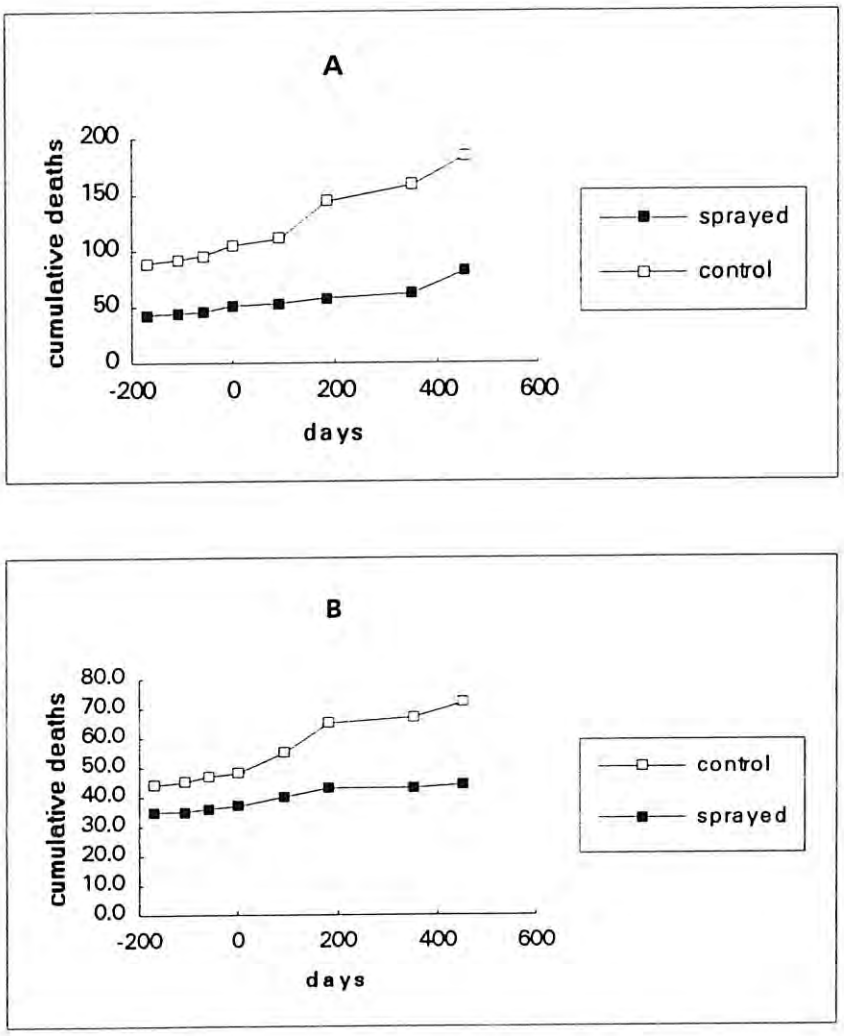
Plant mortality is the ultimate measure of the treatment's effectiveness and in our experiment it has been expressed as cumulative deaths within each plot.
Phosphonate applied aerially reduced plant death in the treated areas (Figure 1) and controlled the disease in all treated plots in all reps in the first twelve months. After one year the number of deaths in treated plots started to increase in reps **A** and **C** and after sixteen months the rate of deaths increase, in treated plots and controls, is similar. In reps **B** and **D** we still have an excellent control of the disease with both treated plots having almost no deaths. The number of dead plants in the control plots in **B** and **D** increased substantially in the same period and at a similar rate as the controls in **A** and **C**.
The *B. coccinea* plants in reps **B** and **D** had much higher initial concentration of phosphite ion in their leaf tissue. The plants in these two blocks were also younger (3 to 8 years old) and therefore their leaf area per unit ground area was significantly smaller than leaf area of plants in rep **A** and **C** (12 to 18 years old). When the chemical was applied to the plots at 60 l/ha, younger, smaller plants in **B** and **D** possibly received a higher dose which resulted in the higher concentration of phosphite ion found in the leaf tissue.

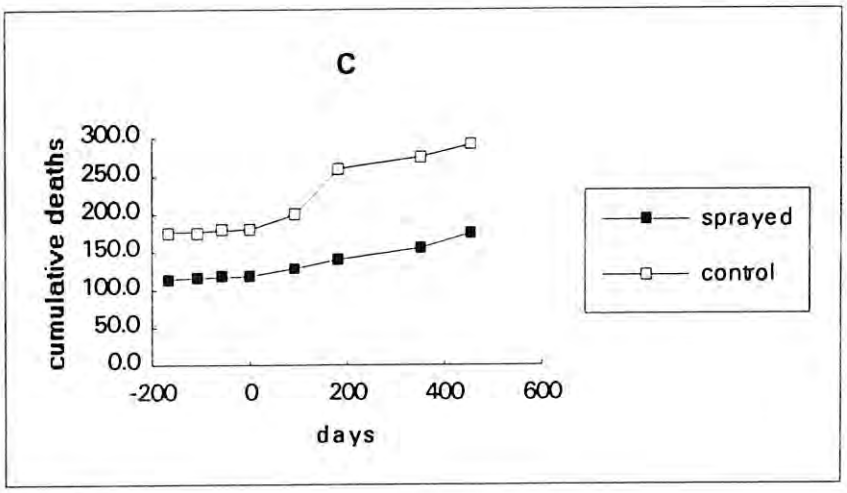
There was a substantial increase in the number of dead plants in all control plots 90 to 200 days after spraying. That increase coincided with a period of very dry conditions of 1993/94 summer and early autumn. Drought put additional stress on plants already affected by the fungus.
No such increase was recorded in the treated plots in the same period, indicating that the sprayed plants were protected by the chemical and therefore, able to better survive difficult environmental conditions because their roots sustained less damage from the activity of the pathogen.

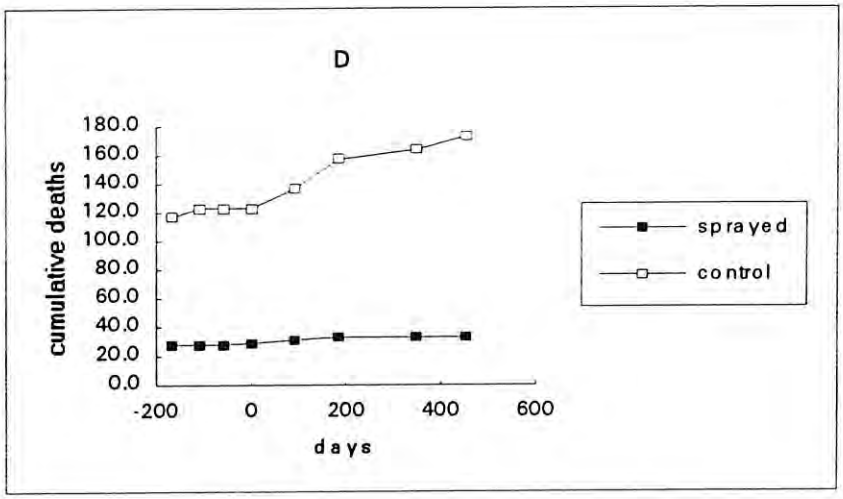
During the last harvest (twelve months after spraying) we also collected root samples from the sprayed plots. The samples were analysed and the concentration of phosphite

ion ranged from 0.2 to 0.7 $\mu g\ g^{-1}$.
This result indicates that phosphonate is distributed in shoots and roots. The new experiments established last year will allow to determine the relative distribution of the phosphite ion in the plant tissue.
Plant growth has been monitored before and after spraying and no significant differences in the rate of growth between treated and untreated plants have been observed. Application of 10 % phosphonate at 60 l/ha did not affect plant growth.

Figure 1 Gull Rock aerial application trial - number of dead plants.







1.3 Aerial spray trial at Millbrook Reserve

Phytophthora cinnamomi has had a high impact on a population of *Banksia brownii* at Millbrook Reserve 30 km north of Albany. A foliar application field trial (using backpack sprayer) carried by CALM demonstrated that phosphonate protected the plants for up to three years (Shearer, *pers. comm.*). In that trial the experimental plots were set up on the edge of active infection front and sprayed with phosphonate. After three years the infection front moved more than three metres past the plots leaving the sprayed plants healthy.

This aerial spray trial is a second one involving *Banksia brownii* and it will allow comparison of the effectiveness of phosphonate application in controlling *Phytophthora* infection in the same plant species but in two different areas. In addition the application of the fungicide at Millbrook will permit saving another population of this endangered plant species from extinction.

Almost the whole population of *B. brownii* (6 ha) has been sprayed aerially, using low volume application. Plants in the Millbrook Reserve and Gull Rock were sprayed on the same days so the follow-up application at Millbrook also involved larger droplet size. The plots were sprayed twice with 10% phosphonate (with 0.5% Synertrol as a wetting agent) in a low volume of 30 l/ha which gave the effective rate of 60 l/ha. The

second spraying in December was done at the same rate. Chemical analysis and plant health measurements have been carried out and the duration of protection will be established. The results are presented in Table 3.
The concentration of the active ingredient was significantly increased by the second application of phosphonate. Improved coverage due to increased droplet size in the follow-up application resulted in significantly higher concentration of phosphite in plant tissue.
The "after 2nd spraying" concentration of phosphite at Millbrook was almost 4 times higher than at South Sister due to the following factors:
- spray concentration was increased from 8.5% to 10%
- application rate was increased from 30 l/ha to 60 l/ha
- droplet size was increased (lower losses through drift and improved coverage).

Table 3 Phosphite concentration ($\mu g\ g^{-1}$) in leaf tissue of *B. brownii*.

Sampling time	PO_3 ($\mu g\ g^{-1}$)
before 1st spraying	0
after 1st spraying	4.5
before 2nd spraying	1
after 2nd spraying	22
twelve months after spraying	0.41

We also collected root samples from the sprayed plots at the last harvest. The samples were analysed and the concentration of phosphite ion ranged from 0.3 to 1.9 $\mu g\ g^{-1}$. This result indicate that phosphonate was distributed in roots as well as shoots.

2. New field experiments

2.1 Aerial application trial at North Dandalup

In October 1994 we established a new phosphonate aerial application trial involving three plant species: *Banksia attenuata, Banksia ilicifolia and Banksia menziesii.* Plots (60 m * 30 m) have been set up on the edge of active infection front (remnant bushland vegetation on private property). An assessment sub-plot was marked (40 m * 20 m) within each sprayed plot. Two plants of each species were marked within each assessment plot and plant samples for chemical analysis are being taken from the same plants at each harvest.
Plants were sprayed with three concentrations (10, 20 and 40 %) sprayed twice at 60 l/ha of phosphonate using aircraft (Giles Aviation). In the North Dandalup aerial application trial we are going to test higher concentration rates than those used in Albany.
40 % phosphonate is currently the highest concentration available and has not been used to treat plants before. The spray was mixed with 2 % Synertrol (wetting agent).

The concentration of Synertrol in all new experiments was higher than in Albany trials because phosphonate was applied in warm temperature conditions.
First spraying was done in early November, second spraying four weeks later.

Measurements

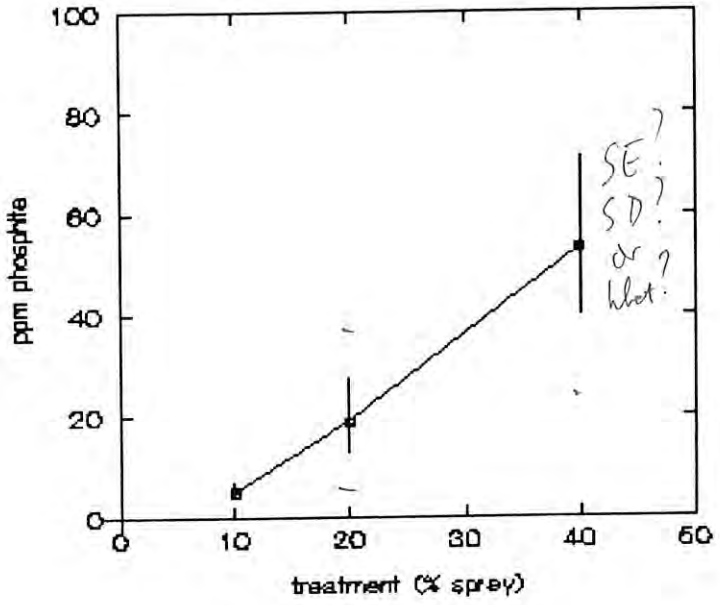
Shoot samples were taken for chemical analysis (phosphonate and major nutrients) immediately after the first and second application of the chemical and will be taken every few months. Root samples were also collected from selected trees sprayed with 20 % phosphonate and will be analysed.
In order to determine the effect of phosphonate on flowering and seed setting we will collect the seed that would be counted and germinated. We will also measure the number of dead plants in sprayed/control plots to establish the duration of the effectiveness of the fungicide.

This field trial will provide information on the following:

- the effectiveness of aerial application of phosphonate applied at various concentrations
- the longevity of phosphonate residues in the roots and shoots
- the longevity of the fungicide in mature plants
- the effect of the treatment on plant nutrition
- the effect of phosphonate on flowering, seed set and seed viability
- the highest concentration that can be used safely with aerial spraying

Leaf samples from the first harvest (after first spraying) were analysed and the data is presented in Figure 2. The average concentration of phosphite in the leaf tissue was 5.3, 18.9 and 53.4 $\mu g\ g^{-1}$ for 10, 20 and 40 % treatment respectively that should provide adequate protection especially in the two higher treatments. The ED_{50} concentration rate for *P. cinnamomi* isolates in culture was found to be less than 5 $\mu g\ g^{-1}$ in low (0.1 mM PO_4) phosphate conditions (Komorek, 1994).
The data was analysed using ANOVA (general linear model) and no significant differences (P=0.01) were found between reps, plant species, trees or samples. The differences between treatments were significantly different. The standard errors presented in Figure 2 are asymmetric because the data for the statistical analysis was log transformed.
The follow up treatment increased the concentration significantly so it is expected that in 20 and 40 % treatments, phosphonate residues will persist in the plant tissue for much longer than in Albany trials due to significantly higher initial tissue concentrations. Further harvests and plant observation will allow to establish how long the plants will remain healthy and stay protected from infection.

Figure 2 Phosphite concentration ($\mu g\ g^{-1}$) in leaf tissue of *Banksia* plants.



2.2 Field trial at Eneabba - West (distribution trial)

A new phosphonate application trial involving was established in November 1994. Small plots (4 m *1 m) containing regenerating seedlings (post fire) were sprayed with three different concentrations of phosphonate (10, 20 and 40 %) sprayed twice using hand-held ultra-low volume sprayer.
Each plot was sprayed at the rate equivalent of 15 l/ha and the spray was mixed with 2 % Synertrol. The hand-held ultra-low volume sprayer allows us to do small scale experiments. The number of droplets per unit area and droplet size were similar to droplet number achieved by aerial application at North Dandalup as determined by water sensitive papers.
The experiment involves 5 destructive harvests. First spraying was done in late October and the follow-up spraying about four weeks later.

Measurements

Plant samples (roots and shoots) for chemical analysis (phosphonate and major nutrients) were taken immediately after spraying and will be taken every two to four months in the future. At the time of sampling we also measure plant height. Plant mortality in all sprayed and control plots is also measured.

This field trial will provide information on the following:

- the duration of protection given to plants by various concentrations of phosphonate
- the longevity and relative distribution of phosphonate residues in the roots and shoots
- the longevity of the fungicide in young plants
- the effect of the spray on plant nutrition
- the highest concentration of phosphonate that can be applied to young plants without causing phytotoxicity

Leaf and root samples from the first (spring) harvest (after two applications) were analysed and the data is presented in Figure 3. The average concentration of phosphite in the leaf tissue was 5.9, 26.3 and 115.4 $\mu g\ g^{-1}$, root tissue 5.2, 19.6 and 91.3 for 10, 20 and 40 % treatment respectively which should provide adequate protection especially in the two higher treatments.

The data was analysed using ANOVA (general linear model) and no significant differences (P=0.01) were found between reps or samples. The differences between treatments were significantly different.

The concentration of phosphite ion in shoots was slightly higher than in roots at all spray concentration but the difference was not statistically significant (P=0.01). Regression analysis (Figure 4) demonstrated that there was a linear relationship between the concentration of phosphite in shots and roots ($R^2 = 0.986$) in harvest 1. Phosphonate as a systemic fungicide is distributed through xylem and phloem (Groussol *et al.*, 1986). It has been demonstrated that the relative seasonal distribution of the phosphite ion is influenced by source-sink relationships in the plant and for example in avocados, the best time for phosphonate application is thought to be late summer when the roots are the main metabolic sink of the plant (Whiley *et al.*, 1986). Further harvests will demonstrate how the concentration and the relative (in shoots and roots) distribution of phosphonate residues changes in time.

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Figure 3 Phosphite concentration ($\mu g\ g^{-1}$) in leaf and root tissue of *Banksia telmetia* plants.

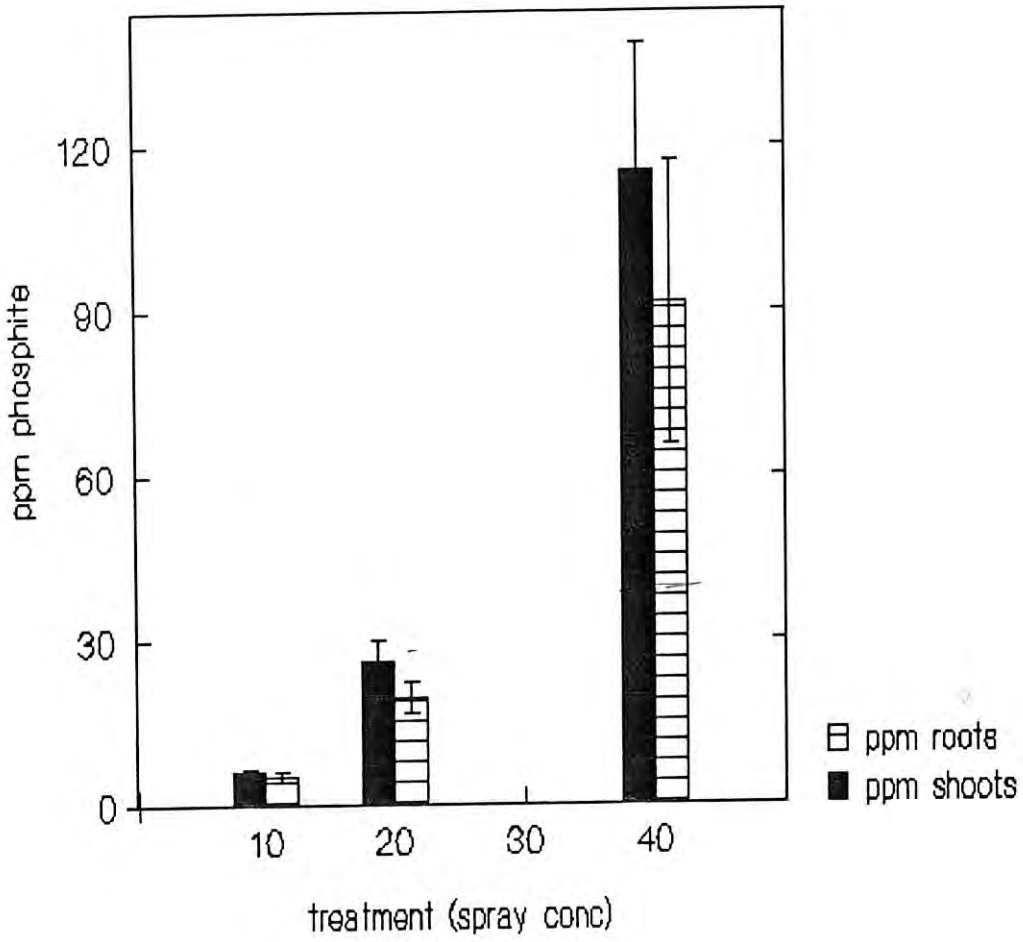
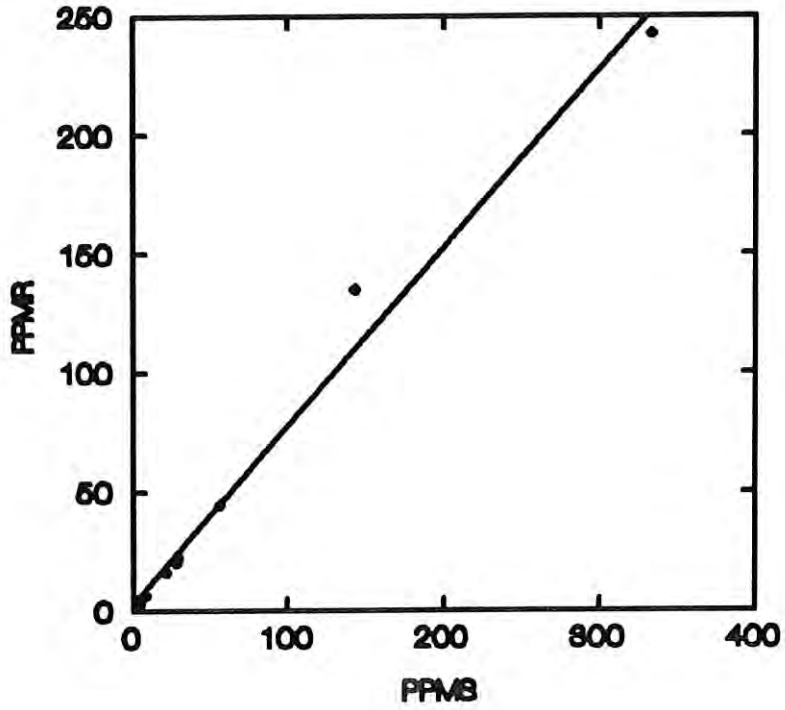


Figure 4 Regression line showing a linear relationship between phosphite shoot and root concentration of *Banksia telmetia* plants at harvest 1. (PPMS and PPMR - shoot and root concentration of phosphite ion in $\mu g\ g^{-1}$).



2.3 Field trial at Eneabba - West (active infection front)

An experiment involving *Banksia atenuata* and *Banksia menziesii* was established in October 1994.
Plots have been set up on the edge of active infection front and ten plants of each species were marked within each plot. Individual plants were sprayed with three different concentrations of phosphonate (10, 20 and 40 %) sprayed once or twice using hand-held ultra-low volume sprayer. The sprayed plant are of various ages, ranging from one year old seedlings to more mature seven year olds.
This experiment has been designed predominantly to monitor plant mortality and to determine the appropriate concentration rate of the chemical that can be applied to plants. The benefits of follow-up application will also be assessed.
First spraying was done in late October and the second spraying about four weeks later. Plant tissue analysis will also be conducted.

This trial will provide information on the following:

- the concentration rate that will give long term protection to plants against infection and can be applied without causing phytotoxicity
- the duration of protection given to plants by various concentrations of phosphonate applied once or twice

- the longevity and relative distribution of phosphonate residues in the roots and shoots in time

2.4 Field trial at Eneabba - South (infection front)

We established phosphonate application trial involving species of *Dryandra, Petrophile and Xanthorrhoea.*
Individual healthy plants (in the area affected by *P. cinnamomi*) were sprayed with three different concentrations of phosphonate (10, 20 and 40 %) sprayed once or twice using hand-held ultra-low volume sprayer
The treatment resulted in plants sprayed with 20 % and 40 % being burnt to some extent.
First spraying was done in late October. Second spraying (treatments 1,3 and 5) was done about five weeks later.
Plant samples (roots and shoots) for chemical analysis (phosphonate and major nutrients) were collected after spraying and will be collected twice in 1995.
At the time of sampling we measure plant height and after flowering, collect the seed that would be counted and germinated.

Aims

- To determine the effectiveness of phosphonate application on different plant species (expressed as plant mortality)
- To determine the duration of protection given to plants by various concentrations of phosphonate
- To establish the longevity of phosphonate residues in shoots
- To determine the effect of 2 vs 1 application of the fungicide
- To determine the effect of the spray on plant nutrition
- To study the effect of phosphonate on flowering, seed set and seed viability
- To establish the highest concentration of phosphonate that can be applied without causing phytotoxicity
- To determine the ability of plants to recover from injury caused by high application rates

2.5 Field trial at Eneabba - South (phytotoxicity trial)

An experiment involving *Lambertia multiflora* was established in November 1994.
Single plants (located in the area affected by *P. cinnamomi*) were sprayed with three concentrations of phosphonate (10, 20 and 40 %) sprayed twice using hand-held ultra-low volume sprayer. The chemical was applied using hand-held ultra-low volume sprayer.
The treatment resulted in plants sprayed with 20 % and 40 % being burnt to some extent.
First spraying was done in late October and the follow-up spraying about four weeks later. The experiment involves five destructive harvests.

Plant samples (roots and shoots) for chemical analysis (phosphonate and major nutrients) were taken immediately after spraying and will be taken every two to four months in the future. At the time of sampling we also measure plant height. Plant mortality in all sprayed and control plots is also measured.

Aims

This field trial will provide information on the following:

- the duration of protection given to plants by various concentrations of phosphonate (expressed as plant mortality)
- the longevity and relative distribution of phosphonate residues in the roots and shoots in time
- To determine the effect of the spray on plant growth and nutrition
- To establish the highest concentration of phosphonate that can be applied without causing phytotoxicity
- the ability of plants to recover from injury caused by high application rates

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Acknowledgments

We wish to thank the staff of Western Australian Chemistry Centre, Agricultural Chemistry Laboratory: Dr Neil Rothnie for his aid and advice in this project and to Wayne Best for his assistance in the quantitive determinations of phosphonate in plant samples.
We also would like to gratefully acknowledge all the helpful suggestions by Dr. Bruce Grant, Department of Biochemistry, Melbourne University.

Too brief

DEVELOPMENT OF A DNA DIAGNOSTIC TEST FOR *PHYTOPHTHORA CINNAMOMI.*

REPORT 3/3/1995

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We have prevously reported on progress for scope items 1 and 2 (Ann. report 10/3/1993). In that report we outlined the approach taken to establish a culture collection of isolates of Phytophthora, and to isolate a species specific DNA sequence from *P. cinnamomi*. These sequences were isolated using RAPD-PCR, and shown by hybridization to be specific for *P. cinnamomi*. These fragments were then sequenced using dideoxysequencing. The nucleotide sequence can be used to construct oligonucleotide primers for *P. cinnamomi*.

SCOPE ITEM 2: Identification of Phytophthora taxon specific DNA sequences.

We have reported previously the isolation of DNA sequences specific for *P. cinnamomi*. These can be used as the basis of a diagnostic test for detection of *P. cinnamomi*. Upon isolation of these sequences we decided to proceed with the development of the diagnostic test. The intention is to isolate sequences specific for other taxa at a later stage.

Sequences specific for other taxa (P.megasperma and P. citricola) will be isolated during 1995/1996, and developed into PCR assays. During the validation period these tests can be validated coincidentally with the test for P. cinnamomi. The devfelopment of PCR tests for these sequences will be greatly facilitated by the techniques developed for the P. cinnamomi test.

SCOPE ITEM 3: Develop a quantitative PCR test for each sequence.

(3.1) Construction of pairs of oligonucleotide primers:
A PCR test has been developed for the *P. cinnamomi* specific DNA sequence. pairs of oligonucleotide primers to sites flanking the specific sequence were constructed and found to function in a PCR reaction.

The conditions required for amplification have now been optimised. Factors investigated for their effect on amplification include: concentration of magnesium chloride, concentration of DNA, and inclusion of DMSO in the reaction.

STATUS: Complete

(3.2) Test primer pairs against target *Phytophthora* taxon:
Several primer pairs were tested with DNA from a range of *Phytophthora* spp, and also with DNA from other fungal spp. Two of the primer pairs were found to give products with all spp tested, ie. are non specific. However other primer pairs based on the same *P. cinnamomi* specific sequence were found to be specific for *P. cinnamomi* in that an amplification product was obtained only with *P. cinnamomi* DNA.

STATUS: Complete

(3.4) Method for extraction of DNA from soil: Soil contains a lot of substances variously referred to as "humic acids". These are extracted along with DNA and they inhibit subsequent enzymatic reactions such as PCR and restriction digestion. A number of methods for extraction of bacterial DNA from soil have

been developed, but these are too cumbersome and not applicable to fungi. Our purpose was to develop a method which is simple and effective and not too laborious. We have investigated a large number of factors which affect the amplification of the DNA from soil, and have developed a method of amplification which works effectively. This same method can be used for extraction of DNA from native plant species.

We are also ivestigating a method of selectively separating the fungal mycelium/zoospore/oospore/ from the soil or plant milieu. This method based on the use of atibodies coupled to magnetic beads will greatly simplify and speed up the analysis of samples, and enable the analysis of much larger samples.

STATUS: Near Completion

(3.4) Development of an internal standard: Use of the diagnostic test under field conditions will require development of an internal standard to preclude false negatives. The standard can be developed from the cloned fragment by insertion of a DNA sequence (150-300bp) into a suitable restriction site within the cloned fragment. This insertion will result in a larger amplification product than would be observed from the original fragment. Addition of the standard to a sample containing *P. cinnamomi* DNA will result in two amplification products observed by gel electrophoresis, the larger from the internal standard and the smaller from the *P. cinnamomi* DNA in the sample. Failure to observe the larger would indicate inhibition of the PCR reaction by components in the sample. The test would need a period of validation under field conditions during which results obtained with the test were compared with results obtained by current procedures.

STATUS: In progress

SCOPE ITEM 4. Validate the test by field trials.

(4.2) Validation of the test by reconstruction experiments with soil ammended with *P. cinnamomi:* This experiment is to determine the sensitivity of the test under field conditions. The approach being taken is to mix dilutions of *P. cinnamomi* DNA with soil extract, and after purification amplify the DNA using the specific primers. The ammended soil samples will be analyzed concurrently with the baiting procedure so that the tests can be accuratly compared for sensitivity.

STATUS: Due to start 4/95.

(4.3) Validation of the test with natural soils and plant material: In this activity samples of soil and plant material are analyzed for *P. cinnamomi* , *P. megasperma*, and *P. citricola* by (a) baiting technique, (b) DNA probe by hybridization analysis, and (c) PCR test so that we can evaluate the performance of the test under field conditions, and compare the effectiviness of the test with the baiting method. We include the hybridization test as an independent confirmation of the results obtained with the PCR test. The field samples are likely to be more heterogeneous than the laboratory samples and contain material from a much wider range of microbial, animal and plant species, it is possible that .the PCR test may interact nonspecifically with some of these species, but it is unlikely that the hybridization test would.

STATUS: Not Yet Started

(4.4) Using the test to study colonization of the host plant. The presence of the fungal mycelium within tissues can be detected by in situ PCR of tissue sections. Sections can be taken at intervals after infection and analyzed. This will enable us to follow the progress of the fungus through the plant. By comparing resistant and sensitive plants we might get a clue as to the factors affecting the sensitivity of the host. We can also use the technique to determine which tissues and which cells within a tissue are colonized.

STATUS: Not Yet Started

This does not seem to have progressed far

PROJECT 3

DEVELOPMENT OF GIS-BASED DECISION-SUPPORT TOOLS AND THE DATABASING OF *PHYTOPHTHORA*-SENSITIVE TAXA.

R. Wills, A. Conacher, A. Chapman, C. Robinson, M. Grant & G. Behn

OBJECTIVE

To provide a reliable, graphical decision-support system for monitoring and controlling the spread of dieback disease.

The project has three elements:

1. assemble, verify and automate access to appropriate data sets;
2. maintain, update and interrogate data sets to answer basic management questions;
3. develop a model with predictive capabilities and test validity of predictions.

OVERVIEW

This project uses a Geographic Information System (GIS) to tie together relevant spatial information about *Phytophthora* to provide both managers and researchers with a means of easily answering questions that were previously impossible or too time-consuming to attempt. The project currently targets the Two People's Bay-Mt Manypeaks area (Fig. 1) in CALM's Albany District. This area is significant because of a long history of *Phytophthora* impact and a high conservation value due to the large representation of susceptible plant species, the presence of 22 plant taxa on CALM's Priority list, including 7 Declared Rare Flora (DRF) and four of which are highly susceptible to *Phytophthora cinnamomi* (*Adenanthos cunninghammi*, *Andersonia* sp (TPB) G Keighery 8229, *Banksia brownii*, and *Banksia verticillata*), vegetation associations of special conservation interest, as well as the presence of a number of endangered animal species including the Noisy Scrub-bird, the Western Bristlebird, the Western Whipbird, and the recently rediscovered Gilbert's Potoroo.

For example, in the 1994 Annual Report we demonstrated how the GIS, based on dieback distributions derived from classified satellite imagery resulting from a joint project by CALM and CSIRO's Division of Mathematics and Statistics, could be used to easily quantify the area of infection, identify what landforms were severely affected by dieback, and quickly identify areas of uninfected high hazard landform, etc.

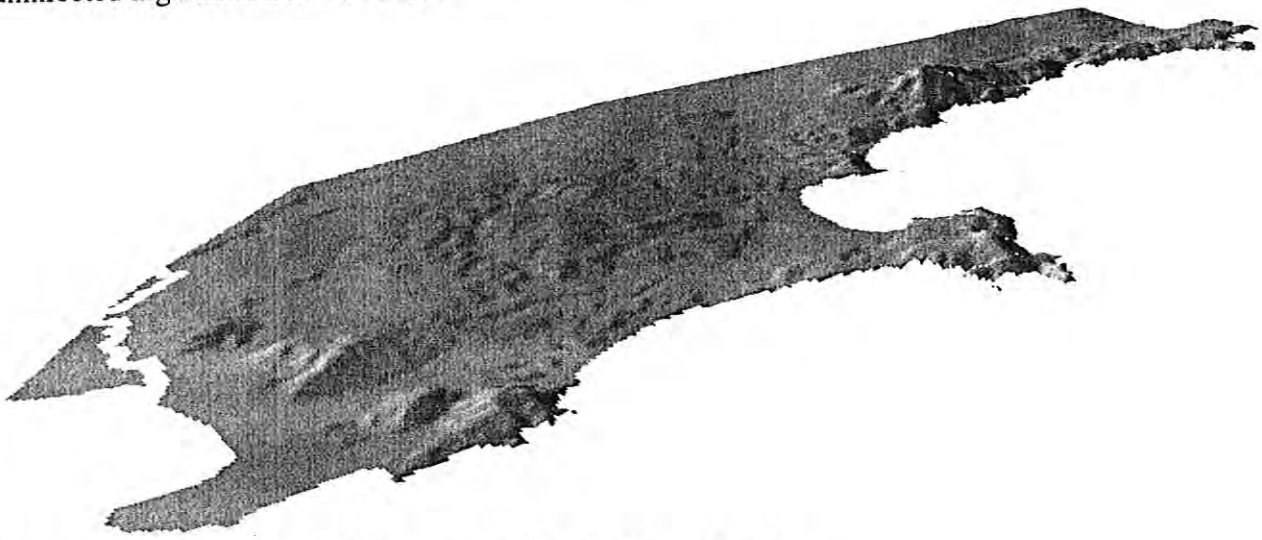

Fig. 1. A view of the digital elevation model for the study area.

12 MONTH *PRECIS*

Work since March 1994 has focused on: validating and improving available data, improving access to available corporate databases and obtaining additional data sets, the development and refinement of the predictive model, the design of a user interface, and seeking out and establishing links with other GIS-based projects both locally and interstate with common objectives and similar data needs.

Data

Data sets which are currently available include cadastre, roads, contour, elevation, slope, aspect, hydrology, dieback (based solely on classified satellite imagery), landform, vegetation, and species distribution based on data accessed from WA Herbarium voucher information. For development purposes, the Herbarium datasets are limited to include only the Proteaceae, Myrtaceae and Cyperaceae. However, it is envisaged that, once dynamic links are established between the Herbarium database and the GIS interface, information on any plant taxon should be accessible.

Validation

The main impediment to further progress in this project is now data quality, particularly data on the distribution and spread of *Phytophthora cinnamomi*. Field observations supported by sampling were used to assess the accuracy of the current dieback map, derived from the classification of two co-registered Landsat images from February and April 1991. Sampling for pathogens was conducted at healthy sites as well as those interpreted as infected to assess the potential for distribution of the pathogen without disease expression. Field work started in December and further work will be carried out in the next four months.

Preliminary results.

Field observations conducted at 85 sites have been assessed, with 71 soil and plant tissue samples taken from 62 locations for cultural detection of *Phytophthora cinnamomi* or other known pathogens (Table 1). A total of 40 of the 43 sites believed infected based on field observations were sampled for the fungus, 33 samples of which yielded positive results for *Phytophthora cinnamomi*. An as yet unidentified species of *Phytophthora* (but not *cinnamomi*) was recovered in one sample, and the native pathogen *Armillaria luteobubulina* was recovered from two other samples. Six out of eight locations believed uninfected (based on field observations) were sampled for the presence of the fungus - one sample yielded a positive recovery of *Phytophthora cinnamomi* from soil baiting. This highlights the uncertainty which can accompany the mapping of the distribution of a pathogen based on assumptions on the presence or absence of disease symptoms.

Table 1. Interpreted dieback status and results of sampling for the presence of pathogens at 85 sites in the Two Peoples' Bay area.

Field interpretation of infection	Number of sites	Number Sampled*	*P. cinnamomi* recovered	(Other pathogens recovered)
Yes	47	44 (+7)	29 (+ 1)	(1 *Armillaria*)
Maybe	5	3	1	
Uninterpretable	8	4 (+1)	1	(1 *Armillaria*)
No	25	11 (+1)	1	(1 *Phytophthora* sp.)
Total	85	62 (+9)	32 (+1)	

* includes duplicate samples at 9 sites

When results of field interpretation of dieback status are compared with those derived from the classification of satellite imagery, some important differences can be found (Fig.2, Table 2.) While the classification yielded errors for both dieback-presence and dieback-absence, it appears the classification was more likely to yield false negatives (no dieback when it is actually present at 45% of sites) than false positives (classified as dieback when it was absent at 24% of sites). Thus, the current classification gives an underestimate of dieback impact.

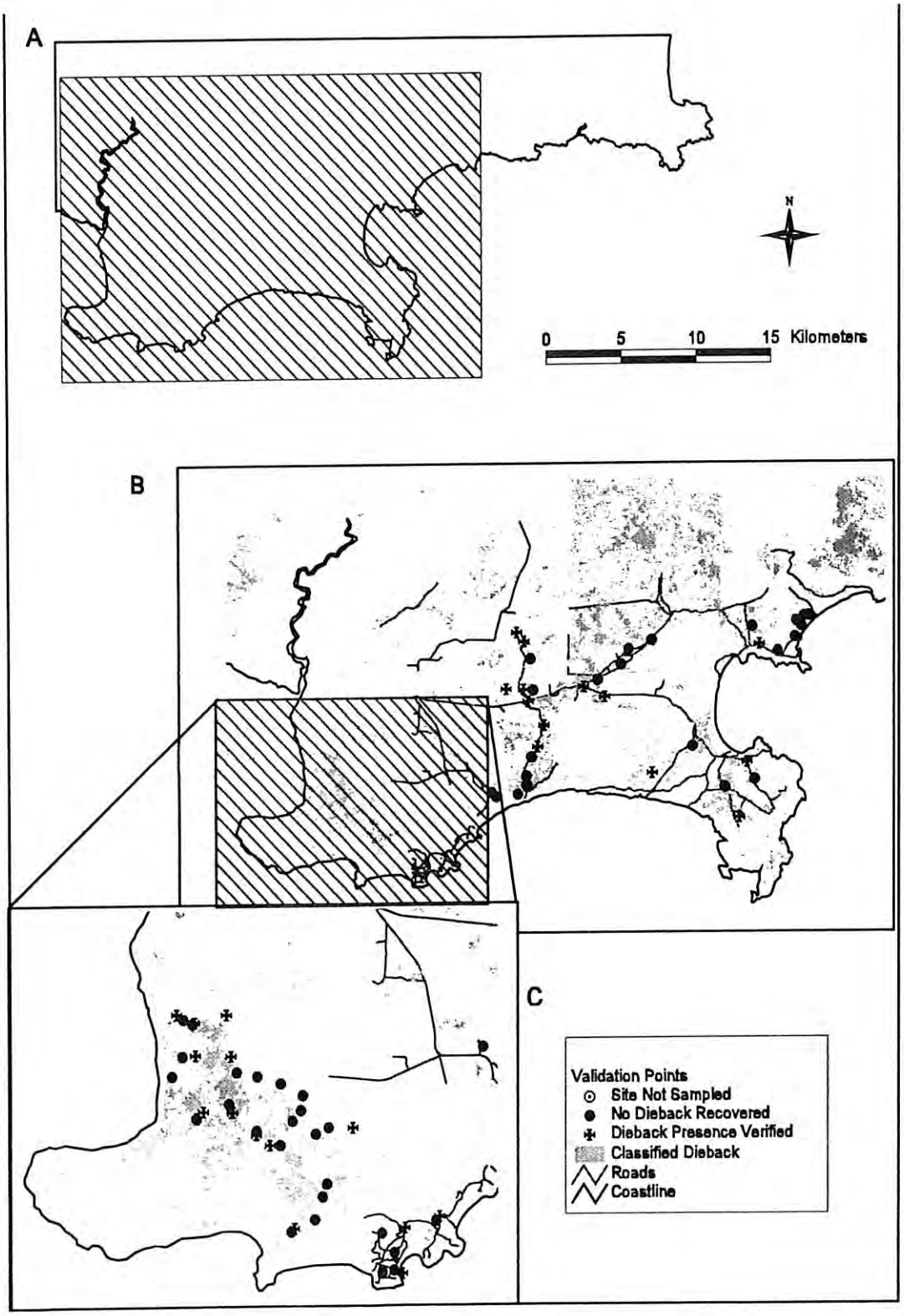

Fig 2. Field sites associated with dieback, as mapped from satellite imagery, from the Two Peoples' Bay.

Table 2. Classified dieback status based on satellite imagery in relation to interpreted dieback status of 85 sites in the Two Peoples' Bay area.

Field interpretation	Total	Classified yes	Classified no
Yes	47	26	21
(Yes - active infection)	(10)	(5)	(5)
(Yes - low impact)	(5)	(2)	(3)
(Yes - old infection)	(30)	(19)	(11)
(Yes - spot)	(2)	(0)	(2)
Maybe	5	1	4
Uninterpretable	8	6	2
No	25	6	19
Total	85	39	46

Observations suggest that areas misclassified as dieback absent (false negative) occur in areas of *Agonis* woodland, broad sedge flats, areas of dense canopy, and in the case of very old infections. Areas misclassified as dieback present (false positive) occurred on the slopes of Mt Gardner, in frequently-burnt areas, in dunes, and granite outcrops (apparently due the effect of topography or substrate altering reflectance).

This work highlights the current dieback classification based on two co-registered Landsat images from February and April 1991 does not adequately define dieback distribution for the purposes of mapping and modelling. A collaborative project with the CSIRO has commenced to determine if improved identification of dieback impact can be derived from remote sensed imagery. Additional analysis of imagery from a greater range of dates (1984-1994) combined with the stratification and masking of those images using data on landform, and layers derived from a Digital Elevation Model (DEM), will greatly improve image classification for the identification of dieback impact.

Work to examine historical information obtained from aerial photography of selected sites from different landforms (scheduled for April) will improve knowledge of the patterns of disease development which will assist greatly in future refinements of the dieback model (see below).

Data linkages

The implementation of dynamic linkages to Herbarium databases was delayed due to the late delivery of Texpress, a SQL-compliant upgrade of the Herbarium's existing RDBMS. However, the arrival of the software and the appointment of a consultant should provide the desired connectivity. The appointed consultant will: integrate existing Texpress databases at the Western Australian Herbarium with Arc/Info GIS and other Departmental databases, beginning with the integration of the *Phytophthora*-GIS project databases and other databases, including the seed storage data sets for the Threatened Flora Seed Centre collection (Project 5); write APIs providing cross-platform access via SQL links to Texpress and Oracle databases; and write ODBC drivers suitable for cross-platform access to Texpress.

The potential for linkages with other projects aimed at facilitating the presentation of information to researchers and managers is being examined, with developments proceeding in concert with other database access initiatives at the Western Australian Herbarium. For example, links with the Herbarium's DATMAP project, a system to allow untrained users to make simple spatial queries that will provide species distribution maps. Additional links are now being developed with projects examining the biology and management of endangered animals such as the Bristlebird and Noisy Scrubbird (Dr Allan Burbidge), the Gilbert's potoroo (Dr Tony Start) (last collected in 1869) in the

Two Peoples' Bay area, and collections-based spatial data analysis with scientists at the WA Museum.

User interface

The objective here is to provide a tool for people unskilled in the operation of Arc/Info that will make data accessible to managers, so that managers can obtain answers to the above questions for specific areas themselves. Interface development will take a two-tiered approach by incorporating ArcView 2 in addition to development within Arc/Info.

In its present form, the interface on Arc/Info provides simple menu-based access to a range of data sets which may then be examined individually or overlayed in any pattern required by the user. Data sets which are currently available include cadastre, roads, contour, elevation, slope, aspect, hydrology, dieback, landform, vegetation, and species distributions. Landsat imagery of the area may also be viewed.

The interface also allows the user to define areas on screen and obtain other information such as the distance between two user-defined points, the area of a selected location and so forth. Finally, the user can add legends so that map output of the interrogation session may be obtained if required.

The interface will continue to be enhanced by further discussion with field managers and by additional assessment of systems developed by other organisations. The use of ArcView 2 as the basis of information presentation is now being investigated to provide PC-based access to data rather than requiring a work station base. ArcView 2 provides a more cost-effective means of accessing native Arc/Info data files, and its relatively simple customisation tools will allow the generation of interfaces closely meeting user requirements. Use of ArcView 2 also provides the opportunity to provide smaller (local) data sets on portable computers for use in the field. However, while ArcView 2 can display model output, it cannot provide access to the modelling capabilities of Arc/Info.

Communications with a number of organisations have been initiated to examine ways in which GIS databases have been used for data presentation and environmental modelling - these include the Western Australian Department of Planning and Urban Development (DPUD); Alcoa Australia; ANU; CSIRO Divisions of: Water Resources, Wildlife & Ecology, Soils, and Mathematics and Statistics; and NRIC. Development of a targeted user-interface for GIS at DPUD (MacDuff 1995) will provide a valuable guide for the further refinement and implementation of GIS in this project. Discussions have been initiated with district and regional managers from the Department of Conservation and Land Management to assess their requirements, and their suggestions will be incorporated by September 1995.

Modelling

The dieback model provides predictions of the spread of *Phytophthora cinnamomi* based on hydrogeomorphic characteristics, and the susceptibility of vegetation and soils (Fig 3). The model can be conceptualised as a broad scale model which utilises available data to provide an indication of possible dieback threats (Fig. 4). The model relies on LANDSAT imagery (described above) to identify sources of infection, from which the algorithm predicts the direction and rate of spread. Hydrological modelling techniques have been facilitated through the raster-based GRID module of Arc/Info which provides the ability to combine numerous 'surfaces' within the same spatial context. The factors affecting dieback at higher resolutions require detailed knowledge of micro-hydrogeological processes and cannot be addressed using existing data; investigations to improve the resolution of available data (described above) is continuing. Validation of model output has high priority and will be undertaken in the continuation of field studies described earlier. Future developments will incorporate linear dispersal and Gaussian dispersal algorithms to assist in the prediction of dieback spread along roads and tracks.

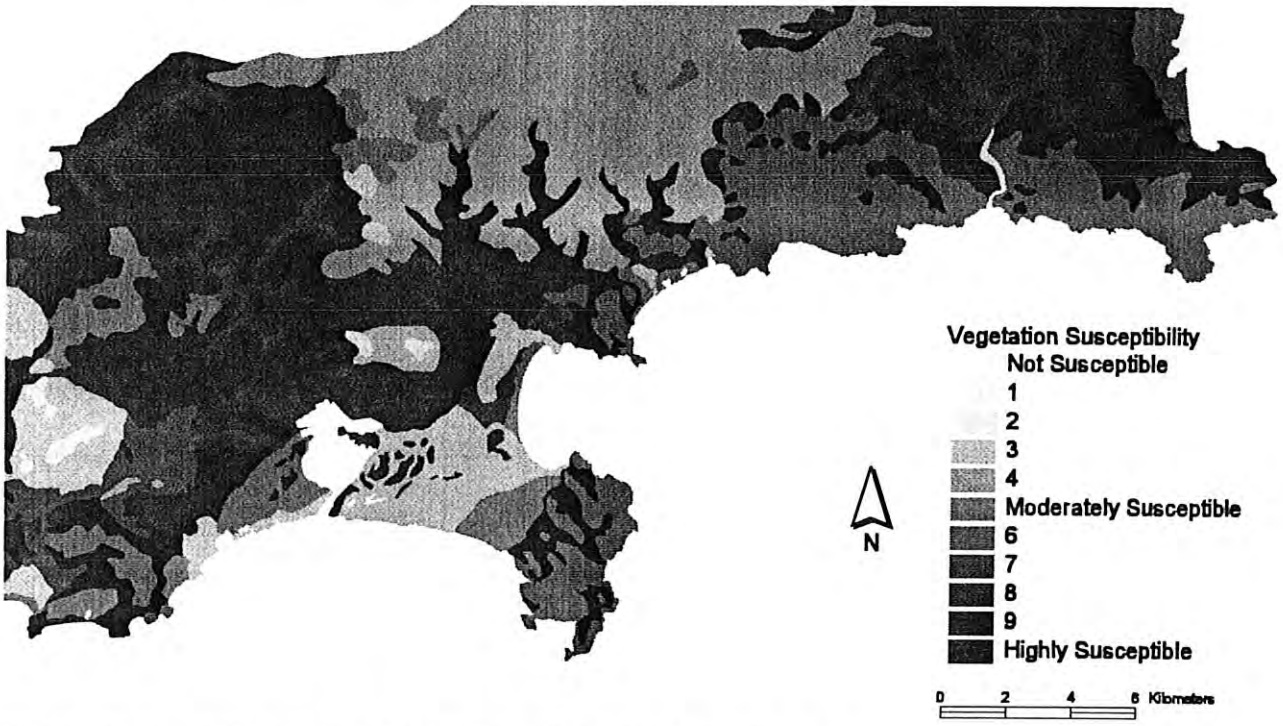

Fig. 3. Susceptibility of vegetation by landform for the study area.

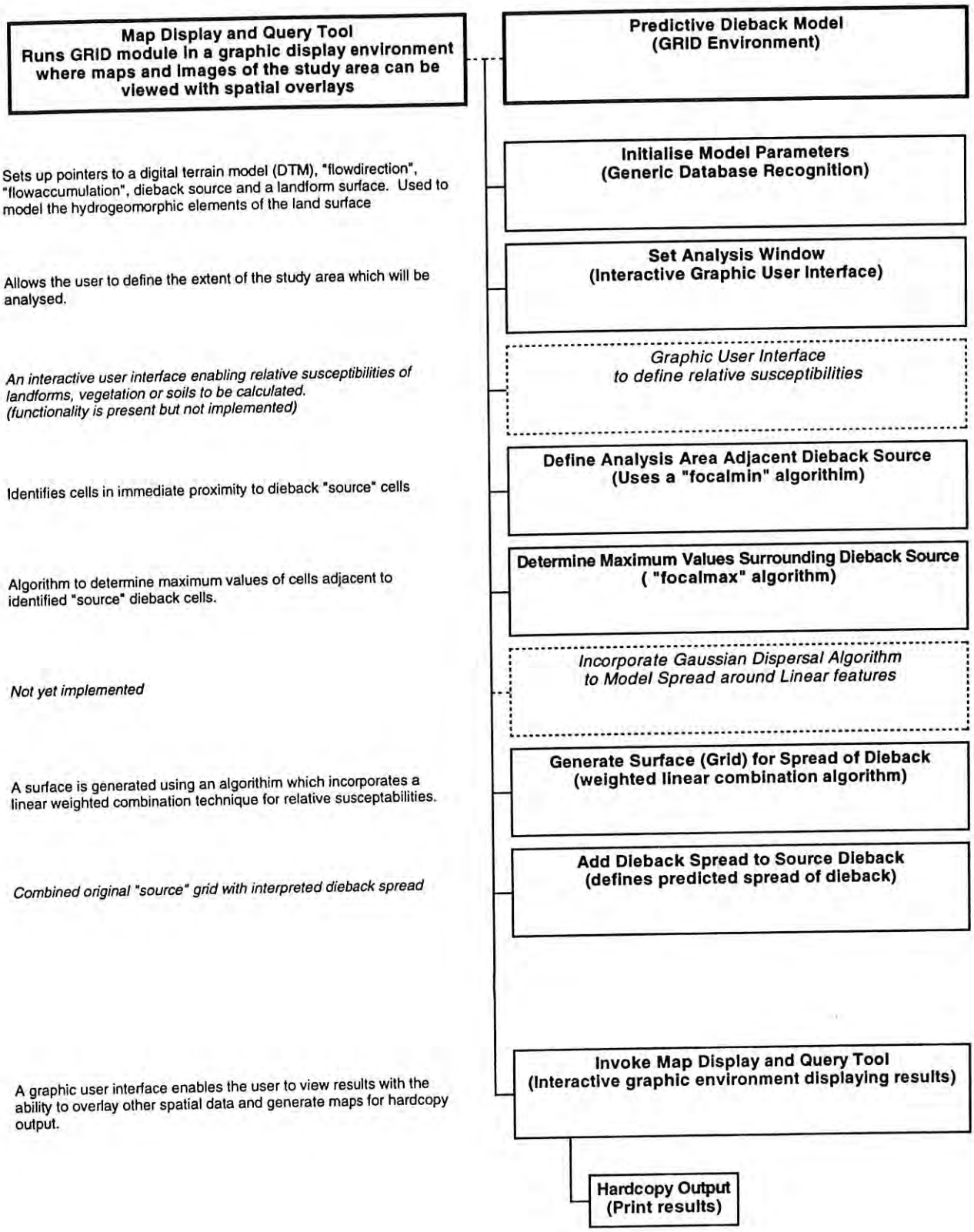

Fig. 4. Flow chart outlining the interface and model.

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Results from these and associated studies are now appearing, with due acknowledgment to ANCA, in the public forum.

PUBLICATIONS

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Wills R. T.; Conacher A. J.; Chapman A. R. & Behn G. (1994) Developing a GIS-model to aid in the management of *Phytophthora cinnamomi*. In: *Ecological Society of Australia 1994 Open Forum and Symposium Conference*. p 90 Ecological Society of Australia, Canberra, Australian Capital Territory.

Associated studies

Khangura R.; Hardy G. E. St. J. & Wills R. T. (1994) Fungi associated with cankers in Western Australian plant communities. In: *Handbook of the Symposium on Plant Diseases In Ecosystems: threats and impacts in south-western Australia.* (eds R. T. Wills & W. A. Cowling) p 26 Royal Society of Western Australia and the Ecological Society of Australia, Perth Western Australia.

Murray D. I. L.; Wills R. T. & Hardy G. E. St. J. (1994) Cankers of native plant species from the south-west of Western Australia. In: *Handbook of the Symposium on Plant Diseases In Ecosystems: threats and impacts in south-western Australia.* (eds R. T. Wills & W. A. Cowling) p 32 Royal Society of Western Australia and the Ecological Society of Australia, Perth Western Australia.

PROJECT 4

ANNUAL REPORT

The control and management of *Phytophthora megasperma* in the native plant communities of s-w Australia

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This report covers the period February 27, 1994 to February 26, 1995.

TABLE OF CONTENTS

ABSTRACT

Phytophthora megasperma is an active plant pathogen in National Parks directly to the north of Perth, extending up to Eneabba, and along the south coast in the Fitzgerald River National Park (FRNP). Where active, *P. megasperma* has high impact on species contributing to habitat structure, e.g. *Banksia attenuata* in the northern sandplain and *B. baxteri* and *B. attenuata* on the south coast.

Twenty-six s-w Australian isolates of *P. megasperma* were screened at three different phosphonate concentrations *in vitro*. The radial growth response varied greatly, with three categories of sensitivity being designated; 1) very sensitive, 2) mildly sensitive, and 3) broadly immune. The calculated ED_{50} value for mycelial inhibition of 482.5 μm/ml was considered to be greater than any previously reported. An aerial spray trial of phosphonate was carried out in two diseased sites in the FRNP: 1) at the foot of East Mount Barren and 2) a gravel pit along Hamersley Drive. After 16 months, there was no significant difference between plant mortality in phosphonate treated and untreated plants. Significantly, all dead plant species belonged to the family Proteaceae. No analyses of tissue levels of phosphonate in aerial plant parts has been carried out to date. These analyses will be carried out over the next three months, and then we will be in a position to identify if phosphonate was taken-up by the plants, and interpret the plant mortality data.

Isozyme analysis of mycelium was carried out on 88 *P. megasperma* isolates using cellulose acetate gel electrophoresis. From the isozyme analysis, 12 putative loci were identified, and twelve electromorphs (i.e. multi-locus genotypes) characterised. The majority of the isolates (approx. 76%) belonged to two electromorphs found only in s-w Australia. Of the six overseas *isotypes*: 1) one was represented with eight s-w Australian homologues, 2) one had a single homologue from South Australia, while 3) the remaining four *isotypes*; were monotypic. No correlation was observed between the morphological categorisation (based upon oogonium diameter) and the allozyme-based classification. The completion of the isozyme analysis necessitates the running of two newly identified electromorphs from overseas. This will be completed over the next three months, and then we will be in a position to interpret the significance of our obtained genetic distances.

Representatives of each of the two south coastal electromorphs were selected and screened for variability in forming lesions in 5 year old stems of *B. baxteri* in an infested site in the FRNP. After three and six weeks, stems were retrieved from the field, and lesion extension assessed. All isolates induced lesions in the stems of *B. baxteri*. Isolates belonging to electromorph 'I' (isolated from the quartzite marine plain soils) proved to be the most virulent of the two south coastal electromorphs screened.

INTRODUCTION AND AIM

Phytophthora megasperma is the most common *Phytophthora* species recovered from dieback affected vegetation of the northern sandplain (Hart *et al.* 1991). The infections extend over a range of approximately 160 km: commencing in Moore River National Park, and extending through areas adjacent to Badgingarra and Nambung National Park to just north of Eneabba (CALM 1990). *P. megasperma* has also been identified as an active pathogen in the area around East Mount Barren within the Fitzgerald River National Park (FRNP) and around the Hopetoun area to the east of the FRNP (CALM 1991). It has now become apparent that *P. megasperma* is active in the western side of the FRNP, e.g. the Point Ann Area, West Mount Barren and Quaalup. In addition, *P. megasperma* has also been recovered from the Esperance area and within Cape Arid National Park to the east of Esperance. Because of its wide geographic distribution, and impact upon geographically restricted flora, it is critical that work on this pathogen continues to determine its occurrence, behaviour and avenues of control. This report details: 1) the results of a phosphonate spray trial to retard the damage caused by the disease; 2) the isozymic variability of the pathogen; and 3) the pathogenicity of *P. megasperma* from the FRNP.

1 CONTROL OF *P. MEGASPERMA*

1.2 Efficacy of aerial applications of phosphonate to retard the damage caused by *P. megasperma* in the FRNP

To date, phosphonate has proven to be the most efficacious fungistatic agent in the control of the dieback disease caused by *P. cinnamomi* in native s-w Australia (Shearer and Fairman 1991). From our previous report, we screened 26 s-w Australian isolates of *P. megasperma* at three different phosphonate concentrations. Statistically significant differences were identified between all of the isolates (ANOVA: F-value = 544.56, p-value = 0.0001). We identified three broad responses to amended phosphonate, with respect to growth rate; 1) very sensitive, 2) mildly sensitive, and 3) broadly immune. In general, the growth rate of individual isolates decreased with increasing phosphonate concentration (Fig. 1.2.1). While 19 isolates demonstrated sensitivity, two isolates actually grew faster at the higher phosphonate levels, e.g. Isolates 29 and 30 (Fig. 1.2.1). The ED_{50} value was calculated to be 482.5 μm/ml: which was much greater than any previously documented concentration. This suggested that our WA *P. megasperma* isolates were less sensitive to phosphonate than other agriculturally-retrieved representatives of *P. megasperma*. Indeed, Dercks and Buchenauer (1987), identified *P. megasperma* as being the most insensitive of four *Phytophthora* species screened *in vitro* to aluminium ethyl phosphite. This apparent insensitivity was attributed to two additional phospholipid membrane components being present in *P. megasperma*.

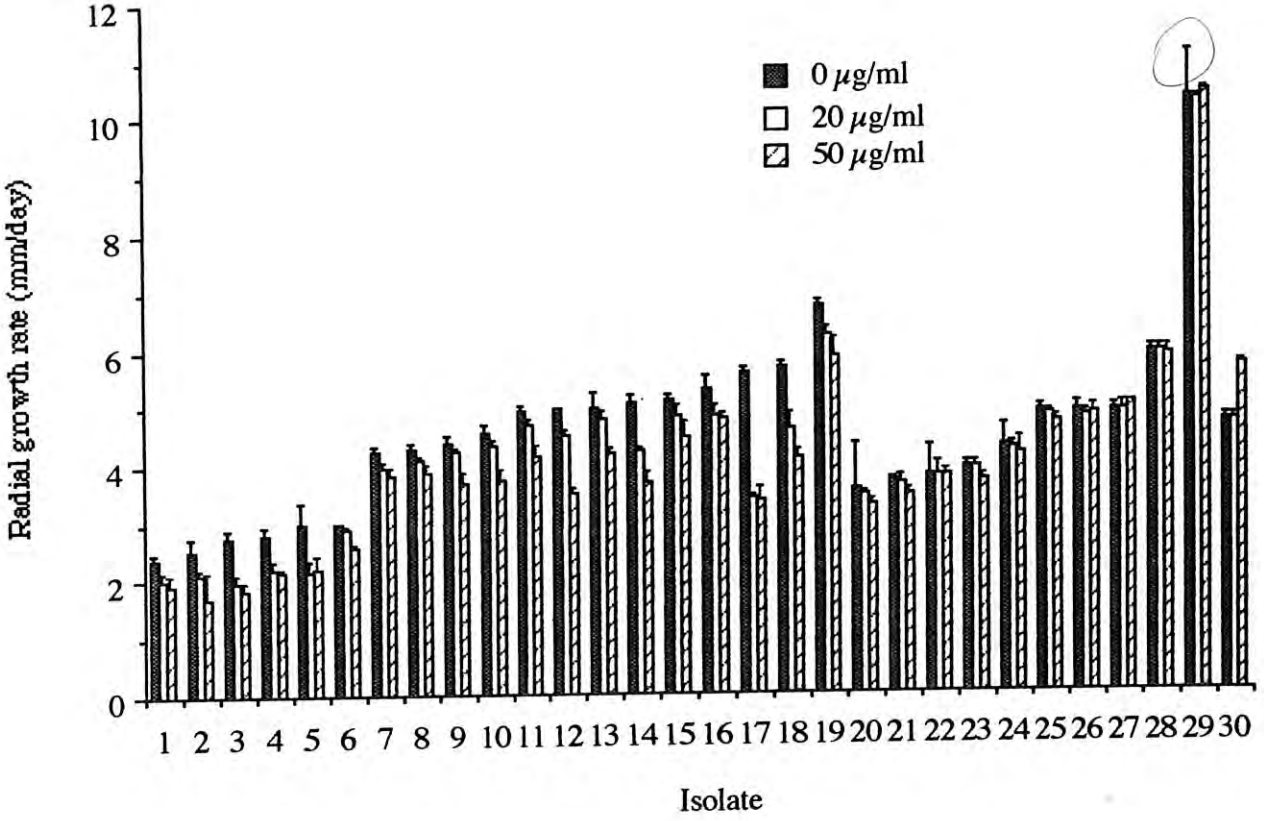


Fig. 1.2.1 The radial growth rate response of thirty s-w Australian isolates of *P. megasperma* to three levels of phosphonate; 0 μg/ml, 20 μg/ml and 50 μg/ml *in vitro*.

In our previous report, we also presented the design and preliminary results of an aerial spray trial carried out in two diseased sites in the FRNP, viz., at the foot of East Mount Barren (EMB) and a gravel pit along Hamersley Drive (GP). The spray trial was commenced 27.10.93. Plants were individually tagged and assessed for disease expression prior to spraying. We then sprayed the plants and monitored the change in disease symptoms in plants treated with two 0.3% doses of phosphonate, and a set of control test-plants sprayed with water. Aerial plants parts of *Dryandra cuneata* were sampled immediately after spraying to determine phosphonate take-up. We now present summary data of plant mortality 16 months after the spray treatment. If the phosphonate treatment was effective, then plant mortality should be lowered in the phosphonate treated plots.

For both sites, there was no significant difference between the number of mortalities in phosphonate treated and control treated plots (Table 1.2.1).

Table 1.2.1 Chi-squared analysis of number of dead and living plants after being aerially spray treated with phosphonate v. control

N.B. ^{A} = Accept null hypothesis.

EMB

	Dead	Alive
+ Phos	9	351
Control	8	352

$\chi^2 = 0.066$, $P = 0.8061^{A}$

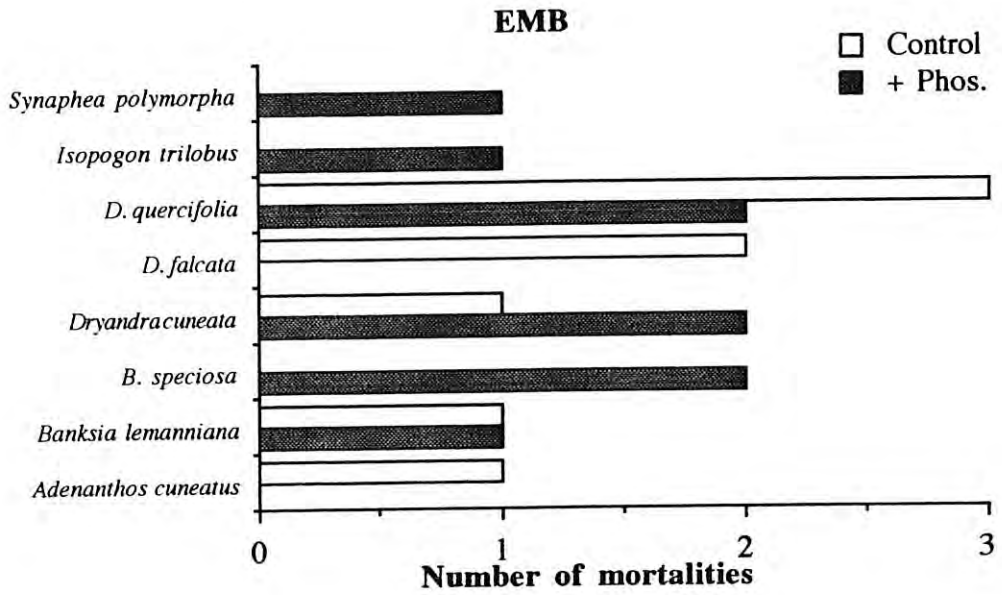
GP

	Dead	Alive
+ Phos	4	356
Control	4	356

$\chi^2 = 0$, $P = 1^{A}$

The plant species which perished over the 16 month period are depicted in the bar graph (Fig. 1.2.2). We retrieved *P. megasperma* from diseased, necrotic roots of dead plant species at both sites. Significantly, all dead plant species belonged to the family Proteaceae.

At this stage, we cannot interpret the mortality data in sprayed v. control-sprayed plants. This is because no leaf-tissue analyses of phosphonate levels have been carried out. Samples have been taken from the spray trial immediately after the spraying, and 15 months after its commencement. These samples will be assayed over the next three months, the results of which are prerequisite for any interpretation of our mortality results.



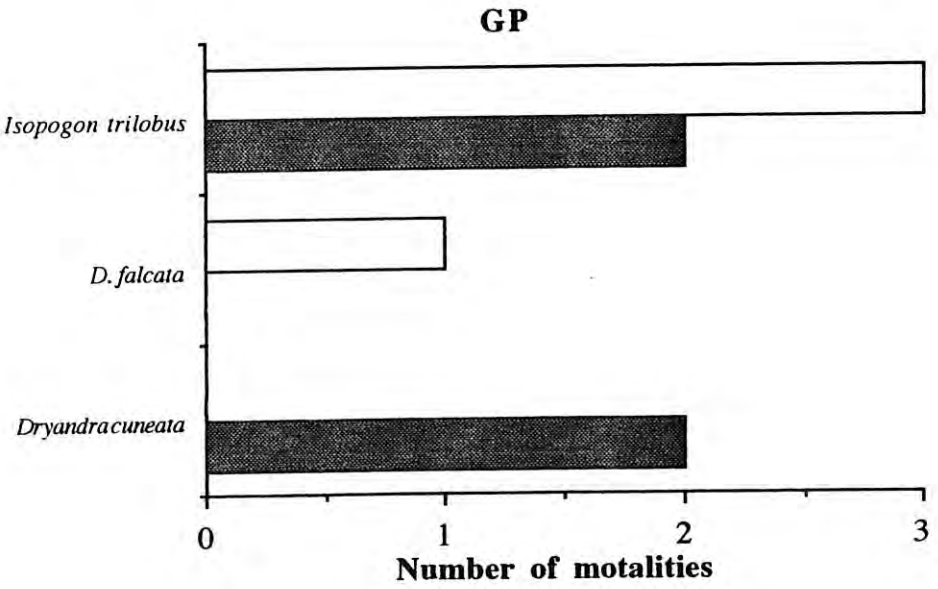


Fig. 1.2.2 Bar graph depicting the number of mortalities of individual species in two sites (EMB and GP) and with two treatments (+ Phos. = sprayed with phosphonate and control = no phosphonate).

2 BIOLOGY AND PATHOGENICITY OF *P. MEGASPERMA*

2.2 Variability of *P. megasperma*

2.2.1 Isozyme variation of P. megasperma from s-w Australia

Phytophthora megasperma Drechs. has been described as an extremely diverse and variable plant pathogen (Hansen and Hamm 1983). Several attempts have been made to define subgroups on the basis of morphology, pathogenicity, and host plant species. Stamps *et al.* (1990), revised the original key of Waterhouse (1963) and proposed the following classification for *P. megasperma* subspecies according to morphometrics and cardinal temperature for colony growth: 1) those with large oogonia (mean size 40-58 μm), moderate growth rate (6-7 mm/day at 25°C) and maximum temperature for growth at approx. 30°C are classified as *P. megasperma* var. *megasperma*; and 2) those with smaller oogonia (mean $\leq$40 μm), slow growth rates (3-5 mm/day at 25°C), and higher cardinal temperatures with a maximum temperature at approx. 34-37°C are classified as *P. megasperma* var. *sojae*.

As knowledge of non-morphological characters has accumulated, the concept of Waterhouse (1963) and Stamps *et al.* (1990) of two *P. megasperma* varieties based on oogonial size has been increasingly challenged. From our previous work (Bellgard *et al.* 1994), we identified 12 morphologically distinct groups on the basis of oogonium diameter. Six *morphotypes* have been described previously (Hansen *et al.* 1986), while, the remaining six *morphotypes* had oogonium diameters smaller than any of the previously described taxa. We undertook isozyme analysis (using cellulose acetate plate electrophoresis of mycelium) of 79 *P. megasperma* isolates from s-w Australia, three from South Australia, and six isolates from overseas which represent the six putative taxa within the *P. megasperma* species complex (Table 2.2.1.1). Genetic distances were determined according to Rogers (1972), and unweighted pair groupings with arithmetic averaging (UPGMA) phenograms were constructed using the computer program BIOSYS-1 (Swofford and Selanger 1981).

On each resulting zymogram, each allozyme band was given a designation relative to the fastest anodally moving band. In this way multi-locus genotypes (i.e. electromorphs) for each isolate were built. Twelve putative loci were identified, and 12 discrete electromorphs were characterised among the *P. megasperma* isolates retrieved from the s-w Australia (Table 2.2.1.2). The majority of the isolates screened (approx. 70%) belonged to electromorph 'I' (Table 2.2.1.1). Electromorph 'II' was the next most represented: comprising 6% of the isolates (Table 2.2.1.1). Six of the electromorphs were monotypic, i.e. had only a single representative (electromorphs; 'III', 'VI', 'VIII', 'IX', 'X', 'XI'). Of the six reference *isotypes*, P471 (i.e. 'VII') was the best represented with eight s-w Australian homologues.

Table 2.2.1.1 Origin and characteristics of the *P. megasperma* isolates used in the isozyme study

Isolate	Isolated from....	Location	Growth rate (mm/day)[1]	Oogonium diam. (±s.e.) (μm)[2]	Sporangium length (±s.e.) (μm)[3]	Sporangium width (±s.e.) (μm)[4]	Sporangium pore (±s.e.) (μm)[5]	Electro-morph
P452*	*Brassica* sp.	Great Britain	2.9	47.5 (0.7)a	60.0 (1.1)	39.3 (0.6)	8.7 (0.2)	IV
TH 7	*Banksia prionotes*	Northern Sandplain, WA	6.0	46.6 (0.6)a	39.4 (0.3)	25.4 (0.3)	10.2 (0.3)	VII
DP17	*B. attenuata*	Wongonderrah, WA	3.5	45.9 (0.6)a	45.7 (1.2)	33.4 (0.7)	8.5 (0.2)	II
TH 1	soil	Cape Arid N.P., WA	3.5	45.8 (0.3)a	55.5 (0.7)	46.7 (0.5)	11.5 (0.3)	I
DC 3215	*B. occidentalis*	Black Point, WA	5.4	45.5 (0.8)a	44.7 (0.6)	32.4 (0.2)	9.1 (0.2)	I
TH 6	*Leptospermum* sp.	Namming N.R., WA	4.7	44.7 (0.5)a	55.2 (0.5)	36.2 (1.0)	11.7 (0.3)	II
SEB 206	*B. attenuata*	FRNP, WA	4.4	44.5 (0.4)	32.3(0.1)	28.5 (0.4)	9.4 (0.1)	I
DP25	*B. attenuata*	Wongonderrah, WA	3.8	43.9 (0.5)b	45.1 (0.6)	32.2 (0.4)	9.3 (0.2)	II
P450*	Clover	Mississippi, USA	2.5	43.6 (0.6)b	46.1 (0.8)	28.5 (0.7)	8.6 (0.2)	III
DC27	*B. attenuata*	Cervantes Rd, WA	4.9	42.6 (0.6)c	42.5 (0.3)	32.0 (0.8)	8.9 (0.2)	I
HSA 1157	*Hakea* sp.	Eneabba, WA	5.1	42.5 (1.0)c	46.9 (1.4)	34.4 (1.0)	9.9 (0.3)	I
DP26	*B. attenuata*	Brand H'way, WA	4.8	42.4 (0.8)c	48.9 (0.7)	33.1 (0.9)	14.9 (0.3)	I
P471*	Apple	California, USA	5.1	42.1 (0.4)c	53.5 (2.4)	34.3 (1.1)	8.7 (0.2)	VII
DC 1612	soil	Hopetoun, WA	6.5	41.9 (0.9)c	38.7 (0.4)	25.2 (0.3)	9.5 (0.3)	VII
SEB 207	*B. attenuata*	FRNP, WA	3.8	41.5 (0.2)	33.4 (0.8)	27.4 (1.2)	6.9 (0.1)	I
DP28	*B. attenuata*	Bibby Rd, WA	3.3	41.3 (0.5)c	53.1 (1.3)	37.1 (0.7)	9.5 (0.2)	I
DCE 441	*B. attenuata*	Minyolo Brk., WA	3.0	41.2 (0.3)c	63.4 (2.8)	36.9 (1.0)	13.3 (0.3)	I

SEB 210	*D. cuneata*	FRNP, WA	3.6	40.8 (0.1)	45.7 (1.1)	28.9 (0.3)	8.8 (0.2)	I
DP24	*H. prostrata*	Hedges, WA	4.9	40.7 (0.7)c	55.6 (1.2)	43.3 (0.6)	12.5 (0.4)	I
P439*	Douglas-fir	Oregon, USA	6.9	40.3 (0.3)d	53.0 (0.9)	36.8 (0.7)	10.1 (0.2)	IX
HSA 1647	soil	Cataby, WA	4.3	40.3 (0.6)	49.9 (0.3)	32.3 (0.4)	10.0 (0.3)	I
SEB 209	*D. cuneata*	FRNP, WA	4.4	40.2 (0.2)	44.5 (0.9)	28.7 (0.4)	9.4 (0.2)	I
HSA 1800	*B. attenuata*	Cataby, WA	3.6	40.1 (0.1)	44.9 (0.3)	34.3 (0.4)	9.0 (0.3)	I
SEB 201	*D. quercifolia*	FRNP, WA	3.7	39.8 (0.0)	29.4 (1.3)	19.8 (0.5)	5.1 (0.1)	I
SEB 203	*D. tenuifolia*	FRNP, WA	3.8	39.6 (0.1)	33.7(1.2)	25.9 (0.6)	7.9 (0.1)	I
SEB 239	*B. attenuata*	FRNP, WA	3.5	39.3 (0.3)	45.3 (0.5)	31.9 (0.2)	8.7 (0.2)	I
SEB 228	*B. attenuata*	FRNP, WA	3.7	39.1 (0.3)	40.4 (0.3)	32.2 (0.3)	8.5 (0.2)	I
DC 3248	soil	Cape Arid N.P., WA	4.8	39.0 (0.5)e	50.2 (2.2)	41.7 (1.6)	14.4 (0.4)	I
SEB 234	*A. cuneatus*	FRNP, WA	3.8	38.7 (0.5)	42.3 (0.2)	32.3 (0.2)	9.9 (0.3)	I
SEB 235	*D. plumosa*	FRNP, WA	3.6	38.7 (0.1)	40.2 (0.4)	35.5. (0.2)	9.9 (0.5)	I
SEB 236	*B. baxteri*	FRNP, WA	3.8	38.6 (0.4)	47.4 (0.4)	35.2 (0.1)	8.5 (0.2)	I
P445*	Soybean	Wisconsin, USA	2.5	38.6 (0.3)e	33.9 (0.5)	24.5 (0.2)	7.7 (0.1)	XI
SEB 242	*B. baxteri*	FRNP, WA	3.8	38.6 (0.1)	40.0 (0.2)	28.9 (0.3)	8.3 (0.2)	I
SA 01	soil	SADPI	3.9	38.6 (0.2)	41.2 (0.3)	29.5 (0.3)	8.8 (0.2)	VI
SEB 246	*H. varia*	FRNP, WA	3.3	38.5 (0.7)	40.9 (0.3)	28.9 (0.7)	8.9 (0.2)	I
SEB 226	*B. baxteri*	FRNP, WA	4.1	38.1 (0.1)	35.7 (0.3)	27.2 (0.3)	8.2 (0.2)	I
SEB 225	*D. cuneata*	FRNP, WA	3.5	38.0 (0.1)	33.6 (0.2)	27.8 (0.2)	8.7 (0.2)	I
TH 5	*B. attenuata*	Jurien Bay Rd, WA	3.8	37.5 (0.8)f	40.4 (0.9)	40.4 (0.9)	12.7 (0.4)	I
SEB 247	*B. media*	FRNP, WA	3.8	37.4 (0.5)	38.9 (0.6)	32.9 (0.3)	10.4 (0.1)	I
SEB 213	*B. lemanniana*	FRNP, WA	3.6	37.0 (0.4)	34.7 (0.4)	29.9 (0.3)	11.9 (0.4)	I
SEB 224	*B. baxteri*	FRNP, WA	2.5	37.3 (0.1)	45.6 (0.4)	34.6 (0.3)	8.8. (0.2)	I
SEB 233	*D. cuneata*	FRNP, WA	3.8	37.2 (0.1)	42.6 (0.3)	35.5. (0.1)	10.0. (0.0)	I
SEB 252	*D. circioides*	FRNP, WA	3.3	36.9 (0.4)	46.7 (0.3)	34.5 (0.3)	12.0 (0.2)	I

SEB 227	*Adenanthos cuneatus*	FRNP, WA	2.2	36.7 (0.3)	43.5 (0.2)	35.5 (0.5)	9.2 (0.3)	I
P484*	Alfalfa	South Africa	2.4	36.6 (0.5)f	42.0 (0.5)	31.9 (0.3)	8.9 (0.2)	X
SEB 114	*B. baxteri*	FRNP, WA	4.8	36.6 (0.7)f	53.4 (1.6)	41.7 (1.3)	12.9 (0.4)	I
SEB 208	*Conospermum distichum*	FRNP, WA	3.8	36.6 (0.3)	38.9 (0.9)	28.4 (1.1)	10.0 (0.3)	I
SEB 244	*B. gardneri*	FRNP, WA	3.0	36.6 (0.2)	44.9 (0.3)	34.0 (0.3)	10.2 (0.2)	I
SEB 250	*Allocasuarina campestris*	FRNP, WA	6.0	36.4 (0.2)	44.6 (0.7)	35.3 (0.9)	11.0 (0.2)	VII
SEB 251	*Xanthorrhoea platyphylla*	FRNP, WA	5.7	36.4 (0.2)	39.0 (0.2)	32.9 (0.2)	10.0 (0.3)	VII
DP21	*D. sessilis*	Cape Arid N.P., WA	3.8	36.3 (0.8)f	45.5 (1.2)	36.9 (1.1)	10.9 (0.3)	I
SEB 243	*B. media*	FRNP, WA	3.5	36.3 (0.1)	44.5 (0.3)	32.9 (0.2)	11.4 (0.2)	I
SA 02	soil	SADPI	3.9	36.3 (0.3)	39.6 (0.1)	30.9 (0.1)	9.9 (0.2)	V
SEB 115	*B. attenuata*	FRNP, WA	4.9	36.1 (0.8)f	51.8 (1.6)	39.7 (0.9)	15.9 (0.1)	I
SA 00	soil	SADPI	5.4	35.9 (0.4)	43.6 (0.3)	31.9 (0.3)	10.2 (0.4)	IV
HSA 1151	*B. attenuata*	Eneabba, WA	5.0	35.7 (1.1)f	43.9 (1.1)	34.1 (1.0)	8.7 (0.2)	I
TH 8	*B. attenuata*	Dandaragan, WA	5.9	35.5 (0.5)f	39.3 (0.3)	26.1 (0.5)	9.3 (0.3)	I
TH 3	*B. ilicifolia*	Wongonderrah, WA	4.4	35.5 (0.6)f	48.7 (0.2)	39.5 (0.2)	8.2 (0.1)	I
SEB 240	*B. media*	FRNP, WA	3.8	35.4 (0.3)	40.7 (0.3)	34.5 (0.2)	9.3 (0.2)	I
SEB 241	*B. media*	FRNP, WA	4.0	35.0 (0.2)	34.5 (0.3)	28.9 (0.3)	9.0 (0.1)	I
SEB 212	*B. attenuata*	FRNP, WA	3.6	34.9 (0.2)	36.8 (0.3)	30.4 (0.3)	8.8 (0.8)	I
SEB 232	*B. lemanniana*	FRNP, WA	4.4	34.3 (0.1)	31.3 (0.2)	30.7 (0.1)	8.1 (0.2)	I
SEB 237	*B. baxteri*	FRNP, WA	3.0	33.9 (0.2)	40.8 (0.4)	32.4 (0.6)	9.5 (0.2)	I
SEB 205	*D. plumosa*	FRNP, WA	4.7	33.6 (0.4)	28.3 (0.6)	25.8 (0.7)	7.9 (0.0)	I
SEB 216	*D. falcata*	FRNP, WA	3.1	33.5 (0.1)	38.9 (0.4)	34.6 (0.3)	9.9 (0.2)	I
HSA 1158	*Hakea* sp.	Eneabba, WA	4.2	32.9 (0.3)g	50.1 (1.7)	38.0 (0.6)	12.4 (0.3)	I
SEB 221	*B. lemmaniana*	FRNP, WA	3.6	32.2 (0.4)	40.4 (0.7)	33.2 (0.1)	10.0 (0.2)	I

SEB 249	*B. baxteri*	FRNP, WA	3.4	32.1 (0.2)	35.8 (0.3)	32.8 (0.9)	10.0 (0.0)	I
DP20	*B. speciosa*	Cape Arid N.P., WA	4.6	31.9 (0.5)h	39.9 (0.5)	34.7 (0.4)	12.1 (0.3)	I
HSA 1666	soil	Cataby, WA	5.6	31.6 (0.2)	45.3 (0.2)	40.8 (0.3)	12.3 (0.1)	VII
DCE 442	*B. attenuata*	Mullering Brk., WA	5.8	31.4 (1.0)h	51.6 (2.2)	32.9 (1.3)	14.2 (0.4)	I
DCE 177	*P.radiata*	Jarrahwood, WA	5.5	31.3 (0.6)h	45.9 (0.7)	31.7 (0.7)	11.9 (0.3)	I
SEB 248	*D. plumosa*	FRNP, WA	2.8	31.0 (0.9)	46.7 (0.3)	30.8 (0.5)	10.2 (0.1)	I
HSA 1638	soil	Cataby, WA	5.3	30.5 (0.2)	43.6 (0.2)	31.3 (0.4)	11.2 (0.4)	VII
HSA 1648	soil	Cataby, WA	5.3	30.2 (0.2)	47.6 (0.3)	33.3 (0.2)	12.1 (0.2)	VII
HSA 1658	soil	Cataby, WA	5.6	29.9 (0.2)	46.5 (0.4)	31.8 (0.2)	10.5 (0.1)	VII
R3-6 283	*P. radiata*	Jarrahwood, WA	5.2	29.8 (0.7)i	48.8 (1.2)	29.4 (0.7)	8.5 (0.2)	I
HSA 1654	soil	Cataby, WA	3.3	29.3 (0.3)	50.8 (0.3)	30.1 (0.3)	8.6 (0.3)	V
R1-2 283	*P. radiata*	Jarrahwood, WA	5.3	29.2 (0.8)i	51.7 (1.4)	30.9 (0.9)	8.7 (0.2)	I
DP18	*B. attenuata*	Wongonderrah, WA	2.8	28.6 (0.6)j	42.9 (1.0)	35.9 (1.0)	10.9 (0.3)	II
SEB 219	*B. lemanniana*	FRNP, WA	3.6	28.5 (0.2)	45.6 (0.4)	33.5 (0.1)	9.4 (0.2)	I
HSA 1655	soil	Cataby, WA	4.2	27.9 (0.3)	46.7 (0.4)	35.0 (0.2)	9.9 (0.3)	II
HSA 1705	soil	Cataby, WA	3.5	26.8 (0.4)	44.6 (0.4)	34.5 (0.1)	10.4 (0.2)	VIII
SEB 220	*D. quercifolia*	FRNP, WA	3.3	26.7 (0.2)	52.3 (0.3)	33.5 (0.4)	8.4 (0.2)	I
SEB 222	*D. falcata*	FRNP, WA	3.8	26.1 (0.1)k	57.4 (0.3)	34.9 (0.1)	11.4 (0.4)	I
SEB 214	*D. falcata*	FRNP, WA	3.6	25.0 (0.1)	40.3 (0.9)	31.2 (0.3)	9.5 (0.5)	I
DCE 440	*C. triplinervum*	Eneabba, WA	3.7	24.8 (0.3)l	57.2 (1.4)	38.5 (0.6)	9.1 (0.3)	XII
SEB 218	*I. formosus*	FRNP, WA	3.9	24.7 (0.1)	50.8 (0.3)	33.5 (0.4)	9.3 (0.2)	I

N.B. WA = Western Australia, N.P. = National Park, N.R. = Nature Reserve, FRNP = Fitzgerald River N.P., Rd = Road, H'way = Highway, Brk. = Brook, SADPI = South Australian Dept. of Primary Industry.

Locations Wongonderrah = 30.56°S115.37°E, Cape Arid N.P. = 33.83°S/123.00°E, Black Point = 34.46°S/119.41°E, Namming N.R. = 31.21°S/115.68°E, FRNP = 33.91°S/120.01°E, Cervantes Rd = 30.37°S/115.23°E, Eneabba = 30.81°S/115.28°E, Brand H'way = 30.72°S/115.49°E, Hopetoun = 33.91°S/120.13°E, Bibby Rd = 30.45°S/115.28°E, Minyolo Brk. = 30.73°S/115.51°E, Hedges = 32.80°S/116.47°E, Cataby = 30.76°S/115.52°E, Jurien Bay Rd = 30.25°S/115.16°E, Dandaragan = 30.24°S/115.76°E, Mullering Brk. = 30.62°S/115.49°E, Jarrahwood = 33.80°S/115.67°E.

* = Isolate described in Hansen *et al.* (1986).

[1] = Fisher's LSD=0.3 (p=0.05).

[2] = Entries followed by the same letter are not significantly different as indicated by the Scott-Knott procedure (λ=500, p=0.002).

[3] = Fisher's LSD=1.2 (p=0.05).

[4] = Fisher's LSD=0.8 (p=0.05).

[5] = Fisher's LSD=0.3 (p=0.05).

Table 2.2.1.2 List of putative loci and diagnostic allozyme profiles (electromorphs)

TpI	Fum	MpI	HK	IDh	ME	6-PGD-1	6-PGD-2	LDh	PgI	MDh-1	MDh-2	Electro-morph	e.g.
AA	BB	CC	AB	BB	CC	BB	BB	DD	CC	EE	BB	I	SEB 236
AA	BB	CC	AB	BB	CC	BB	BB	DD	BB	FF	AA	II	TH 6
CC	AA	CC	BB	CC	AA	BB	BB	AA	BB	BB	BB	III	P 450
DD	AA	DD	DD	AC	CC	BB	BB	CC	BD	DD	DD	IV	P 452
DD	AA	DE	DD	CC	CC	BB	BB	CC	DD	DD	DD	V	SA 02, HSA 1654
DD	AA	EE	DD	CC	CC	BB	BB	CC	DD	DD	DD	VI	SA 01
FF	AA	FF	DD	CC	DD	BB	BB	CC	DD	CC	DD	VII	P 471, SEB 250
FF	CC	DD	CC	EE	CC	BB	BB	BB	CC	EE	CC	VIII	HSA 1705
EE	AA	BB	BB	DD	BB	AA	AA	CC	CC	DD	CC	IX	P 439
EE	BB	AA	EE	EE	AA	CC	CC	DD	AA	DD	FF	X	P 484
FF	CC	CC	DD	CC	EE	BB	BB	DD	AA	AA	FF	XI	P 445
CC	BB	CC	AB	BB	CC	BB	BB	AA	BB	FF	AA	XII	DCE 440

N.B. TpI = Triphosphate Isomerase, Fum = Fumarase, MpI = Mannose-6-phosphate Isomerase, HK = Hexokinase, IDh = Isocitrate Dehydrogenase, ME = Malic Enzyme, 6-PGD = 6-Phosphogluconate Dehydrogenase, LDh = Lactate Dehydrogenase, PgI = Phosphoglucose Isomerase, and MDh = Malate Dehydrogenase.

P452 (i.e. 'IV') had a single homologue from South Australia, while the remaining four overseas *isotypes* (i.e. 'III', 'IX', 'XI', 'X') were monotypic (Table 2.2.1.1).

Two phenograms were generated from the BIOSYS output. The first (Fig. 2.2.1.1) describes the association among the WA *P. megasperma* isolates. This phenogram dictates a primary dichotomy between electromorphs 'I', 'II', 'XII' and 'VIII' from electromorphs 'V' and 'VII' (Fig. 2.2.1.1). The branch comprising electromorphs 'V' and 'VII' being separated from the others by a genetic distance of 0.42, with 'V' a distance of 0.32 from 'VII' (Fig. 2.2.1.1). Contained in the other branch of the phenogram are electromorphs 'I', 'II', 'XII' and 'VIII' (Fig. 2.2.1.1). 'VIII' was a distance of 0.68 from either 'I', 'II', or 'XII' (Fig. 2.2.1.1). 'II' and 'XII' are a distance of 0.21 from 'I' (Fig. 2.2.1.1).

The second phenogram (Fig. 2.2.1.2) describes the associations between WA isolates versus South Australian and the reference isolates of Hansen *et al.* (1986). The isolate representing *P. medicaginis* (i.e. 'X') was a genetic distance of 0.90 from the rest of the 87 isolates assayed (Fig. 2.2.1.2). In the bulk of the phenogram, 'IX' was separated from the others by a distance of 0.82 (Fig. 2.2.1.2). The remainder of the phenogram contains three of the reference isolates (Hansen *et al.* 1986). Of these three, the isolate representing *P. sojae* (i.e. 'XI'), was the most dissimilar, with a genetic distance of 0.67 (Fig. 2.2.1.2).

The remaining isolates are separated into two broad groups: (i) a clump associated with two of the reference isolates (i.e. 'VII' and 'IV'), and (ii) a group of WA electromorphs 'I', 'II', 'XII' and 'VIII' (Fig. 2.2.1.2). Of group (i), 'VII' was the most dissimilar with a genetic distance of 0.36, while 'IV' was a distance of 0.12 from the remainder (Fig. 2.2.1.2). 'I', 'II', 'XII', and 'VIII' are classed together (a genetic distance of 0.58) in much the same way as that depicted in Fig. 2.2.1.1

Electromorph 'I' comprised approximately 70% of the *P. megasperma* isolates assayed for isozyme variability, and was distinct from any of the overseas isolates. Oogonium diameters within this electromorph ranged between 45.8 μm (isolate TH 1 from Wongonderrah, approx. 150 km north of Perth) and 24.7 μm (isolate SEB 218 from FRNP, approx. 650 km s-e of Perth). Isolates with this allozymic profile were recovered from throughout the range where *P. megasperma* is recovered from s-w Australia.

In a similar way, Electromorph 'II' was not similar to any of the overseas isolates, and comprised 6% of the total number of samples assayed, with oogonium diameters ranging between 45.9 μm (isolate DP 17 from Wongonderrah) and 27.9 μm (isolate HSA 1655 from Cataby, approx. 130 km north of Perth). However, isolates with allozymic profile 'II', were restricted geographically to sites north of Perth, and were not found south of Perth. These

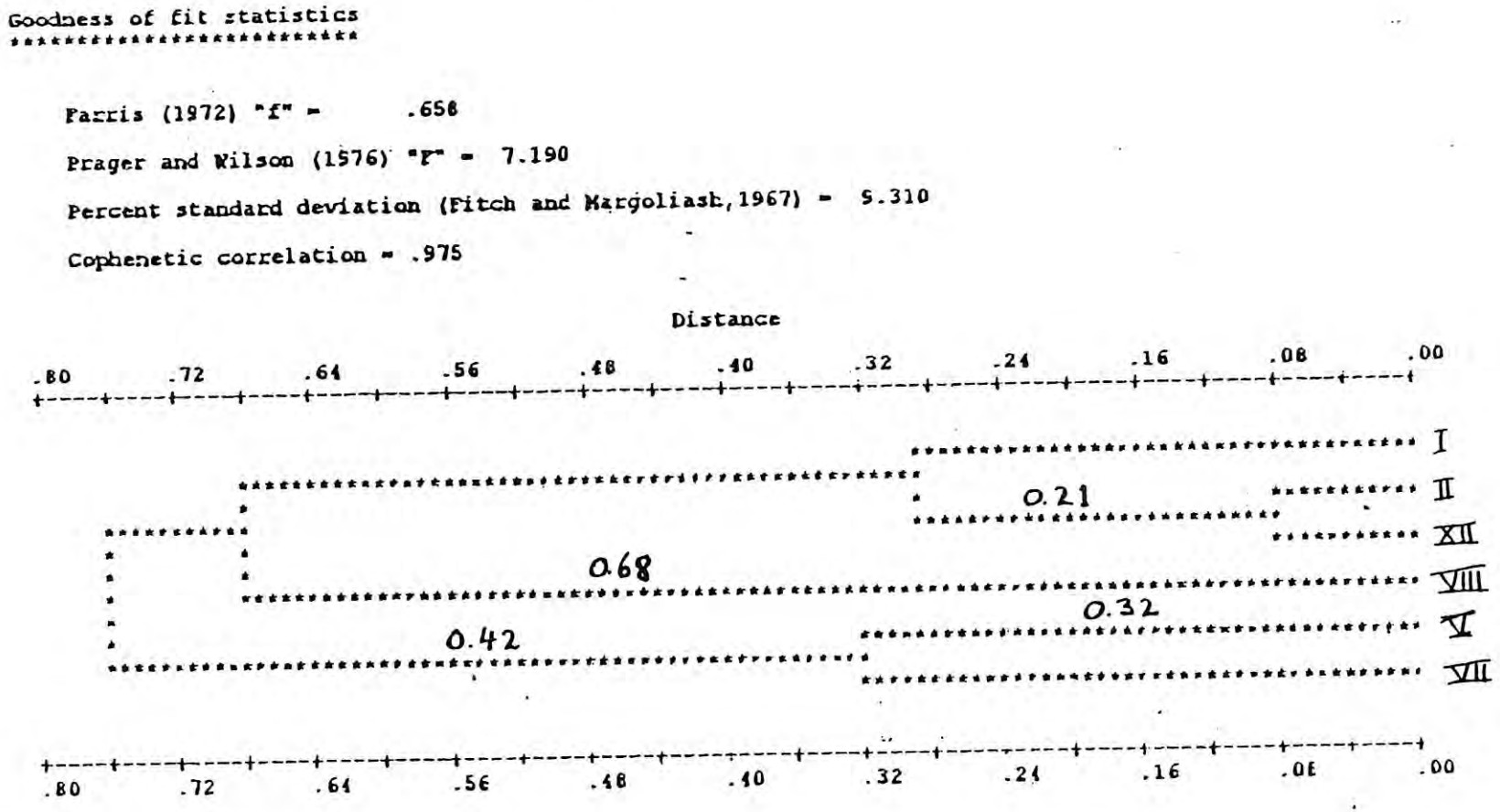


Fig. 2.2.1.1 Phenogram describing the associations among WA isolates of *P. megasperma* (N.B. Roman numerals correspond to the those presented in Table 2.2.1.2).

Goodness of fit statistics

Farris (1972) "f" = 2.047

Prager and Wilson (1976) "P" = 6.970

Percent standard deviation (Pitch and Margoliash, 1967) = 8.871

Cophenetic correlation = .970

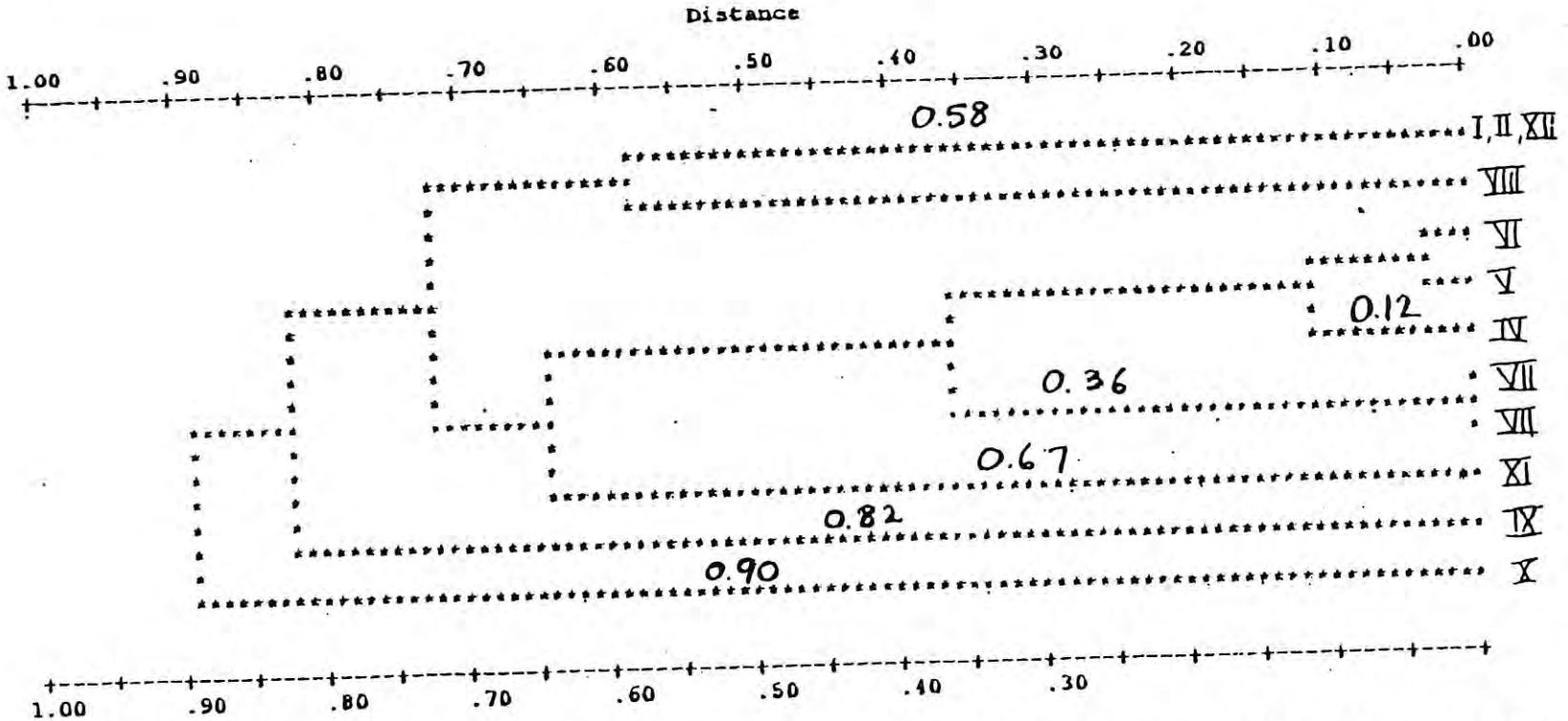


Fig. 2.2.1.2 Phenogram describing the association among WA versus South Australian and reference isolates of Hansen *et al.* (1986). Again, roman numerals correspond to those presented in Table 2.2.1.2.

two electromorphs highlight the local disparity between diagnostic allozymic profile and oogonium diameter, i.e. splitting isolates arbitrarily by oogonium diameter, serves to divide isolate groups that are otherwise isozymically similar.

This apparent disparity between allozyme profile and oogonium diameter is further compounded when we consider the homologues of *isotype* 'VII': one of the overseas isolates, P471. 'VII' had a total of eight s-w Australian homologues. Of the eight, two were recovered from the FRNP. Interestingly, they possess a profile distinct from the other isolates recovered from the FRNP which all possess profile 'I'. DC 1612 possessed an oogonium diameter statistically similar to that of P 471, i.e. approx. 42 μm, while TH7 had an oogonium diameter statistically equivalent to P452 , i.e. *isotype* 'IV'. These two isolates could be classified as classic *P. megasperma* var. *megasperma* on the basis of oogonium diameter alone. The six remaining 'VII' homologues had oogonium diameters less than 37μm, placing them into *P. megasperma* var. *sojae* (as per Stamps *et al.* 1990): while isozymically they are equivalent to 'VII'. This pattern is repeated with P 452 i.e. *isotype* 'IV' (oogonium diam. = 47.5 μm), with its single South Australian homologue SA 00, having a statistically dissimilar oogonium diameter of 35.9 μm.

Kuan and Erwin (1980) argued for a broad concept of the species *P. megasperma*, interpreting oogonial size distribution as a continuum. Due to the range of oogonium diameters observed within each discrete electromorph, we suggest that oogonium morphology is phenotypically plastic even within a discrete genotype. Alternatively, we need not expect correlation between the structural genes characterised and oogonium diameter, as the determination of oogonium diameter may be under the control of an unrelated suite of genes, not necessarily assayed and characterised in our current study.

Electromorph II and XII were only recovered from areas to the north of Perth. Additionally, they were closely associated (genetic distance = 0.08) on the phenogram (Fig. 2.2.1.1). In comparison, electromorph I was recovered from throughout WA. This apparent geographical disjunction in the distribution of the three electromorphs could be an artefact of the sampling procedure. The isolates collected from field-material, were sampled because the plant host displayed dramatic symptoms of infection/decline. In this way, a number of less-virulent strains (e.g. electromorph 'VIII') may not have been sampled because they did not induce dramatic disease symptoms, and hence, the plant would not have been sampled and the isolate not retrieved as frequently.

The completion of the isozyme analysis necessitates the running of two newly identified electromorphs from overseas. This will be completed over the next three months, and then we will be in a position to interpret the significance of our obtained genetic distances.

2.2.2 *Comparative pathogenicity of P. megasperma from s-w Australia*

Greatest impacts of *P. megasperma* in the FRNP are associated with post-fire regenerating (< 5 years old) Proteaceae-Myrtaceae communities (heaths-shrublands-low open woodlands), in sites with impeded drainage. The isolates of *P. megasperma* from infested south coastal sites fall into two electromorph groups. Electromorph 'VII' was recovered from sites on the Archaean Yilgarn Block, while isolates representing electromorph 'I' were recovered from the more recent quartzite marine plains: extending up to 250 km from the present coastline (Aplin and Newbey 1990a). For management purposes, *functional* differences are of significance. Morphological and isozymic analysis have described and defined the variation in *P. megasperma* from the FRNP, and have established a basis upon which functional variability can be interpreted. Representatives of each of the two south coastal electromorphs were selected and screened for variability in pathogenicity in 5 year old stems of *Banksia baxteri* in an infested site in the FRNP.

A gravel pit infected with *P. megasperma* 9.5 km along Point Ann Rd (34°10'36.2"S/119°31'01.6"E) was the site for the *in situ* field experiment. *B. baxteri* (5 year old post-fire regen.) skirts the edge of one of the northern pits, in the form of discontinuous clumps of varying size. Six isolates of *P. megasperma* were screened in this trial (Table 2.2.2.1). All six isolates were isolated from various locations from within the FRNP and are representative of the two electromorphs occurring in the FRNP (Table 2.2.2.1). The experiment was a Randomised Complete Block design. Each stem of *B. baxteri* was inoculated with a single isolate of *P. megasperma*, using a modified method of Smith and Marks (1985). Control stems (within each block) received an uncolonised agar plug. After three and six weeks, stems were retrieved from the field, and lesion extension assessed.

Table 2.2.2.1 *Phytophthora megasperma* isolates used in the comparative pathogenicity stem inoculation trial

N.B. * = Diseased plant sampled by Mal Grant, all isolates retrieved from diseased plant roots

Isolate	Host	Electromorph	Retrieved from.....
SEB 250	*Allocasuarina campestris**	'VII'	Hamersley Dve
SEB 251	*Xanthorrhoea platyphylla**	'VII'	Bell Track
SEB 201	*Dryandra quercifolia*	'I'	Point Ann Rd
SEB 242	*Banksia baxteri*	'I'	West Mt Barren
SEB 209	*Dryandra cuneata*	'I'	HIGP
SEB 234	*Adenanthos cuneatus*	'I'	East Mt Barren

The data presented summarises the total area of lesions quantified after six weeks (i.e. the second harvest). The ANOVA identified that there were significant differences between isolates (Table 2.2.2.2). Additionally, the contrast between electromorphs 'VII' and 'I' also identified a significant difference (Table 2.2.2.2).

Table 2.2.2.2 Analysis of Variance table of total lesion area

Source	df	MS	P-value	Significant
Replicate	9	508388	0.16	No
Isolate	5	980920	0.021	Yes
Elect. 'VII' v. Elect. 'I'	1	2513471	0.0083	Yes
Residual	45	329633	-	n/a

N.B. Electromorph 'VII' isolated from diseased plants inhabiting Archaean Yilgarn Block soils, while Electromorph 'I' isolated from quartzite marine plain. Any P-value < 0.05 is considered significant.

Isolates SEB 250 and 251 (i.e. electromorph 'VII'), produced significantly smaller lesions than either SEB 201, 209 and 234 (i.e. electromorph 'I') (Fig. 2.2.2.1).

From this study, the most virulent isolates of *P. megasperma* were isolated from diseased plants growing in the more recent quartzite coastal plain soils. Significantly, this comparatively more pathogenic form (i.e. electromorph 'I') is the most frequently isolated, and broadly distributed *P. megasperma*-form isolated from plants exhibiting disease expression in a number of sites on the South Coast region (e.g. FRNP; East Mount Barren, Point Ann, West Mount Barren and Quaalup and Cape Arid NP).

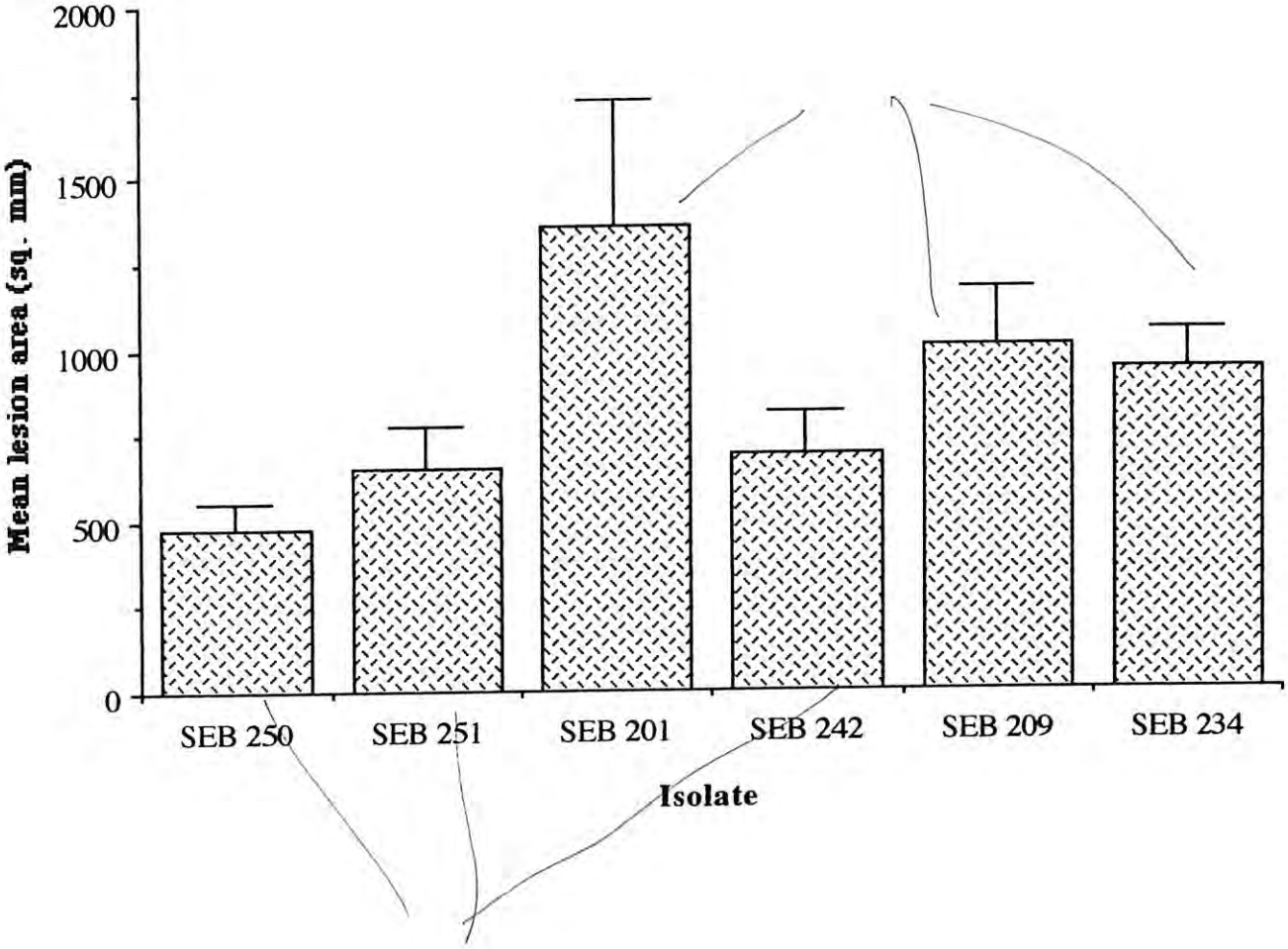


Fig. 2.2.2.1 Mean lesion area (±s.e.m., n=15) developed on *B. baxteri* stems after six weeks, by six isolates of *P. megasperma* retrieved from the FRNP. Isolates SEB 250 and 251 were recovered from diseased plants inhabiting Archaean Yilgarn Block soils, while the remainder retrieved from quartzite marine plain.

4 LIAISON

- Fitzgerald Advisory Committee - update of the results of the current research going on in the FRNP. Presented by Colin Crane, Nov. '94.
- Symposium on Plant Diseases in Ecosystems: threats and impacts in south-western Australia - Poster/paper presented entitled 'Variability in *Phytophthora megasperma* retrieved from diseased areas of WA' (Bellgard *et al.* 1994).
- Copy of report forwarded to Moora District (CALM) and South Coast Region (CALM). We would like to acknowledge the continuing commitment of the staff of the South Coast Region for co-operation and facilitation of research in the FRNP. Additionally the N.P. Rangers based at the FRNP for their continuing on-site help and input.
- On a recent visit to the US, Dr Stan Bellgard had the opportunity of discussing the significance of the isozyme results with Dr E. Hansen (Plant Pathology, Oregon State University). Dr Hansen has recently revised the taxonomy of *P. megasperma* resulting in the elevation of three putative taxa to the species level. Dr Hansen had two suggestions to improve the manuscript: 1) compare our allozymic profiles to two new putative taxa recently described by Förster and Coffey (1993) on the basis of mitochondrial and nuclear DNA polymorphisms, and 2) analyse colony growth morphology (on defined media) in terms of the allozymic groups we have described.
- We would like to acknowledge: 1) the isozyme expertise provided by Steve Carstairs; 2) the statistical expertise provided by Matthew Williams; 3) the *P. megasperma* isolates from the northern sandplain provided by Roz Hart (Hart, Simpson & Associates); and the volunteer assistance of Francine de Gruchy.
- Publication: Bellgard, S., Shearer, B., Crane, C. and Smith, B. (1994). Morphological variability exhibited by *Phytophthora megasperma* retrieved from diseased areas of Western Australian bushland. *Symposium on Plant Diseases in Ecosystems* (Ed. by R.T. Wills and W.A. Cowling). p. 20. Royal Society of WA and ESA, Press Printers, South Perth, WA.
- Publications in preparation: 1) Bellgard, S.E., Crane, C.E. Shearer, B.L. and Smith, B. The effect of phosphonate on radial growth and oogonium formation in isolates of *Phytophthora megasperma* retrieved from diseased national parks of s-w Australia, and 2) Bellgard, S.E., Carstairs, S., Crane, C.E. Shearer, B.L. and Smith, B. Isozyme variation of *Phytophthora megasperma* retrieved from diseased national parks of s-w Australia.

CONCLUDING DISCUSSION AND FUTURE DIRECTIONS

Control

- *P. megasperma* displayed variation in mycelial inhibition to phosphonate *in vitro*. Some differing responses were observed, with some isolates showing little or no growth inhibition to increasing concentrations of phosphonate *in vitro*. The calculated ED_{50} value for mycelial inhibition was considered to be greater than any previously reported.
- Aerial applications of phosphonate to five year old diseased post-fire regeneration has been associated with very few deaths in either the treated or untreated plants. The only documented deaths were of proteaceous plant species. Significantly, the documented susceptible species; *Banksia lemanniana*, *B. speciosa*, *Dryandra cuneata*, *D. falcata*, *D. quercifolia*, and *Isopogon trilobus*, are geographically restricted to the Eyre phytogeographic region (Aplin and Newbey 1990b). These plant species contribute significantly to the composition and structure of the Proteaceae/Myrtaceae mixed rock heath associated with East Mount Barren. Indeed, the faunal richness of the northern uplands, is attributed, in part, to the habitat diversity generated by the tight mosaic of soil/vegetation types resulting from the presence of granitic outcrops (CALM 1991). Consequently, removal of plant species contributing to habitat structure, e.g. *B. speciosa* by *P. megasperma*, could result in the loss of refugia for smaller animals.

> It is imperative that tissue analyses of phosphonate levels in treated plants be carried out soon. This will allow the interpretation our current mortality results, and determine if phosphonate was indeed taken-up by the plants sprayed.

> Within the FRNP other sites have been identified as being infested by *P. megasperma*, e.g. Point Ann and areas within the West Mt Barren region. Another fully replicated spray trial is required to take into account site, host and pathogen variability.

Biology and Pathogenicity of *P. megasperma*

- Twelve discrete allozymic profiles were identified in the 88 *P. megasperma* isolates examined. We have compared our isolates to six of the eight currently recognised groups within the *P. megasperma* species complex.

> Representative isolates have been obtained from Coffey, and we need to compare our local taxa against the two most recently described forms to ensure that our profiles have not been previously described. This will serve to complete our study and allow us to comment on the significance of the genetic distances we have identified between the various *P. megasperma* forms. Additionally, we need to analyse the colony growth morphology of our

allozyme groups. Diagnostic colony morphology plus allozymic profiles could provide a more definitive classification system than the current system based on the alpha-taxonomic characters of oogonium diameter and sporangium morphometrics.

- We have demonstrated variability in the pathogenicity of two *P. megasperma* electromorphs. Variability in pathogenicity in relation to the location from where the pathogen was retrieved (i.e. electromorph VII v. I) was identified.

> We need an integrated study to assess the implications of the observed pathogenic variability. Permanent study sites should be established within diseased areas on the coastal landforms in the FRNP to monitor soil water and temperature. Contemporaneously, plants should be individually tagged and disease expression assessed. These plots could also provide the sites for the additional aerial spray trial of phosphonate. Disease fronts should be delineated, and oospore numbers quantified and correlated with seasonal climatic and soil data. In this way, we could test the efficacy of phosphonate, and also pin-point what environmental conditions are likely to trigger germination and subsequent outbreaks in infested sites.

Occurrence

- We have assessed six sites in the FRNP and one site in Cape Arid N.P. for impacts/effect of *P. megasperma* upon the community structure.

> We need more site typing especially in and around Cape Arid N.P., to get a complete distribution map of *P. megasperma* in s-w Australia, host susceptibility to infection, and also to retrieve any other local variants of the pathogen.

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PROJECT 5: ANNUAL REPORT TO ANCA
March 1995

IDENTIFYING, GERMPLASM STORAGE AND *IN VITRO* PROPAGATION OF *PHYTOPHTHORA* AND CANKER THREATENED TAXA

A. Cochrane, D. Coates and C. McChesney

1. Identification of rare and threatened flora at risk from dieback disease

The Department of Conservation and Land Management Declared Rare (Threatened) and Priority Flora List for Western Australia (Ken Atkins 14/09/94) is currently being used as the basis for all collections. Continued consultation with CALM dieback interpretation officers has led to greater knowledge of species observed to be susceptible to *Phytophthora* and canker dieback with many genera appearing to be quite variable in their response to these diseases. Collections are occurring on a species by species basis, dependant on site characteristics as well as species susceptibility.

2. *In vitro* propagation

Discontinued (see Year 1 report)

3. Cryostorage

Discontinued (see Year 1 report)

4. Seed collection

Two hundred and twenty-two accessions of threatened or priority taxa have been incorporated into the Threatened Flora Seed Centre (TFSC) as of 20th February, 1995 (See Appendix 1). This represents 94 taxa in 10 families. Collaborative field trips with other CALM staff and collectors from Kings Park and Botanic Gardens continue to yield good results. A number of new populations of threatened taxa have been discovered on routine collection trips. Communication between CALM districts and the TFSC continues to be excellent with assistance being given for collections and the provision of field advice.

Collaboration with local community groups has been very successful with a local farmer being contracted to collect seed from seed traps erected in 1993 adjacent to his property. This was to determine whether a weekly collection was more cost-effective than monthly collections by the TFSC manager and whether the incidence of seed predation would be reduced. Three collections over the 1993/1994 fruiting season yielded 94 seeds only from 10 seed traps, whereas 9 weekly collections over the 1994/1995 fruiting season have yielded 238 seeds. It is impossible to categorically state that weekly collections have increased the seed yield as seasonal differences may have attributed to the greater number of seeds collected from traps in the 1994/1995 season. However, it was noted that both 1993/1994 and 1994/1995 were good flowering years for the taxa concerned. It is hoped

that this strategy will be continued for the next collecting season and possibly expanded to involve local community members in other areas where long term continuous seed collections are needed.

5. Seed storage, viability testing and inventory system

Pre and post-storage viability data on the effects of moisture content reduction and storage of germplasm in carbon dioxide at sub-zero temperatures is now available for a number of taxa held in the TFSC's genebank.

Storage in general reduces the germination rate as indicated by a tendency for post-storage seed to take longer to reach the same percentage germination of pre-storage seed (Figures 1-8). Furthermore, it appears that a proportion of pre-storage seed tends to germinate simultaneously whilst post-storage seed has a tendency to germinate at a less uniform rate. A slower rate of germination is to be expected as carbon dioxide is known to reduce or halt metabolism of germplasm. It is possible that secondary dormancy is being imposed on the seed through the storage process. However, storage in carbon dioxide does not appear to reduce the seed's ability to germinate in the long term.

Maximum germination of *Banksia verticillata* was similar for both pre- and post-storage seed (Figures 1-4), while maximum percentage germination of the *Dryandra* seedlots was in fact greater for post-storage than pre-storage seed (Figures 5-8). Other species tested showed less clear trends and in some cases storage may have affected the viability of the seed although other factors such as fungal and bacterial infection may have attributed to the apparent loss of seed viability. It is hoped that more appropriate sterile conditions can be attained in the future so that accurate data comparisons can be made.

Accessions will continue to be monitored on a yearly, then 5 yearly basis until adequate knowledge of the flora's response to sub-zero storage is attained. A monitoring regime of ten years will then be implemented subject to viability figures. A number of difficult-to-collect species have had seed incorporated into the TFSC, with some success forthcoming on their germination. Continued collaboration with the Research unit at Kings Park and Botanic Gardens is an integral part of this success particularly in relation to their use of seed cryostorage techniques. A great deal of work is required in the future to look at practicable means to collect and germinate some of these difficult taxa, as well as research into the seed biology and pollination mechanisms for a wide range species..

The fruiting-time database continues to be updated as new information and new species are added to the list of flora being targeted by the TFSC.

6. Future collections and proposed new developments

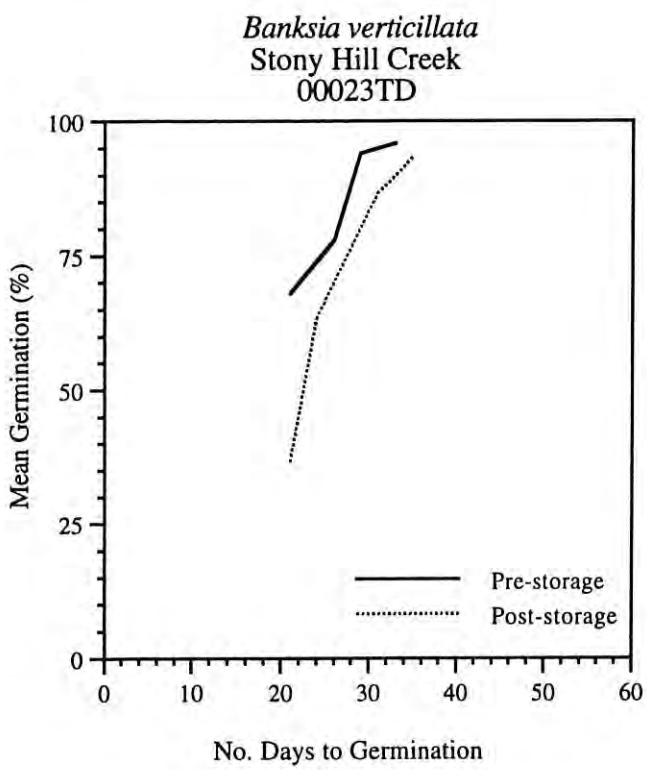
The coming year will see continued collections of threatened flora from the south-west and resampling of previously collected taxa. Additional populations of targeted flora will be added to the accessions where necessary to consolidate the gene pool of that taxon.

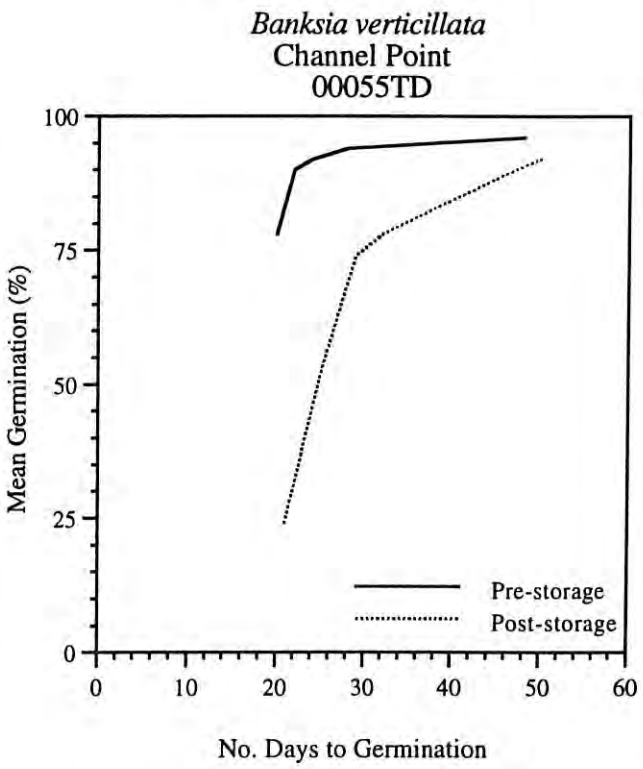
Research into the after-ripening processes in *Dryandra ionthocarpa* is well under way although final data is unavailable at present. It appears from a preliminary assessment that the use of ethylene is not suitable for emulating dry storage after ripening.

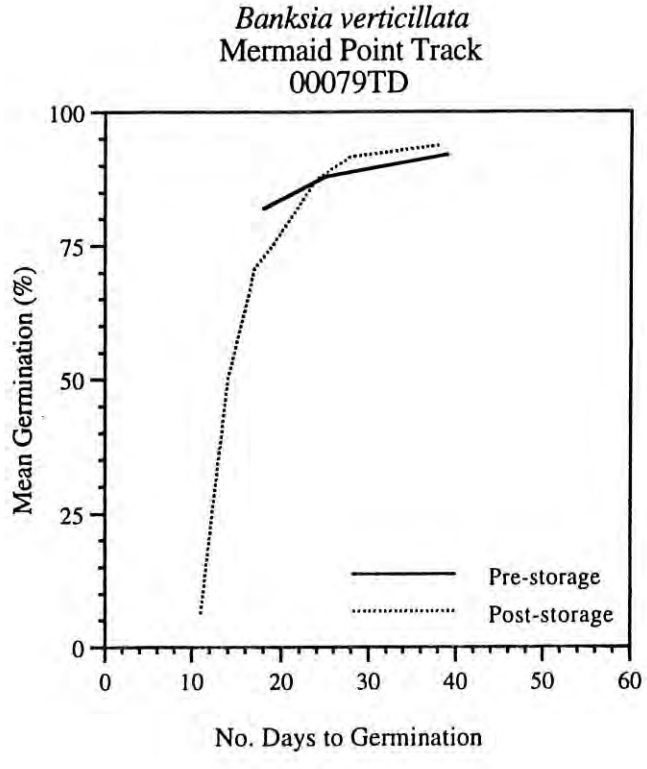
A number of new research projects are currently being developed. These will include research into desiccation tolerance of some common Proteaceous species from the south-west which will commence this autumn/winter and will be extended to a number of the threatened species. It is also proposed to begin research into aspects of the viability of seed from endangered *Banksia* species sprayed with phosphonate, as soon as suitable sites are found.

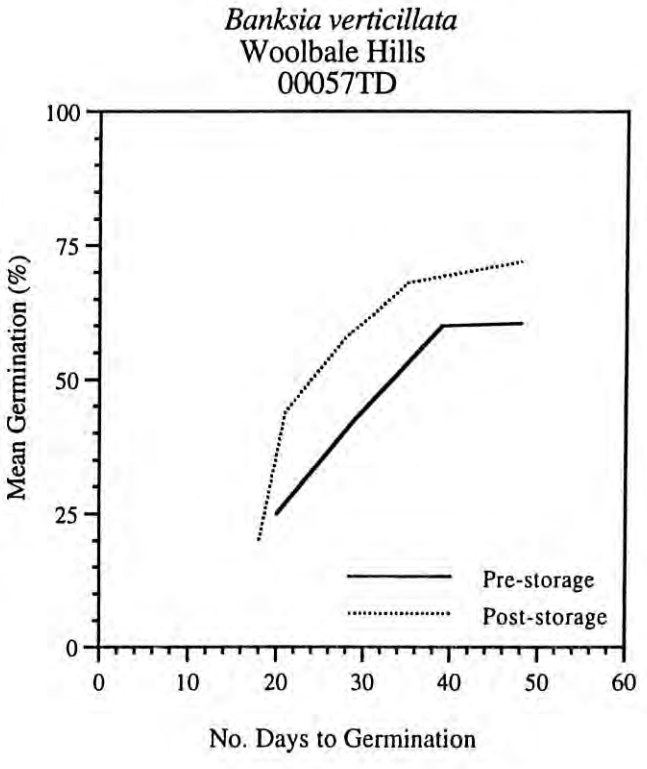
Currently, funds are being sought externally for a visit to the Kew Seed Bank (Wakehurst Place) to work in collaboration with scientists on genebanking and to attend a Plant Conservation Techniques course held at Kew Botanical Gardens.

Figures 1-4. Comparison of pre- and post-storage mean percentage germination.









Figures 5-8. Comparison of pre- and post-storage mean percentage germination.

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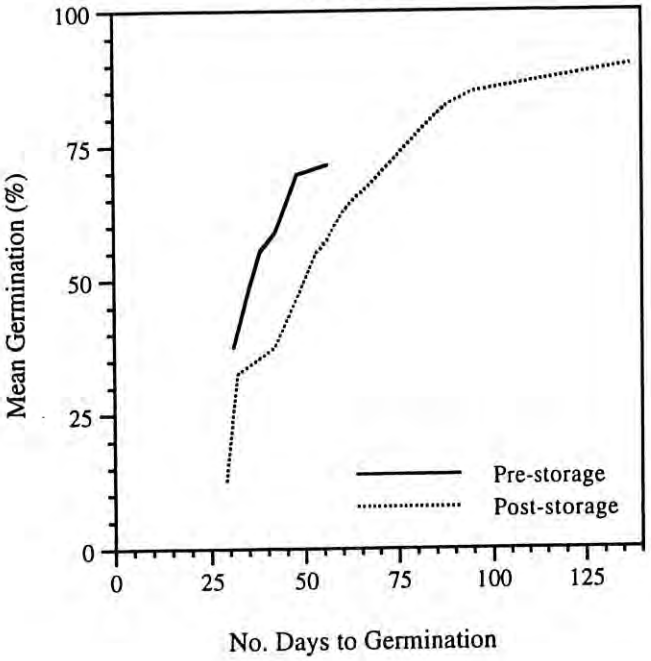
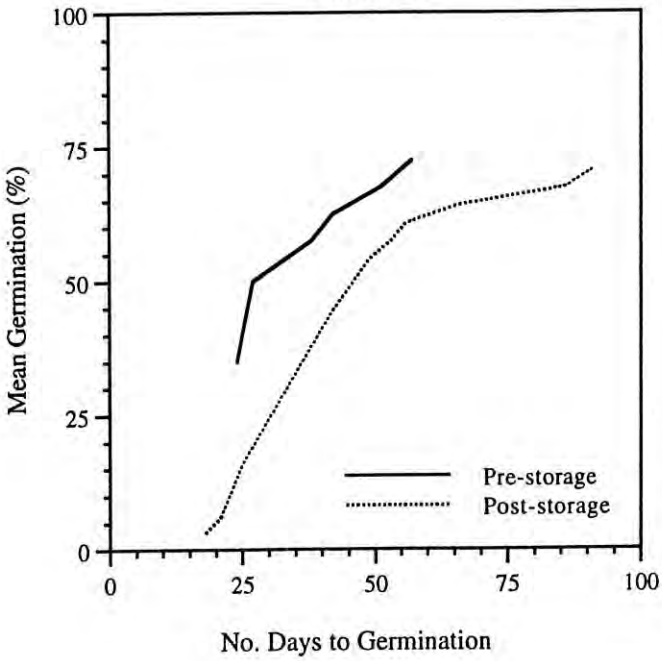
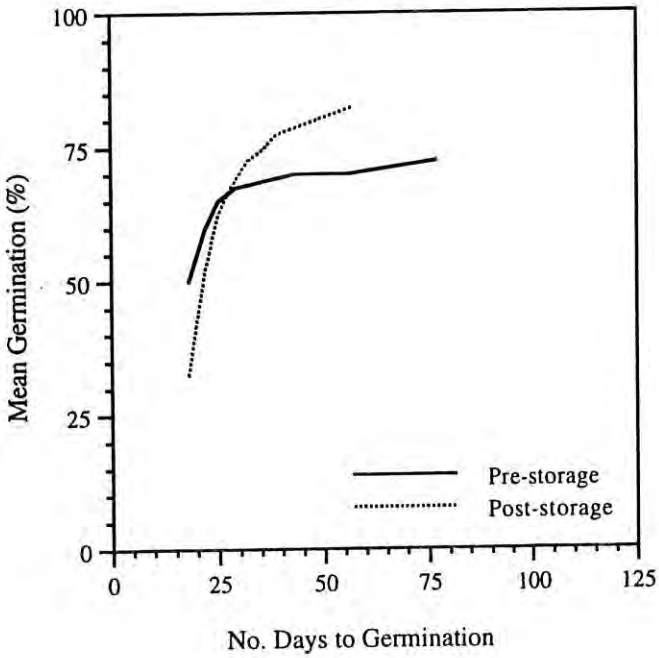
Stirling Range Drive
00071TD



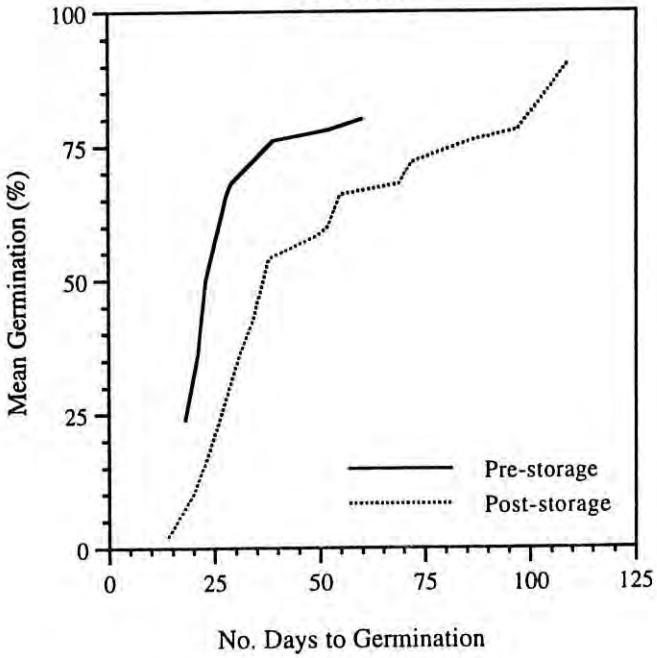
Dryandra erythrocephala var. inopinata
Hopkins Nature Reserve
00085TD



Dryandra sqarrosa ssp. argillacea
Wonnerup Road, Ruabon
00062TD



Dryandra sqarrosa ssp. argillacea
Smith Road, Wicher
00063TD



APPENDIX 1: ACCESSIONS LIST

Seedlot	Collection no	Date	Species	Location	Type
00001 TD	DJC	9/08/87	*Banksia brownii*	Mt Hassell	I/4
00002 TD	DJC	2/11/88	*Banksia brownii*	Bluff Knoll	I/17
00003 TD	DJC	9/02/88	*Banksia cuneata*	Lake Mears Road	I/6
00004 TD	DJC	9/02/88	*Banksia cuneata*	Swamp Road	I/10
00005 TD	DJC	9/02/88	*Banksia cuneata*	Badjaling NR	I/13
00006 TD	DJC	10/06/85	*Banksia brownii*	Cheyne Beach	I/38
00007 TD	DJC		*Banksia brownii*	Quaranup	I/29
00008 TD	DJC	24/08/89	*Banksia brownii*	Cheyne Bch Road	I/49
00009 P2	DJC	26/04/88	*Banksia epica*	Toolina Cove	I/45
00010 TD	DJC	9/02/88	*Banksia cuneata*	Water Res. 12397	I/30
00011 TD	DJC	9/02/88	*Banksia cuneata*	Simpsons Farm	I/15
00012 TD	DJC	8/02/88	*Banksia cuneata*	Bruce Rock Road	I/30
00013 TD	DJC	23/05/88	*Banksia cuneata*	Quairading	I/23
00014 TD	DJC	24/09/90	*Banksia cuneata*	Laze Away	I/29
00015 TD	DJC	1/06/86	*Banksia verticillata*	Mt Hopkins	I/6
00016 TD	DJC	10/05/86	*Banksia verticillata*	The Gap,Torndirrup	I/11
00017 TD	JAC1016	15/03/94	*Verticordia attenuata*	Elgin Rd, Capel	B/20
00018 TD	DJC	8/03/88	*Banksia oligantha*	Wongeling N	I/10
00019 TD	DJC	8/03/88	*Banksia oligantha*	Wongeling W	I/20
00020 TD	DJC	8/03/88	*Banksia oligantha*	Wongeling E	I/10
00021 TD	DJC	26/11/86	*Banksia verticillata*	Mermaid Point Tk	I/20
00022 TD	DJC	9/06/85	*Banksia verticillata*	Poison Hill	I/9
00023 TD	DJC	10/05/86	*Banksia verticillata*	Stony Hill Creek	I/13
00024 TD	DJC	28/11/90	*Dryandra ionthocarpa*	Kambellup S	I/19
00025 TD	DJC	9/08/90	*Banksia oligantha*	Marribank site 1	I/14
00026 TD	DJC	9/08/90	*Banksia oligantha*	Marribank site 2	I/11
00027 TD	DJC	9/08/90	*Banksia oligantha*	Marribank site 3	I/10
00028 TD	DJC	9/08/90	*Banksia oligantha*	Marribank site 4	I/30
00029 TD	DJC	7/06/85	*Lambertia orbifolia*	Narrikup 1	I/9
00030 TD	MM1490	13/12/92	*Lambertia orbifolia*	Narrikup 2	B/15
00031 TD	MM1453-1469	12/12/92	*Isopogon uncinatus*	Muttonbird	I/16
00032 TD	MM1473-1482	12/12/92	*Banksia verticillata*	Stony Hill Creek	I/10
00033 TD	MM1484	13/12/92	*Andersonia* sp. Two Peoples Bay	Boulder Hill	B/50
00034 TD	MM1489	14/12/92	*Andersonia echinocephala*	Mondurup	B/30
00035 TD	MM1492-1503	15/12/92	*Andersonia grandiflora*	Toolbrunup	I/12
00036 TD	MM1507	18/12/92	*Adenanthos pungens ssp effusa*	Tambellup	B/10
00037 TD	MM1528-1540	22/01/93	*Dryandra seneciifolia*	Red Gum Pass	I/13
00038 TD	MM1541-1550	22/01/93	*Andersonia grandiflora*	Stirling Drive	I/10
00039 TD	MM1551-1560	23/01/93	*Dryandra ionthocarpa*	Kambellup N	I/10
00040 TD	MM1575-1596	25/01/93	*Banksia brownii*	South Sister	I/22
00041 TD	MM1612-1614	29/01/93	*Lambertia echinata ssp. echinata*	Lucky Bay	I/3
00042 TD	MM1615-1624	30/01/93	*Isopogon alcicornis*	Mt Burdett	I/10
00043 TD	MM1638	31/01/93	*Dryandra serra*	South Sister	B/20
00044 TD	RWills	18/09/92	*Banksia verticillata*	Jimmy Newhills Harbour	B/6
00045 P3	MM1565-1569	24/01/93	*Thomasia solanacea*	Limeburners Lane	I/5
00046 P3	MM1573	24/01/93	*Eucalyptus acies*	South Sister	B/5
00047 P2	MM1625	30/01/93	*Melaleuca coccinea ssp. eximia*	Mt. Burdett	B/10
00048 V	MM1626-1630	30/01/93	*Eucalyptus merrickiae*	Kent Road	I/5

00049 TD	JAC1047	16/03/94	*Verticordia attenuata*	Bussel Hwy	B/30
00050 TD	JAC1045	16/03/94	*Verticordia attenuata*	Ruabon Rd, Tutunup	B/50
00051 V	MM	19/05/93	*Banksia goodii*	Millbrook NR	I/5
00052 V	DJC	9/05/86	*Banksia goodii*	Millbrook NR	I/12
00053 TD	JAC1020-1029	16/03/94	*Verticordia plumosa var ananeotes*	Ambergate NR	I/10
00054 G	DJC	1/06/87	*Banksia semi-nuda ssp remanens*	Mt. Hopkins	I/7
00055 TD	MM1654-1678	4/04/93	*Banksia verticillata*	Channel Point	I/25
00056 TD	MM1680-1694	6/04/93	*Banksia verticillata*	Stony Hill	I/15
00057 TD	MM1695-1719	7/04/93	*Banskia verticillata*	Woolbale Hills	I/25
00058 TD	MM1720	15/05/93	*Lambertia orbifolia*	Dennis Road	B/15
00059 TD	MM1721	15/05/93	*Lambertia orbifolia*	Brennans Ford	B/21
00060 TD	MM1724-1739	18/05/93	*Banksia brownii*	Quaranup Road	I/16
00061 TD	MM1740-1780	19/05/93	*Banksia brownii*	Millbrook Nat. Res.	I/41
00062 TD	JAC251-260	20/07/93	*Dryandra squarrosa ssp. argillacea*	Ruabon/Tutunup	I/10
00063 TD	JAC261-277	21/07/93	*Dryandra squarrosa ssp. argillacea*	Smith Rd, Wicher R.	I/17
00064 G	JAC278-294	22/07/93	*Banksia semi-nuda ssp remanens*	Thompsons Cove	I/17
00065 G	JAC295-308	22/07/93	*Banksia semi-nuda ssp remanens*	Aldridge Cove SW	I/14
00066 G	JAC309-311	23/07/93	*Banksia semi-nuda ssp remanens*	Long Point carpark	I/3
00067 TD	JAC312-329	23/07/93	*Banksia seminuda ssp remanens*	Poison Hill SW face	I/18
00068 TD	JAC330-354	24/07/93	*Banksia brownii*	Mt. Hassell	I/25
00069 TD	JAC363	6/08/93	*Isopogon scaber*	44mp Brookton Hwy	B/16
00070 TD	JAC368	18/08/93	*Dryandra anatoma* (sp. 48)	Mt. Tayluberlup	B/15
00071 TD	JAC372-387	19/08/93	*Dryandra seneciifolia*	Lookout-Stirling Range Dr	I/16
00072 TD	JAC388-404	19/08/93	*Dryandra sp. 49* (aff.ferruginea)	Lookout-Stirling Range Dr	I/17
00073 TD	JAC405	20/08/93	*Andersonia sp. nov* (Mt.Lindesay)	Mt. Lindesay	B/10
00074 TD	JAC406	21/08/93	*Dryandra serra*	Hunwick Rd(East)	B/32
00075 TD	JAC409-419	21/08/93	*Dryandra serra*	Hunwick Rd(West)	I/11
00076 TD	JAC421-424	21/08/93	*Banksia brownii*	Hazzard Rd	I/4
00077 TD	JAC425-439	22/08/93	*Banksia brownii*	Hassell Beach Rd	I/15
00078 TD	JAC441-448	23/08/93	*Banksia verticillata*	Mermaid/Channel Pt	I/8
00079 TD	JAC449-464	23/08/93	*Banksia verticillata*	Mermaid Pt Track	I/16
00080 TD	JAC466-475	24/08/93	*Dryandra seneciifolia*	Hamilla Hills	I/10
00081 TD	JAC477-488	14/09/93	*Dryandra acanthopoda* (sp.1)	Carter Rd, Woodanilling	I/12
00082 TD	JAC489	14/09/93	*Dryandra lepidorhiza* (sp. 41)	Orchard/Dinwoodie Rds, Woodanilling	B/20
00083 TD	JAC490	15/09/93	*Dryandra sp. 45*	Katanning-Nyabing Rd	B/10
00084 TD	JAC491-510	15/09/93	*Dryandra octotriginata* (sp. 38)	Nyabing-Kukerin Rd	I/20
00085 TD	JAC512-532	16/09/93	*Dryandra erythrocephala var. inopinata* (sp. 44)	Hopkins NR	I/21
00086 TD	JAC533-551	16/09/93	*Dryandra epimicta* (sp. 37)	Hopkins NR	I/21
00087 TD	JAC552-568	17/09/93	*Dryandra sp. 31*	Corrigin-Quarading Rd	I/17
00088 TD	JAC569	21/09/93	*Lambertia fairallii*	Stirling Range Drive, SRNP	B/100
00089 TD	JAC572	9/11/93	*Dryandra mimica*	Old Kent Rd, Wicher Range	B/1?
00090 TD	JAC574	9/11/93	*Lambertia orbifolia*	Dennis Road	B/10
00091 TD	JAC577-596	10/11/93	*Astroloma* sp. Nannup	Scott River Rd. Scott River	I/20
00092 TD	JAC598-612	11/11/93	*Dryandra ionthocarpa*	Kambellup N	I/16
00093 TD	JAC613-628	11/11/93	*Dryandra ionthocarpa*	Kambellup S	I/15
00094 TD	JAC630-639	11/11/93	*Lambertia fairallii*	Stirling Range Drive, SRNP	B/50
00095 E	BHaberley	3/11/93	*Daviesia microcarpa*	Norseman	B/12
00096 TD	JAC644-654	25/11/93	*Daviesia pseudaphylla*	Site 1, East Pillenorup Track, SRNP	I/11
00097 TD	JAC655	25/11/93	*Daviesia pseudaphylla*	Site 2, East Pillenorup Track, SRNP	B/25
00098 TD	JAC643	25/11/93	*Daviesia pseudaphylla*	Site 1/2, East Pillenorup Track, SRNP	B/20
00099 TD	JAC656-677	26/11/93	*Andersonia echinocephala*	Stirling Range Dr., SRNP	I/22

00100 TD	JAC679-698	28/11/93	*Banksia laevigata ssp.laevigata*	Dorrie Hill, Twertup, FRNP	I/20
00101 TD	JAC702	30/11/93	*Adenanthos dobagii*	Quoin Head, FRNP	B/12
00102 TD	JAC700	30/11/93	*Adenanthos ellipticus*	East Mt. Barren	B/8
00103 TD	JAC704	1/12/93	*Daviesia megacalyx*	Elverdton Rd.	B/30
00104 TD	JAC706-718	1/12/93	*Dryandra corvijuga* (sp. 36)	Elverdton Rd.	I/13
00105 TD	JAC719-728	1/12/93	*Dryandra folisissima*	Mt. Desmond	I/10
00106 TD	JAC729-738	2/12/93	*Dryandra folisissima*	Ravensthorpe 2	I/10
00107 TD	JAC739-748	2/12/93	*Dryandra corvijuga* (sp. 36)	Ravensthorpe 2	I/10
00108 TD	JAC749-762	2/12/93	*Daviesia megacalyx*	Ravensthorpe 2	I/14
00109 TD	JAC763/1-5	2/12/93	*Daviesia megacalyx*	Floater Rd.	I/5
00110 P3	JAC767-789	3/12/93	*Dryandra viscida* (sp. 16)	Hatter Hill	I/23
00111 V	JAC791-810	3/12/93	*Banksia sphaerocarpa var. dolichostyla*	South Ironcap	I/20
00112 TD	JAC811-818	4/12/93	*Daviesia oxylobium*	Wamenusking NR	I/8
00113 V	DWO	1/11/90	*Eucalyptus crucis ssp. crucis*	Sandford Rocks NR	B/?
00114 TD	BHammersley	8/09/93	*Dryandra serra*	Mt. Hallowell	I/10
00115 R	SVL	1/10/92	*Lepidium catapycnon*	Garden Gorge, Hammerslay Range	I/1
00116 TD	JAC905	6/01/94	*Adenanthos pungens ssp effusa*	Tambellup	B/3
00117 TD	JAC906	6/01/94	*Adenanthos pungens ssp pungens*	Hamilla Hill, SRNP	B/12
00118 TD	JAC907-916	6/01/94	*Adenanthos pungens ssp pungens*	Hamilla Hill, SRNP (traps)	I/10
00119 TD	JAC821-824	7/01/94	*Adenanthos velutinos*	Geekabee Hill	I/4
00120 TD	JAC828-840	9/01/94	*Banksia verticillata*	Mermaid Point	I/13
00121 TD	JAC841	10/01/94	*Adenanthos ellipticus*	East Mt Barrren	B/40
00122 TD	JAC842-851	11/01/94	*Adenanthos dobagii*	Quoin Head Track	I/10
00123 TD	JAC852-861	11/01/94	*Adenanthos dobagii*	Quoin Head T/O (traps)	I/10
00124 TD	JAC865-867	14/01/94	*Lambertia echinata ssp echinata*	Lucky Bay	I/3
00125 E	JAC868-877	14/01/94	*Myoporum turbinatum*	Heywood Rd	I/10
00126 TD	JAC880	15/01/94	*Astroloma* sp. Grass Patch	Ridley Rd, Grass Patch	B/40
00127 TD	JAC884-904	16/01/94	*Daviesia campophylla*	Griffith/Edwards Rd	I/21
00128 TD		7/07/93	*Banksia cuneata*	Johnston's property (Pop10)	B/?
00129 TD	JAC925-944	9/02/94	*Andersonia echinocephala*	Baby Barnett SRNP	I/20
00130 TD	JAC948-957	11/02/94	*Dryandra sp. Fitzgerald*	Drummond Track west FRNP	I/10
00131 TD	JAC958-969	13/02/94	*Dryandra sp. Fitzgerald*	Hammersley Dr east FRNP	I/12
00132 TD	JAC973-984	15/02/94	*Andersonia* sp. Two Peoples Bay	Boulder Hill, Two Peoples Bay	I/12
00133 TD	JAC985-994	15/02/94	*Andersonia sp. nov* (Mt. Lindesay)	Mt Lindesay	I/10
00134 TD	JAC996-1013	16/02/94	*Banksia occidentalis ssp. formosa*	Black Point, Scott River	I/17
00135 TD	TSwarten	12/93-1/94	*Daviesia spiralis*	Wongan Hills	B/25
00136 P4	ABrown?	1/09/93	*Eucalyptus carnabyi*	York	B/5
00137 TD	JAC1018	15/03/94	*Petrophile latericola*	Williamson Rd, Wicher	B/40
00138 TD	JAC1056-1068	20/04/94	*Banksia verticillata*	Stony Hill	I/13
00139 TD	JAC1069-1073	20/04/94	*Dryandra montana*	Bluff Knoll, SRNP	I/5
00140 TD	JAC1074-1089	21/04/94	*Verticordia harveyi*	South Stirling	I/16
00141 G	JAC1090-1099	22/04/94	*Lambertia echinata ssp. propinqua*	Cheyne Beach Rd.	I/10
00142 G	JAC1100-1105	22/04/94	*Lanbertia echinata ssp. propinqua*	Boulder Hill	I/6
00143 TD	JAC1106	26/04/94	*Dryandra aurantia* ("Little Darkin Swamp")	Little Darkin Swamp	B/15
00144 TD	JAC1108	12/05/94	*Lambertia fairallii*	Below Ellen Peak	B/8
00145 V	JFS1	1/05/85	*Eucalyptus rhodantha*	Three Springs 1	I/6
00146 V	JFS3	1/04/85	*Eucalyptus rhodantha*	Watheroo 3	I/21
00147 V	JFS4	1/04/85	*Eucalyptus rhodantha*	Watheroo 4	I/32
00148 V	JFS5	1/08/85	*Eucalyptus rhodantha*	Watheroo 5	I/14
00149 V	JFS6	15/04/85	*Eucalyptus rhodantha*	Watheroo 6	I/8
00150 V	AK1	12/07/93	*Eucalyptus rhodantha*	Three Springs 1	I/11

00151 V	AK2	13/07/93	*Eucalyptus rhodantha*	Three Springs 2	I/6
00152 V	AK4	2/09/93	*Eucalyptus rhodantha*	Watheroo 4	I/35
00153 V	AK5	23/06/93	*Eucalyptus rhodantha*	Watheroo 5	I/34
00154 V	AK6	13/07/93	*Eucalyptus rhodantha*	Watheroo 6	I/4
00155 V	AK1(92)	17/12/92	*Stylidium coroniforme*	Wongan Hills 1	B/6
00156 V	AK1(93)	16/12/93	*Stylidium coroniforme*	Wongan Hills 1	B/9
00157 V	AK2(93)	16/12/93	*Stylidium coroniforme*	Wongan Hills 2	I/13;B/15
00158 V	AK3(93)	15/12/93	*Stylidium coroniforme*	Maya 3	I/11
00159 V	AK4(93)	15/12/93	*Stylidium coroniforme*	Maya 4	I/25
00160 V	AK5(93)	16/12/93	*Stylidium coroniforme*	Wongan Hills 5	I/14;B/27
00161 TD	DJC	1/06/87	*Banksia verticillata*	Mt. Hopkins	I/19
00162 TD	JAC1131-1144	26/07/94	*Dryandra fraseri var. oxycedrus* (sp. 23)	Kadathinni NR	I/14
00163 TD	JAC1146	26/07/94	*Dryandra borealis ssp. elatior* (sp .20)	Kadathinni NR	B/20
00164 TD	JAC1148-1153	27/07/94	*Leucopogon obtectus*	Beekeeper Rd	I/6
00165 TD	JAC1157-1167	27/07/94	*Dryandra stricta* (sp 15)	Willis Rd	I/11
00166 TD	JAC1127	25/07/94	*Dryandra serratuloides*	Gillingara Pop9A	B/12
00167 TD	JAC1170-1184	28/07/94	*Dryandra serratuloides*	Marchagee Track (Pops5)	I/15
00168 TD	JAC1186-1195	28/07/94	*Dryandra pteridifolia ssp. vernalis* (sp. 22)	Marchagee Track	I/10
00169 TD	JAC1196-1208	28/08/94	*Banksia verticillata*	Isthmus Hill, Torndirrup	I/13
00170 TD	JAC1210	1/09/94	*Dryandra anatoma* (sp.48)	Mt. Talyuberlup	B/6
00171 V	AK3	1/06/94	*Eucalyptus rhodantha*	Watheroo 3	I/5
00172 TD	JAC1147-1156	28/09/94	*Lambertia echinata ssp. propinqua*	Cape Riche	I/10
00173 V	Jens Olesen	Sept-Dec 93	*Anigozanthus humilis ssp. chrysanthus*	Mogumber	I/44
00174 TD	JAC1160-1169	18/11/94	*Melaleuca ordinifolia*	Salt River RD, SRNP	I/10
00175 TD	JAC1170	19/11/94	*Daviesia pseudaphylla*	E. Pillenorup/Sth Bluff Knoll Tracks	B/30
00176 E	Ray Smith	16/11/94	*Rulingia* sp. Trigwell Bridge	Loc 3271 Arthur Shire	B/4
00177 TD	JAC1175	20/11/94	*Banksia brownii*	SE of Ellens Peak	B/10
00178 TD	JAC1179-1191	20/11/94	*Andersonia echinocephala*	SE of Ellens Peak	I/13
00179 TD	JAC1193	21/11/94	*Darwinia lejostyla*	Mt. Trio	B/100
00180 TD	JAC1195	21/11/94	*Darwinia oxylepis*	Below Baby Barnett	B/100
00181 TD	JAC1197	21/11/94	*Darwinia wittwerorum*	Below Mt. Talyuberlup	B/100
00182 TD	JAC1200	22/11/94	*Verticordia helichrysantha*	Cape Riche	B/200
00183 P2	JAC1201-1211	22/11/94	*Melaeuca sculponeata*	Mallee Rd, Jerramungup	I/11
00184 TD	JAC1212 (1-9)	23/11/94	*Daviesia megacalyx*	Floater Rd.	I/9
00185 TD	JAC1213	23/1194	*Daviesia megacalyx*	Elverdton Rd.	B/10
00186 TD	JAC1214	23/11/94	*Daviesia megacalyx*	Sth Ravensthorpe Range (new)	B/50
00187 TD	JAC1221	14/12/94	*Grevillea mccutcheonii*	Princefield Rd, Wicher	B/5
	JAC1294	28/12/94			
00188 TD	JAC1222-1229	14/12/94	*Hakea aff. varia*	Princefield Rd, Wicher	I/8
00189 TD	JAC1231-1240	14/12/94	*Dryandra squarrosa ssp. argillacea*	Tutunup Rd, Wicher	I/10
00190 TD	JAC1247-1255	15/12/94	*Dryandra aff. nivea*	Williamson Rd, Wicher	I/9
00191 TD	JAC1257	15/12/94	*Petrophile latericola*	Williamson Rd, Wicher	B/25
00192 TD	JAC1260	15/12/94	*Hakea aff. varia*	Williamson Rd, Wicher	B/30
00193 TD	JAC1265	16/12/94	*Andersonia aff. latiflora*	Smith Rd, Wicher	B/100
00194 TD	JAC1272-1283	16/12/94	*Grevillea elongata*	Princefield Rd, Wicher	I/22
	JAC1284-1293	28/12/94			
00195 TD	JAC1243	15/12/94	*Brachysema papilio*	Williamson Rd, Wicher	B/5
	JAC1297	28/12/94			
00196 C	MRoddy+DP017	4/12/94	*Acacia awestoniana*	Chester Pass Rd, SRNP	B/7
	DP017	12/12/94			
00197 TD	JAC1301	28/12/94	*Grevillea elongata*	Tutunup Rd, Wicher	B/8

00198 TD	DP009	14/12/94	*Andersonia* sp. Two Peoples Bay	Boulder Hill - south face	B/10
00199 TD	DP010	14/12/94	*Andersonia* sp. Two Peoples Bay	Boulder Hill - east face	B/10
00200 TD	DP011	14/12/94	*Andersonia* sp. Two Peoples Bay	Goodga River	B/10
	JAC1308	11/01/95			
00201 TD	Seed traps	14/11/94-29/01/95	*Adenanthos pungens ssp. pungens*	Hamilla Hills	I/10 (traps)
00202 TD	JAC1303	9/01/95	*Lambertia fairallii*	Stirling Range Drive, SRNP	B/50
00203 TD	JAC1304	9/01/95	*Andersonia grandiflora*	Below Mt. Gog	B/100
00204 TD	JAC1322	12/01/95	*Isopogon uncinatus*	Torndirrup (CJR pop B+C)	B/20
00205 TD	JAC1324	12/01/95	*Isopogon uncinatus*	Torndirrup (CJR popD)	B/50
00206 TD	JAC1325	13/01/95	*Andersonia sp. Mt Lindesay*	Mt. Lindesay	B/20
00207 TD	JAC1327	13/01/95	*Verticordia fimbrilepis ssp. australis*	Kent River	B/200
00208 R	JAC1328	11/01/95	*Hibbertia sp. Porongorups*	Devils Slide	B/6
00209 G	JAC1310-1320	11/01/95	*Lambertia propinqua*	WAWA reserve Two Peoples Bay	I/11
00210 V	AK7	14/12/94	*Eucalyptus rhodantha*	Watheroo 7	I/8
00211 V	AK8	14/12/94	*Eucalyptus rhodantha*	Watheroo 8	I/9
00212 V	AK9	14/12/94	*Eucalyptus rhodantha*	Watheroo 9	I/11
00213 V	AK11	14/12/94	*Eucalyptus rhodantha*	Watheroo 11	I/23
00214 TD	DP023	3/01/95	*Verticordia albida*	Three Springs	B/8
00215 TD	JAC1342	28/01/95	*Verticordia bifimbriata*	Dryandra State Forest	B/12
00216 TD	JAC1340	28/01/95	*Andersonia bifida*	Dryandra State Forest	B/35
00217 TD	JAC1343-1369	13/02/95	*Verticordia plumosa var pleiobotrya*	Mundijong	I/27
00218 TD	JAC1370	13/02/95	*Verticordia attenuata*	Elgin Rd, Capel	B/30
00219 TD	JAC1373-1382	13/02/95	*Dryandra* aff. *nivea*	Tutunup Rd, Wicher	I/10
00220 TD	JAC1384	14/02/95	*Verticordia plumosa var ananeotes*	Ambergate NR, Busselton	B/30
00221 TD	JAC1389	15/02/95	*Verticordia endlicheriana var angustifolia*	Mt Barker Hill	B/50
00222 R	JAC1395	17/02/95	*Kunzea pauciflora*	Mt Melville, Cape Riche	B/50

*Status refers to:

I = seedlot comprised of seed from individual parent plants kept separate

B = seed from individual parent plants bulked as one seedlot due to sporadic or sparse seed production

/ number indicates plants represented in the seedlot.

CONTROL AND MANAGEMENT OF *CRYPTODIAPORTHE MELANOCRASPEDA* CANKER THREATENING *BANKSIA COCCINEA*

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SUMMARY

Canker, incited by *Cryptodiaporthe melanocraspeda*, was first acknowledged as a potential threat to the survival of *Banksia coccinea* stands in 1989. Since then the disease has been recorded throughout the geographic range of *B. coccinea* and has been responsible for the rapid decline of stands. Symptoms of disease are initially small cankers at leaf nodes which quickly expand to girdle branches and eventually kill the plant. The lifecycle of *C. melanocraspeda* is typical of an ascomycete, having a sexual stage in which wind-borne ascospores are produced and an asexual stage in which splash-borne conidia are produced. Ascospores and conidia are released after moist conditions, however the quantity of inoculum involved and dispersal gradients of inoculum are unknown. The role of alternative hosts and seed-borne infections as sources of inoculum have not been determined.

Inoculation experiments were conducted to determine the pathogenicity of *C. melanocraspeda* in non-wounded and wounded stems. The pathogen was able to infect both types of tissue, however the frequency of lesion development in wounded stems was greater than that in non-wounded. The ability to colonise non-wounded tissues would provide the fungus with a much larger reservoir of susceptible tissues than if infection was merely restricted to wounds and may explain the success of this organism as a pathogen. The fungus colonised tissues prior to lesion development suggesting the early stages of disease development may be unnoticed in *B. coccinea* stands. Disease progress was monitored in four stands. The infection rate was greatest during spring and summer and may have been influenced by increased water use as temperatures increased in spring. The infection rate over two years was rapid and comparable to that of chestnut blight. The duration of the study was insufficient to determine the length of the incubation period of the disease.

Fire was evaluated as a possible management tool for diseased stands. A site in the Stirling Range containing moderate and high levels of mortality was burnt. The quantity of litter fuel was over three times greater in the high mortality area and is likely to have influenced fire intensity which was greater in this part of the stand. High fire intensity in stands with high mortality may lead to incineration of cones on dead plants. Monitoring of survival of inoculum in cankers 10 months after burning revealed a substantial decline in viable ascospores, however complete removal of viable inoculum was not achieved. The importance of unburnt remnants of old stands in the formation of infection foci was assessed by recording disease incidence at intervals from remnants of the old stand at four sites. Disease levels were greatest

This is what we generally aim to do

within 25 m of the old stand and declined with distance from the old stand. Fire regimes which create mosaics of small patches of unburnt vegetation could exacerbate the disease problem.

B. coccinea relies upon seed stored in the canopy for regeneration following burning. A survey was conducted to determine the relationship between stand age and disease intensity and cone storage. Canker was present in 70% of the stands surveyed and all stands over 14 years of age. Stand age accounted for most of the variation in cone storage, however large variations occurred between stands of similar age. The seed bank dynamics of three old and three young stands was assessed. The canopy seed store increased exponentially with age in all the stands, however in one stand seed storage declined after an initial exponential increase as a result of high levels of infection in the stand. Seed loss from cones increased in all stands with cone age and averaged 2.5% in one year old cones and 92% in 9 year old cones. Estimated seed loss is expected to reach 50% within 5 years once canker has initiated death. Monitoring of branch health and cone storage in stands showed that stands may lose seed reserves rapidly once branch death exceeds 50%, due to the rate of seed loss exceeding the rate of cone addition. Patterns of seed increase in stands suggest disease is the main cause of senescence and yield loss in stands.

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1. INTRODUCTION

1.1 *BANKSIA COCCINEA*

Banksia coccinea R. Br. or Scarlet Banksia is a distinctive species with no close relatives (George, 1981). *B. coccinea* grows as a shrub or small tree, usually 2 to 5 m high, but sometimes reaching 8 m. It is found in areas with deep white or grey sand, usually as a component of tall shrublands, along the south coast of Western Australia. The species is famous for its unique scarlet flower, which is valued by the cut flower industry (Burgman and Hopper, 1982). Like many species which grow in the sclerophyllous shrublands of Australia, *B. coccinea* is fire sensitive and regeneration is from seed stored in large woody infructescences (cones) in the canopy (George, 1981).

1.2 CLIMATE OF THE SOUTH-COAST

The climate of the Albany region is typically mediterranean. Average summer temperatures are less than 30°C (Table 1.2.1), however extreme temperatures over 40°C may occur during the summer. Almost 75% of rain falls between May and October at Mettler whereas Albany is wetter throughout the year. There is a steep rainfall gradient from south to north in the area (Fig 1.2.1). The Stirling Range strongly influences rainfall patterns to the north of the area.

Table 1.2.1. Long term average monthly rainfall; and maximum and minimum temperatures at Albany and Mettler.

Month	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEPT	OCT	NOV	DEC
A. Albany-long term averages												
Temp-max	25.4	24.8	23.8	21.8	19.4	17	16.1	16.3	17.7	19.7	21.7	23.9
Temp-min	13	13.9	12.4	10.6	8.4	7	6.2	6.2	6.6	8.1	9.9	11.6
Rainfall	31	28	37	49	64	70	80	65	57	58	48	23
B. Mettler-long term averages												
Temp-max	25.2	25.1	24.2	21.7	18.7	16.6	15.7	15.9	17.4	18.9	20.8	23.5
Temp-min	13.5	14.3	13.3	11.6	9.8	8.1	7.5	7.4	7.9	9.1	10.6	12.3
Rainfall	28	25	28	64	101	104	129	104	81	79	46	24

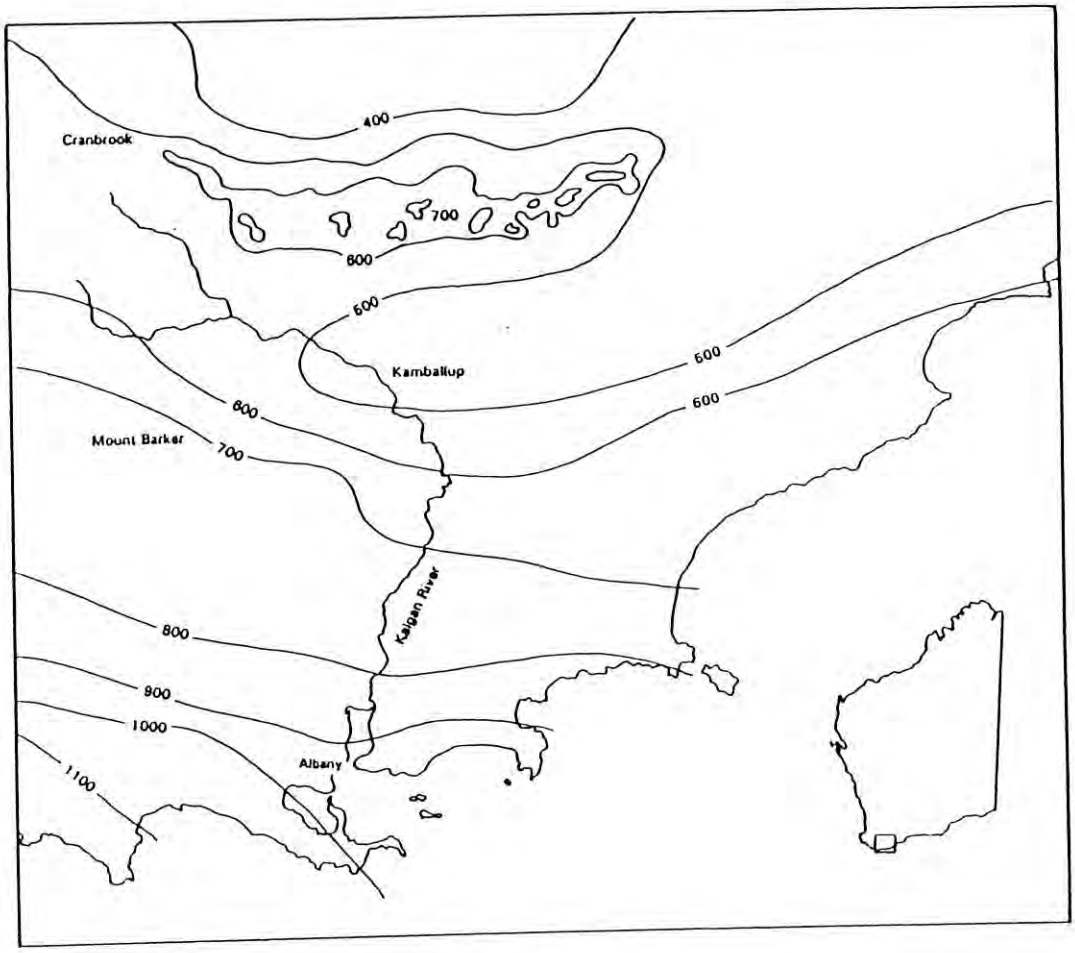

Figure 1.2.1 Annual rainfall isohyets in the Albany region.

1.3 INTRODUCTION TO THE DISEASE

Large numbers of *B. coccinea* were observed dying in 1989. Initially it was thought that plants were being killed by *Phytophthora cinnamomi* Rands, which often occurs in areas where *B. coccinea* grows. Failure to recover *Phytophthora* species and the later observation that plants were dying from the top down, suggested a canker pathogen was probably involved (Shearer and Fairman, 1991). A survey of *B. coccinea* stands revealed that cankers were widespread throughout the geographic range of the species. Four fungi, *Cytospora* sp., *Botryosphaeria ribis* Gross. & Dug., *Diplodina* sp. and *Zythiostroma* sp. were commonly isolated from cankers, with *Bo. ribis* occurring most commonly followed by *Diplodina* sp. Pathogenicity tests confirmed that *Diplodina* sp. was most likely associated with death of *B. coccinea* (Shearer *et al.*, 1995). In recognition of the serious threat the disease poses to *B.*

coccinea and its ability to rapidly kill stands, bush picking of *B. coccinea* was banned from all Crown land from 1991.

Symptoms of disease are initially the drying of leaves on shoot apices or branches and the formation of small dark brown necrotic lesions at leaf nodes. Lesions gradually enlarge, becoming reddish brown, and eventually girdle the stems, causing branch dieback and eventual death of the plant. Small black conidiomata are produced beneath the outer bark in necrotic areas. In moist weather during summer and autumn, pale pink spore tendrils are frequently seen on recently killed portions of stems. Small clusters of perithecia are produced beneath the bark in older cankers.

1.4 RESEARCH OBJECTIVES AND SCOPE OF REPORT

In 1992, funding was obtained from the Australian Nature Conservation Agency to conduct research on canker of *B. coccinea,* with the objective of providing a scientific basis for the management of canker in *B. coccinea* stands. This report details research undertaken between June 1992 and December, 1994 on this project.

2 CAUSAL ORGANISM AND LIFECYCLE

The asexual form of the pathogen was identified by Dr E. Punithalingam of the International Mycological Institute as a species of *Diplodina* Westd. Further work was required to identify the fungus to the species level. The sexual stage of the fungus had not been recorded prior to the commencement of the project.

Cankered branches bearing conidioma of *Diplodina* sp. were examined in 1992. Perithecia of a diaporthaceous fungus were found in cankers. Cultures derived from single ascospores produced the same *Diplodina* sp. previously isolated from cankered tissues, thus establishing an anamorph-teleomorph connection (Kendrick & DiCosmo, 1979). The species was identified as belonging to *Cryptodiaporthe* Petrak on the basis of its valsoid ascomata, prosenchymatous stromatic tissues and thin walled, hyaline, two-celled, ellipsoid ascospores using keys of Barr (1990).

Cryptodiaporthe spp. are known as saprobes and weak parasites of angiosperms. Species tend to be host specific or restricted to closely related species. No *Cryptodiaporthe* spp. have been identified on *Banksia* spp. or other genera within the Proteaceae. The fungus was considered to be a new species, differing from other *Cryptodiaporthe* spp. in host, ascus and ascospore dimensions and stromatic development and was given the new binomial *Cryptodiaporthe melanocraspeda* Bathgate, Barr and Shearer (Bathgate *et al.*, 1995).

The general life cycle of the fungus is shown in Figure 2.1. Fruiting bodies are produced beneath the bark of cankers. Conidioma (asexual fruiting bodies) are produced soon after the death of the plant tissues whereas the development of perithecia is restricted to older cankers. Moisture plays a major role in stimulating spore release in Ascomycetes and Coelomycetes (Ingold, 1971). The conditions required for infection and production of symptoms on *B. coccinea* are unclear, although there is evidence that wounding is not a requirement for infection (see Chapter 4).

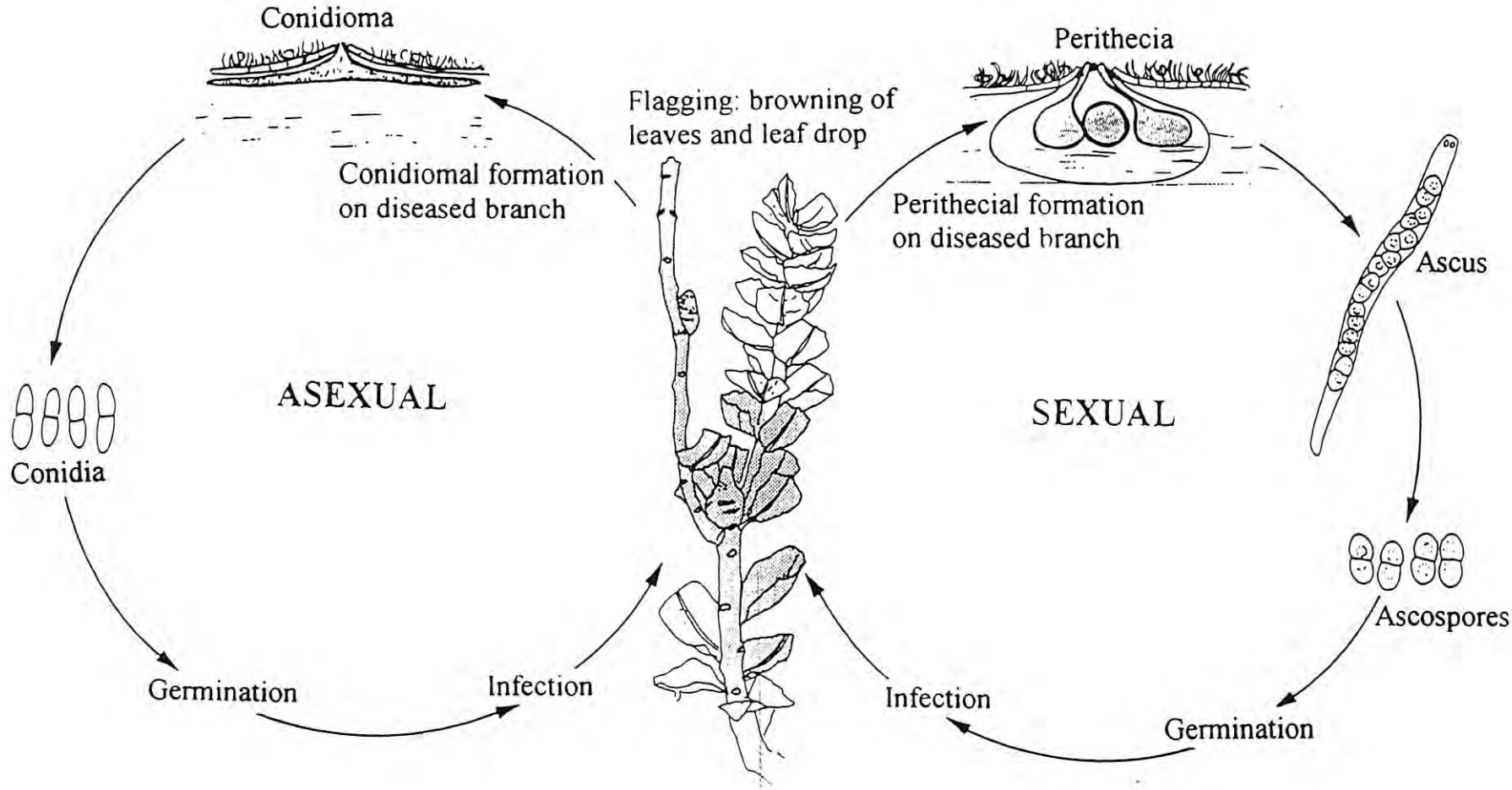

Figure 2.1 The lifecycle of *Cryptodiaporthe melanocraspeda* on *Banksia coccinea*.

3 INOCULUM DYNAMICS

A successful pathogen must be able to sustain high levels of reproduction and possess an efficient means of dispersal and survival prior to infection. Important determinants of disease severity are the type and quantity of inoculum produced, sources of inoculum, and timing and means of dispersal. An understanding inoculum dynamics is fundamental for the planning and implementation of disease management strategies, especially when considering management strategies based on sanitation, such as burning.

3.1 Types of inoculum produced.

The production of two types of inoculum is common in Ascomycetes.
C. melanocraspeda produces splash borne asexual spores called conidia, and wind or splash borne sexual spores called ascospores. Conidia are thought to function as secondary inoculum, whereas ascospores may be important as primary inoculum due to their ability to become airborne. The conidiomal stromata is produced soon after death of the plant tissues. Conidia are exuded in pale pink masses from the conidioma following wetting. Development of the perithecial stromata is delayed and probably does not occur until at least 12 months after death of the tissue. The productive life of the perithecia is not known, nor whether successive generations of perithecia are produced on dead stems.

3.2 Conditions favouring spore release.

Moisture is required for the discharge of ascospores and conidia. Spore trapping was conducted in the Stirling Range National Park to determine a) the importance of airborne inoculum in the epidemiology of the disease; b) whether there was a seasonal pattern of ascospore discharge and if so, c) what was the relationship between spore discharge and environmental conditions. Further work is required to complete this study.

3.3 Other sources of inoculum.

C. melanocraspeda has been isolated from a number of hosts (Table 3.3.1). It is commonly found on *Dryandra cuneata* and *D. falcata* in the Stirling Range National Park, however the incidence of infections on other hosts which grow within the geographic range of *B. coccinea* has not been actively studied. It is not known

whether the teleomorph of *C. melanocraspeda* occurs on alternative hosts. Spread of inoculum from alternative hosts could be important in the introduction of disease into regenerating areas.

Table 3.3.1. Plant species and locations from which *C. melanocraspeda* has been isolated.

Plant species	Location	Source
Banksia. attenuata R. Br.	South west coast, Kings Park	This study
B. baxteri R. Br.	South west coast	This study
B. coccinea R. Br.	South west coast	This study
B. grandis Willd.	Sthn. jarrah forest, south west coast	Shearer *et al.*, 1995
B. menziesii R. Br.	-	Shearer *et al.*, 1995
B. speciosa R. Br.	South west coast	Shearer *et al.*, 1995
Dryandra cuneata R. Br.	South west coast	This study, Shearer *et al.*, 1995
D. falcata R. Br.	South west coast	Shearer *et al.*, 1995
D. sessilis (Knight) Domin	Northern sandplain	Shearer *et al.*, 1995

Seed-borne infections are another possible source of inoculum. Seven percent of seed from cankered *B. coccinea* branches collected from Waychinicup National Park contained *C. melanocraspeda*. *C. melanocraspeda* was not isolated from seeds from healthy branches. The presence of the fungus did not prevent germination, it may therefore be possible for seed to develop into systemically infected seedlings, which later succumb to disease and act as larger inoculum sources. Seed borne infections are important in the epidemiology of diseases caused by Diaporthe species on soy beans (Backman *et al*, 1985) and lupin (Wood and Petterson, 1985).

4 INFECTION OF *BANKSIA COCCINEA* BY *CRYPTODIAPORTHE MELANOCRASPEDA*

Many plant pathogens, especially canker fungi, gain entry into plants through wounds, although direct penetration or penetration through natural openings, such as lenticels and stomata, is possible. Age and growth stage of the host and environmental conditions can also influence the ability of a pathogen to cause disease.

The objective of this study was to determine the pathogenicity of *C. melanocraspeda* in wounded and non-wounded *B. coccinea* stems and to investigate seasonal differences in the susceptibility of plants to infection and lesion formation.

METHOD

Isolation and Culture.

The *Diplodina* sp. culture (JAB63) was isolated from infected *B. coccinea* stem tissue by plating surface sterilised pieces of bark and wood onto half strength potato dextrose agar (HPDA: 7.5g agar, 19.5g potato dextrose agar, 1 L water). Cultures were induced to sporulate by incubating under continuous near-ultra violet (NUV) light at 25°C. For inoculum production, the fungus was cultured on 10% Banksia HPDA (HPDA made with 100 ml banksia extract and 900 ml water; banksia extract: 500g dried chopped *B. coccinea* stems, 1.5 L distilled water, boiled for 60 mins and filtered) and incubated as for the isolations. After 3-4 weeks, conidioma were scraped from the surface of the cultures and macerated either using a pestle and mortar or a homogeniser. The macerate was suspended in 0.1% agar and filtered through 4 layers of gauze. The spore concentration was determined using a haemocytometer and adjusted to 1×10^6 spores per ml. The suspension was refrigerated until used (usually the next day).

Experiment 1

An abandoned *B. coccinea* plantation near Wanneroo, 40 km north of Perth, was used for the first experiment. Plants were 10 years old, 2-3 m high and had many branches. An inspection of the site prior to the study failed to detect *C. melanocraspeda* in the stand, although the fungus had been isolated from Banksia woodlands in Kings Park, Perth. Shoots from the current year's growth were inoculated in January, April, August and October, 1993. At each inoculation date, eight plants were randomly selected. Nine shoots from the most recent growth were selected for inoculation (only six plants were selected in January) within each plant. Six of the shoots were wounded by removing a leaf, the remaining three stems were not wounded. Pieces of cotton wool (approx. 5 mm diameter) which had been dipped in the spore suspension

were placed over the leaf scar on three of the branches. On the three non-wounded branches, similar pieces of cotton wool were placed in the leaf axil. For the controls, a piece of cotton wool which had been dipped in sterile 0.1% water agar was placed over the leaf scars on the remaining shoots. The cotton wool was bound in place with polyethylene wrap or flagging tape. The diameter of inoculated shoots ranged from 3 to 16 mm. Wounds were superficial and did not penetrate to the depth of the cambium. Stems were harvested 2, 6 and 12 months after inoculation.

Experiment 2

A 10 year old natural stand of *B. coccinea* growing in the Stirling Range National Park (Figure 6.2.1, Site 28, SET2) was used in the second experiment. The plants were approximately 1 to 1.5 m high with 2 to 4 branches per plant. Cankers caused by *C. melanocraspeda* were present on 5% of the plants in 1993. Shoots from the current year's growth were inoculated in June, September and December, 1993 and March, 1994. At each inoculation date, 48 healthy plants were randomly selected and within each plant two shoots selected (only 42 were selected in June). Twenty four of the plants were randomly selected and wounded on each shoot, the remaining 24 plants were not wounded. Inoculation of wounded stems was as for experiment 1, with one stem per plant being randomly selected and receiving the spore suspension and the other the control inoculation. For the non-wounded plants, one stem was inoculated with the spore suspension, as in experiment 1, the other stem was untreated to assess the level of natural infections. The diam. of inoculated branches ranged from 3 to 11 mm. Eight wounded and eight non-wounded plants were harvested 2, 6 and 12 months after inoculation.

Assessment

Shoots were removed and examined for cankers. The outer bark around the inoculation site was rubbed with a scourer soaked in 70% ethanol to remove the dense mat of hairs on the surface. The total lesion length (including leaf scar) and length of necrosis above and below the point of inoculation was measured, and tangential spread at the inoculation point was estimated. Zero values are excluded from lesion extension measurements. Isolations from the bark and xylem were made at the inoculation point, lesion margins and 10 to 20 mm beyond the lesion margin. In non-wounded treatments petioles and leaf tissue was also sampled. Tissue pieces were surface sterilised in 70% ethanol for 1 minute, blotted dry, separated at the cambium into xylem and bark portions and plated onto HPDA. Plates were incubated at 25°C for 10 days under continuous NUV light.

Data Analysis

Mean total lesion length of inoculated and control wounds was compared by t-test. The frequency of infection and lesion extension in inoculated stems was compared by chi-square tests. The frequency of lesions in non-wounded treatments was low and analysis of lesion length data was restricted to the wounded treatments. Lesion extension, total lesion length and tangential spread for the inoculated, wounded treatments was compared by analysis of variance. Lesion length and width data were log transformed to satisfy assumptions of normality of data.

RESULTS

Experiment 1

Lesion lengths of inoculated stems were significantly greater than controls (t_{142}=4.21, P<0.001) and *C. melanocraspeda* was only isolated from the phloem and xylem of inoculated stems.

The frequency of reisolation and lesion extension was significantly lower in non-wounded stems than wounded ($\chi 2_{1,1}$=85.0 and 81.4 respectively, P<0.001). Lesions developed in only 11 of 81 non-wounded stems assessed, compared with 63 of 73 wounded stems. Lesions also took longer to develop in the non-wounded stems. Lesions had not developed in non-wounded stems by 2 months and only 24% of those assessed at 12 months had lesions (Table 4.1). Non-wounded stems inoculated in summer did not produce lesions. 83% of wounded stems had produced lesions by two months. *C. melanocraspeda* was reisolated from only 6% of inoculated non-wounded stems after 2 months and 21% of stems at 12 months. (Table 4.1)

Table 4.1 Percent reisolation of *Cryptodiaporthe melanocraspeda* on, and lesion formation in, *Banksia coccinea* stems 2, 6 and 12 months after inoculation at Wanneroo. Data from 4 inoculation dates are combined.

	Non-wounded			Wounded		
Harvest	No. Inoc.	Reisolation %	Lesion %	No. Inoc.	Reisolation %	Lesion %
2 months	30	3	0	29	90	83
6 months	22	27	18	22	87	77
12 months	29	21	24	22	95	100

Inoculation date and harvest time had significant effects on lesion extension, total lesion length and tangential spread in wounded stems (Table 4.2). Lesions were largest in stems inoculated in April and August (Table 4.3). Average lesion extension

in August reached 40.3 mm by 12 months (Table 4.3). Lesions expanded rapidly within 2 months in stems inoculated in April. All wounded stems inoculated in April were harvested after 2 months to prevent spread of the disease into the plantation. Stems inoculated in January and November did not show increases in lesion length or width from 2 to 12 months.

Table 4.2 Analysis of variance for the effect of inoculation time (January, April, August and October) and harvest date (2, 6 and 12 months after inoculation) on (A) lesion extension; (B) total lesion length; and (C) tangential spread after wound inoculation with *Cryptodiaporthe melanocraspeda*.

	df	Sum of squares	Mean square	*F* value	*Pr* > *F*
(A) Lesion Extension					
Time	3	19.3	6.43	9.52	0.0002
Tree(Time)	26	22.3	0.86	1.27	0.2783
Harvest	2	11.3	5.64	8.35	0.0017
Time*Harvest	4	13.1	3.27	4.85	0.0049
Error	25	16.9	0.68		
(B) Total Lesion Length					
Time	3	14.5	4.84	9.53	0.0001
Tree(Time)	27	20.8	0.77	1.51	0.1256
Harvest	2	11.6	5.78	11.38	0.0002
Time*Harvest	4	12.7	3.16	6.22	0.0007
Error	34	17.3	0.51		
(C) Tangential spread					
Time	3	1.82	0.61	5.37	0.0046
Tree(Time)	27	4.22	0.16	1.38	0.1980
Harvest	2	4.35	2.17	19.19	0.0001
Time*Harvest	3	1.66	0.55	4.89	0.0071
Error	34	10.09	0.30		

Experiment 2

Lesion length of inoculated stems was significantly greater than the controls (t_{160}=3.99, P<0.001). *C. melanocraspeda* was isolated from 4% of the control inoculations and 65% of inoculated stems. The fungus was isolated from both bark and xylem tissues.

Table 4.3 Untransformed mean for (A) lesion extension; (B) total lesion length; and (C) tangential spread in *Banksia coccinea* inoculated with *Cryptodiaporthe melanocraspeda* at Wanneroo in January, April, August and October, 1993 and harvested 2, 6 and 12 months after inoculation. Values in parentheses are 95% confidence intervals. na = not assessed.

	Month Inoculated			
	January	April	August	October
(A) Lesion Extension (mm)				
2 months	2.6 (1.4, 4.7)	27.9 (16.2, 48.1)	4.2 (0.9, 20.1)	6.4 (2.9, 14.3)
6 months	2.4 (1.5, 3.8)	na	2.2 (1.5, 3.2)	5.0 (2.1, 11.8)
12 months	2.8 (1.0, 8.6)	na	46.1 (20.3, 105.0)	7.7 (4.6, 12.9)
(B) Total Lesion Length (mm)				
2 months	4.6 (4.2, 5.0)	23.1 (11.5, 46.5)	6.1 (3.0, 12.6)	10.0 (5.5, 18.0)
6 months	5 (5, 5)	na	5.0 (2.9, 8.8)	9.2 (5.1, 16.4)
12 months	7.1 (3.8, 13.3)	na	33.5 (12.0, 93.9)	10.6 (7.2, 15.6)
(C) Tangential Spread (degrees)				
2 months	na	121.5 (91.7, 161.0)	70.2 (56.5, 87.2)	79.7 (67.3, 94.5)
6 months	60.5 (49.5, 74.1)	na	56.6 (43.5, 73.6)	84.5 (75.4, 94.7)
12 months	93.0 (54.3, 159.4)	na	131.9 (81.4, 2136)	103.8 (87.4, 123.3)

The frequency of reisolation of *C. melanocraspeda* and lesion extension was significantly less in non-wounded than wounded stems ($\chi 2_{1,1}$=28.3 and 52.2 respectively, P<0.001). Lesions had developed in only 10% of non-wounded stems 2 months after inoculation, however this increased to 29% by 6 months and 50% by 12 months (Table 4.4). The proportion of unwounded stems from which *C. melanocraspeda* was reisolated was always higher than the proportion which developed lesions, being 17% at 2 months and 73% at 12 months (Table 4.4).

Lesions developed in 83% of wounded stems and *C. melanocraspeda* was reisolated from 85% of inoculated wounded stems during the 2 to 12 month period. There was no trend of increasing frequency of lesion formation or reisolation of the pathogen with time since inoculation in wounded stems (Table 4.4).

Lesion length was highly variable in wounded stems, and ranged from 2 - 900 mm over all inoculation times and harvests. Time, harvest and their interaction had no effect on lesion extension, however there was a significant effect of time on total lesion length and tangential spread (Table 4.5). Stems inoculated in winter produced the longest and widest lesions, averaging 37 mm in length and 140° in tangential spread (Table 4.6). Stems inoculated in summer had the smallest lesions.

Table 4.4 Reisolation of *Cryptodiaporthe melanocraspeda* on, and lesion formation in, *B. coccinea* stems 2, 6 and 12[A] months after inoculation at Stirling Range National Park. Data from four inoculation dates: June, September and December, 1993 and March, 1994, are combined.

	Non-wounded			Wounded		
Harvest	No. Inoc.	Reisolation %	Lesion %	No. Inoc.	Reisolation %	Lesion %
2 months	30	17	10	30	93	90
6 months	31	52	29	29	93	77
12 months	15	73	53	15	87	87

[A]Data for 12 month harvests of plants inoculated in December and March not included.

Table 4.5 Analysis of variance for the effect of inoculation time (June, September, December and March) and harvest date (2,6 and 12[A] months after inoculation) on (A) lesion extension, (B) total lesion length and (C) tangential spread after wound inoculation with *Cryptodiaporthe melanocraspeda*.

	df	Sum of squares	Mean square	F value	*Pr > F*
(A) Lesion Extension					
Time	3	16.811	5.604	2.50	0.069
Harvest	2	1.176	0.588	0.26	0.770
Time*Harvest	4	20.085	5.021	2.24	0.077
Error	52	116.501	2.240		
(B) Total Lesion Length					
Time	3	26.054	8.685	5.23	0.003
Harvest	2	1.767	0.884	0.53	0.590
Time*Harvest	4	13.249	3.312	1.99	0.106
Error	64	106.293	1.661		
(C) Tangential spread					
Time	3	2.548	0.849	2.83	0.046
Harvest	2	0.514	0.257	0.86	0.430
Time*Harvest	4	1.659	0.415	1.38	0.251
Error	64	19.237	0.301		

[A] Data for 12 month harvests of plants inoculated in December and March not included in analysis

Location of infection

The fungus was isolated from both necrotic and healthy tissue in experiment 1 and 2. In experiment 2, the proportion of stems with infections 10-20 mm from the lesion margin increased from 20% at 2 months to 32% at 6 months and 80% at 12 months. Recovery of the fungus from necrotic tissue varied from 93% after 2 months to 87% after 12 months. *C. melanocraspeda* was isolated from bark and xylem tissues in both wounded and non-wounded stems.

Table 4.6 Untransformed mean (A) total lesion length and (B) tangential spread in *Banksia coccinea* inoculated with *Cryptodiaporthe melanocraspeda* in the Stirling Range National Park in June, September, December 1993[A] and March, 1994. Values in parentheses are 95% confidence intervals.

	Month Inoculated			
	June-93	Sept-93	Dec-93	March-94
(A) Tot. Lesion Length (mm)				
All harvests	37.5 (3.4, 409.2)	15.7 (0.7, 340.1)	5.6 (0.5, 58.3)	15.9 (1.7, 146.3)
(B) Tangential spread (degrees)				
All harvests	140.2 (50.4, 390.0)	109.0 (27.4, 433.9)	79.7 (29.1, 218.2)	107.1 (58.0, 197.8)

[A] Data for 12 month harvests of plants inoculated in December and March not included.

DISCUSSION

C. melanocraspeda was pathogenic in both wounded and non-wounded *B. coccinea* stems. Consequently, infections could be initiated simply by a combination of viable inoculum and suitable conditions for infection. Lesion formation was more frequent in wounded tissue after 12 months, however lesion development in wounded stems would be limited by the number of suitable wounded sites and the age of wounds (Biggs, 1989). Observations at field sites confirmed this. Cankers developed as commonly from non-wounded inflorescences as from those which had been wounded by moth larvae (Bathgate, unpublished). *C. melanocraspeda* differs from pathogens such as *Leucostoma* species, that attack peach and other stone fruits, which cannot invade healthy, intact branches. These fungi are considered weak parasites despite their ability to aggressively colonise tissues once they have produced infections (Biggs, 1989).

It is not known how *C. melanocraspeda* infects non-wounded tissues. The petiole stem junction may provide natural weaknesses in the epidermal tissues, however direct penetration of tissues in the leaf, petiole or stem may also be possible. Most

canker pathogens colonise the host plant through open wounds, dead branches, branch stubs, twigs and leaf scars (Biggs, 1992). Infection through non-wounded tissue has been reported for a few canker diseases of perennial plants. For example: Arnold, (1970) showed dieback and cankers formed from inoculation of leaf scars of yellow birch with *Diaporthe alleghaniensis*. The fungus was readily isolated from inoculated tissues after several years. Infection of *Populus* by *C. populea* occurs through leaf scars and bud scale scars and results in bark necrosis developing at the shoot junctions (Gremmen, 1978). Infection of peach trees by *Botryosphaeria dothidea* occurs both in non-wounded tissue, through lenticels, and in wounded tissue (Weaver, 1974), however moisture stress was required for substantial lesion development (Pusey, 1989). There is little histological evidence for infection of non-wounded tissue (Biggs, 1992).

The extent of lesion development in stems was highly variable. Many lesions on wounded stems had been walled off by 12 months, however frequent isolation of the fungus from healthy tissue ahead of the visible lesion margin suggests the response of the host was insufficient to contain the pathogen. The increased isolation frequency with time from non-wounded stems suggests active growth of the fungus within stem tissue rather than dormancy prior to lesion development. Infections at two months may have been superficial and easily removed during surface sterilisation. Increased detection of the fungus at 6 and 12 months corresponded with isolation from both xylem and bark tissues and suggests radial penetration as well as longitudinal growth of the fungus. Measurements of the incidence of canker in *B. coccinea* stands are likely to underestimate the actual incidence of infection by *C. melanocraspeda*, due to the ability of the pathogen to produce symptomless 'latent' infections.

The histopathology of canker development is unknown, however *Eutypa lata* which has a long incubation period in grapevine and apricot may provide a model for further study. Symptoms of branch dieback caused by *Eutypa lata* are not evident until several years after infection (English and Davis, 1978; Moller and Kasimatis, 1978), due to the gradual colonisation of vascular tissues prior to invasion of cambial and cortical tissues to produce a canker (English and Davis, 1978). The 12 month duration of our experiments may have been inadequate to assess the importance of delayed lesion development. This should be considered in the design of future trials using both wounded and non-wounded plants.

Seasonal variations in infection and lesion production in *B. coccinea* may be due to differences in moisture conditions at the time of inoculation. Fungal spores require a period of wetness for germination. Differences in the rate at which moisture evaporated from the inoculum, may have influenced the success of infection.

5 FACTORS INFLUENCING DISEASE INTENSITY

An epidemic may be defined as an increase of disease limited in time and space in a plant population (Zadocks and Schein, 1979). By studying the pattern of epidemics in time and space, the structure and behaviour of rate determining elements can be analysed. In addition, predictions of future trends in disease development and the efficacy of control measures can be assessed through the comparison of disease progress curves.

The aim of this study was to monitor disease progress in *B. coccinea* populations, and to interpret the observed patterns of disease progress in terms of possible rate determining elements.

METHODS

Permanent strip transects were set up in four stands of different age in 1992 (Table 5.1). Transects were 50 m long and 2 to 3 m wide. Incidence of canker and severity of limb dieback were recorded at approximately 2 to 4 month intervals for 2 years. Canker incidence was assessed as the percentage of plants with cankers per stand. Branch dieback was assessed as the percentage of dead limb area per plant.

Values for severity of limb dieback (y) were transformed using the logit transformation $\log_e(y/(1-y))$. Regression analysis was performed to determine the apparent infection rate, r (Vanderplank, 1963), ie. rate of disease increase at each site. Slopes and intercepts of regression lines were compared by analysis of covariance.

Table 5.1 Location of stands and dates of last fires.

Stand Name	Stand No. (Fig. 6.2.1)	Location	Stand age (1994)
CB2	30	Waychinicup National Park	6
HH	51	Hassell National Park	8
CB1	29	Waychinicup National Park	15
SET1	27	Stirling Range National Park.	23

RESULTS

Disease incidence, severity and mortality increased over time in all sites. The rate of increase within years was not constant. The rate of plant death increased substantially between October and January in 1992 and 1993 at SET1 (Fig 5.1 (A)). A similar, but not so pronounced trend occurred at CB1. Increases in mortality at the other sites were small (less than 5% during the study). Disease incidence and severity increased at greater rates during spring and summer at CB1 and SET1 (Fig 5.1 (B) & (C)).

Analysis of the transformed data showed the apparent infection rates were similar at CB2, CB1 and SET1 (Table 5.2, Fig 5.1 (D)). The apparent infection rate at HH was significantly lower, 0.001. Intercepts of CB2, CB1 and SET1 differed.

Table 5.2 Linear regressions of Logit transformed severity of limb dieback against time (days) in four *Banksia coccinea* stands.

Stand	Intercept	Slope	r^2
CB2	-5.314	0.003	0.960
HH	-3.722	0.001	0.707
CB1	-1.406	0.003	0.981
SET	0.181	0.003	0.967

DISCUSSION

A wave-like pattern of disease progress, as seen in Fig 5.1 A-C, was also reported by Shearer *et al.* (1995) in *B. coccinea* stands. The low rates of increase in disease intensity during winter were also confirmed by observations of cankers which in many cases had distinct ridges of callus at their margins. The rate of disease progress increased during spring and summer and may have been related to increased water use by plants as temperatures increased in spring (Table 1.2.1). Death of apricot branches caused by *Eutypa armeniacae* commonly occurred when the transpiration rate was high and was a result of colonisation of the functional xylem (English and Davis, 1978).

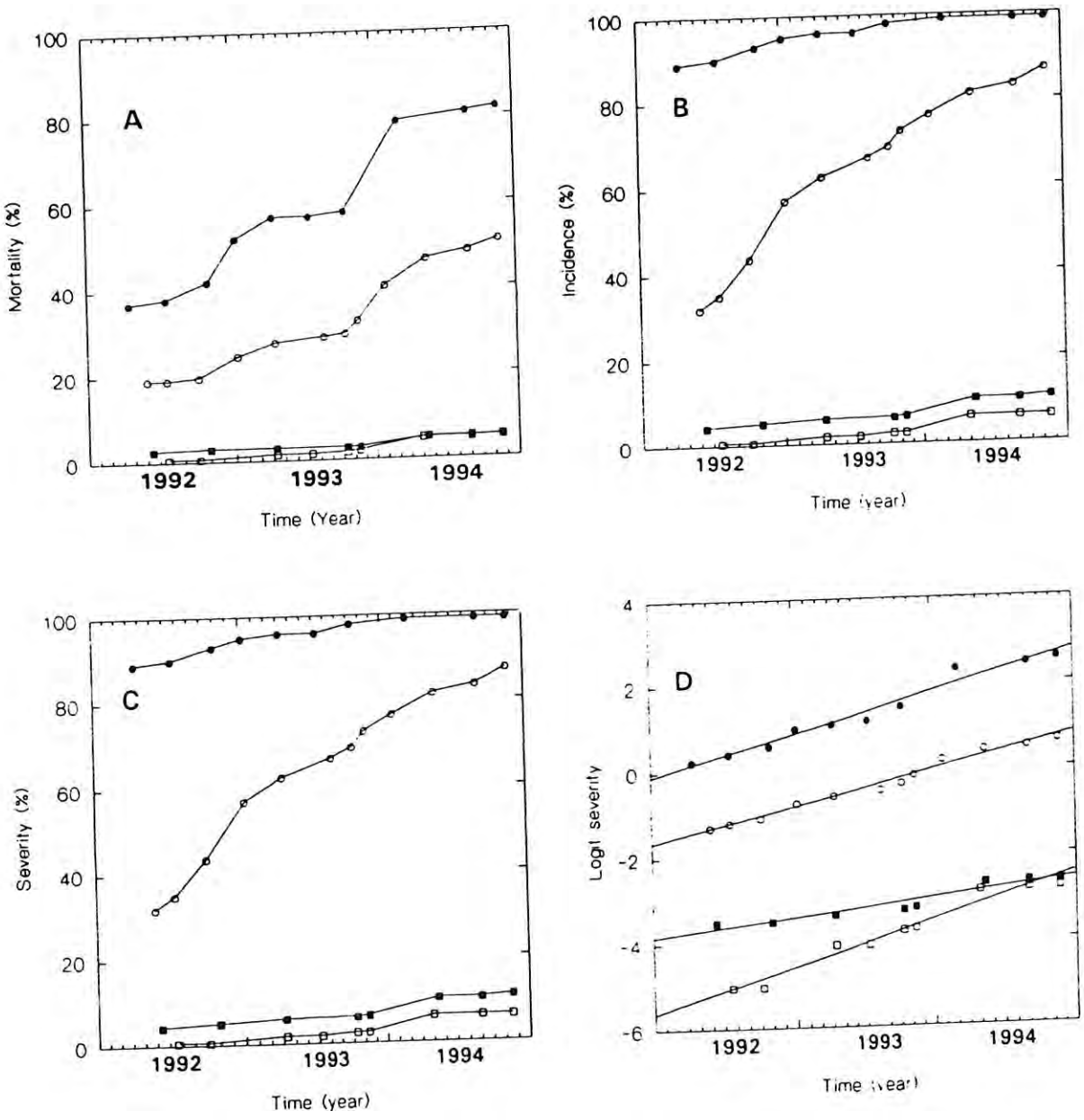

Figure 5.1 Progress in (A). mortality, (B). incidence of limb dieback, (C). severity of limb dieback and (D). Logit transformed severity in four *Banksia coccinea* stands. ● = SET1, O = CB1, ■ = HH and □ = CB2

Disease progress in *B. coccinea* stands was rapid, when compared with progress of Dutch elm disease and comparable with that of Chestnut blight. Berger (1977) presents data comparing the progress of Dutch elm disease at several sites. The maximum rate of progress was r=0.09 per unit per month. The maximum rate of disease progress in *B. coccinea* was r=0.11 per unit per month (=1.28 per unit per year). Roane *et al.* (1986) reviewed research which had calculated rates of chestnut blight progress. The maximum infection rate per year was 1.42 per unit per year. The pathogen has several characteristics which make it a successful pathogen (Schmidt, 1978). Cankers remain infectious for a long time, possibly > 2 years, initially producing secondary inoculum but later primary inoculum. It has a high genetic potential for infection (no sources of resistance in *B. coccinea* have been identified) and it is able to infect without the presence of a wound. Large even-aged *B. coccinea* would also display little functional diversity in terms of the amount and distribution of susceptible tissue (Schmidt, 1978).

The disease progress curves represent only small parts of the entire epidemic. Further monitoring is needed to determine whether (and when) CB2 and HH will enter the exponential phase of disease development and whether the infection rate will remain constant throughout the epidemic.

Many studies have shown drought stress to be a predisposing factor in the development of canker diseases (eg Shoenweiss, 1981; Bachi and Peterson, 1985; Old *et al*, 1990; Vannini and Scarascia Magnozza, 1991). The pattern of disease progress in *B. coccinea* stands suggests that drought stress is unlikely to be a cause of the epidemic. The rate of increase of disease incidence and severity consistently rose during spring and early summer when soil moisture would have been high (Lamont and Bergl, 1991). Studies of other *Banksia* species show that banksias have deep root systems and have access to water stored at depth in the soil, allowing them to maintain high rates of transpiration during the summer and autumn when water is at least supply (Lamont and Bergl, 1991; Dodd and Bell, 1993). We measured pre-dawn xylem pressure potential (XPP) of *B. coccinea* at 11 sites in March, 1993 and found relatively high XPP, -1.10±0.09 MPa, despite the summer having been particularly hot and dry. *B. coccinea* is probably also deep rooted and able to access moisture from deep in the soil, thus maintaining high XPP even under very dry surface conditions. It is unlikely that plants would be under drought stress during autumn except in times of extreme drought (Hnatiuk and Hopkins, 1980).

Wills and Keighery (1994) attributed the disease on the south coast caused by *C. melanocraspeda* to the extreme heat wave which occurred in February 1991. The steady decline of *B. coccinea* stands over the period of this study, and also since 1989 at Cheyne beach (Shearer *et al.*, 1995), provides evidence to the contrary. Outbreaks of canker caused by *Bo. ribis* are, however, strongly linked to plant stress, as was demonstrated by the widespread outbreak of *Botryosphaeria* canker following the 1991 heatwave (Shearer, 1994; Wills and Keighery, 1994). While the episodic occurrence of *Botryosphaeria* canker has caused extensive damage to large stands of vegetation, there is evidence that affected species such as *B. speciosa* (Shearer, 1994) and *B. coccinea* (our own observations) are able to recover after the initial invasion. Such recovery has not been observed in *B. coccinea* stands affected by *Cryptodiaporthe* canker.

A knowledge of the rate of disease development is essential for determining the success of control programs on subsequent disease development. Management strategies, such as burning, interrupt the current disease cycle of the pathogen by consuming inoculum and stimulating regeneration of the stand. Development of disease following fire depends upon the quantity of inoculum and its proximity to the regenerating stand . The aim of management should be to maximise the time between germination and first signs of disease in the stand. Improvements in stand health can only be assessed through long term monitoring of disease intensity.

6. DISEASE MANAGEMENT

6.1 Fire management of diseased stands.

There are two major reasons why fire may offer an effective means of managing diseased *B. coccinea* stands. Firstly, *B. coccinea* is killed by fire and regenerates solely from seed stored in the canopy (George, 1981). Providing seed borne infections are not high, the new generation of plants will commence growth in a generally disease free condition. Secondly, fire offers a means of reducing the amount of infectious plant material, thereby leaving fewer sources of inoculum.

The major factor that may limit the effectiveness of fire management, is the degree to which it reduces inoculum levels. If stands regenerate in an environment where inoculum densities are high, disease development will occur at an earlier stand age than if inoculum densities are low or nil. Fire regimes must be based on a knowledge of what burning conditions are required to eliminate infectious plant material. Components of the fire regime, such as fire intensity, season and patchiness, can be altered to address an aim such as inoculum removal.

The aim of this section was to a) provide an interpretation of fire behaviour in a *B. coccinea* stand in terms of disease and fuel characteristics; b) determine whether burning reduced the viability of inoculum of *C. melanocraspeda*; c) determine the critical temperature × time regimes for death of inoculum within host tissue; and d) determine the importance of unburnt remnants of *B. coccinea* stands in the formation of infection foci in regenerating stands.

6.1.1 RED GUM PASS BURN

The aim of this study was to interpret fire behaviour at Red Gum Pass in terms of stand and fuel characteristics.

HYPOTHESIS → (clear statement of null hyp)

METHODS

An area (RPN1) near Red Gum Pass Road in the Stirling Range National Park (Site 41, Fig 6.2.1) was burnt on 26 October 1993. The area was last burnt in 1969, making the stand about 23-24 years old at the time of the study. A younger *B. coccinea* stand (RPN2, Fig 6.2.1, Stand 42), which was last burnt in 1983, and was

9-10 years old was located to the south of the 24 year old stand. Disease levels ranged from moderate (42% mortality) to high (100% mortality) in different parts of RPN1. These parts of the stand are referred to as RPN1M and RPN1H hereafter. Percent mortality in RPN2 was 17%. Pre-fire weather conditions and calculated surface moisture contents (Sneeuwjagt and Peet, 1979) are shown in Table 6.1.1.1

Table 6.1.1.1 Summary of weather data used to calculate surface moisture content (SMC) at RPN1.

Date	Max. Temp. °C	Rel hum Min %	Rel hum Max %	Rainfall mm	SMC %
22/10/94				7.2	60[A]
23/10/94	20	41	100	0	35
24/10/94	23	41	100	0	23
25/10/94	23	39	100	0	20
26/10/94 [B]	22	41	100	0	19

[A] assumed following 7.2 mm rain.

[B] day of burn.

Estimates of litter and standing fuel biomass were made from within 0.49 m^2 quadrats in representative parts of RPN1M, RPN1H and RPN2 which were not burnt and in the unburnt younger stand after the fire. Biomass of fuels remaining after the fire were also estimated and the quantity of fuel consumed was calculated. Parts of the stand adjacent to Red Gum Pass Road had very high levels of disease and fuels were assessed separately in this part of the stand. Height and cover of the shrub layer were estimated visually. Fire intensity was estimated from average flame heights using equations from Burrows (1994). Flame height was assumed to be equal to char height for these calculations.

RESULTS AND DISCUSSION

Fuel characteristics are presented in Table 6.1.1.2. RPN1H had significantly more litter fuel than the other sites ($F_{2,11}$= 28.6, P<0.001). The biomass of litter at this site was twice that of the RPN1M and over three times that of RPN2. The differences in shrub (aerated) fuels was less pronounced although there was still a significant difference between stand types ($F_{2,12}$=6.6; P=0.01). Fire consumed all litter fuels. Consumption of aerated fuels was almost three times higher in RPN1H than RPN1M. The intensity of the fire varied from low (400 kW m-1) in RPN1M to moderate (877 to 1380 kW m-1) in RPN1H. The predicted SMC (Table 6.1.1.1) is probably an

overestimate of actual SMC at the site. This relationship was developed for Jarrah forest with 60% canopy cover. The vegetation at the site was a low open woodland and would probably have been drier than predicted by tables for standard Jarrah forest conditions (McCaw, pers. comm.).

Table 6.1.1.2. Mortality and density of *Banksia coccinea* and fuel characteristics at the Red Gum Pass Road sites.

	Stand name		
	RPN1M	RPN1H	RPN2
Last burnt	1969	1969	1983
B. coccinea mortality (%)	42	100	17
B. coccinea density (stems/ha x 1 000)	103.8	103.8	20.4
Shrub height (m)	1.5-3	1.5-3	1-2
Shrub cover (%)	75	75	25
Shrub biomass (T/ha)	30.7	20.1	18.9
Litter biomass (T/ha)	11.7	23.2	6.5
Litter fuel consumed (%)	100	100	-
Shrub fuel consumed (%)	14.0	40.9	-
Flame height (m)[A]	1	2-3	-
Scorch height (m)	4-6	8-10	-

[A] assumed equal to char height.

Variations in the quantity of litter fuel in the moderate and high disease areas was probably a major determinant of fire intensity in the stand. High mortality and density of *B. coccinea* combined to create a litterbed dominated by a large quantity of dead stems and leaves. Spatial variability in fire severity, related to variations in fuel distribution and type, is common in shrub and woodland communities in the south-west of Western Australia (Hobbs and Atkins, 1988).

In terms of managing diseased stands of *B. coccinea,* high fire intensity in stands containing high proportions of dead plants may have a detrimental effect on seed survival. Seed may be exposed to lethal temperatures and cones may be incinerated, leading to greater seed losses than in stands with low levels of mortality. High levels of mortality occurred in *Hakea dactyloides* seed when fruits were directly exposed to flames and experienced external temperatures higher than 400°C (Bradstock *et al.*, 1994). Incineration of cones was found to contribute to seed loss in *B. burdettii*, with

cone incineration increasing from 0% in 1 year old cones to 90% in 8 to 12 year old cones (Lamont and Barker, 1988). As disease increases in *B. coccinea* stands, the proportion of the total seed store in older cones would rise as the production of new cones declines, in addition loss of seed reserves from natural release of seed and increased susceptibility of older cones to incineration could lead to stands which fail to regenerate following fire. This point will be examined further in 6.2.

6.1.2 INOCULUM SURVIVAL IN BURNT STANDS

The aim of this study was to determine i) whether burning reduced the viability of inoculum of *C. melanocraspeda* and ii) the critical temperature × time regime for death of inoculum within host tissue.

Hypothesis?

METHODS

Experiment 1

This experiment was carried out at RPN1, in burnt and unburnt sections of the stand (see Section 6.1.1). Survival of inoculum was assessed in August 1994, 10 months after the fire, in burnt and unburnt parts of the stand. Only plants where flame height had reached 1-1.5 m were sampled in the burnt part of the stand as prior sampling had indicated that inoculum survival in charred stems was nil. Cankered stems which appeared to have fruiting bodies of *C. melanocraspeda* were collected from uncharred regions of the plants.

Stems (30 cm lengths) were prepared for assessment of inoculum viability by moistening with tap water 1-3 hrs before examination. The presence of conidioma and perithecia was assessed by scraping off the outer bark in areas where ostioles were protruding through the bark. Perithecia were assessed as active if they contained a clear mucousy fluid or inactive if the contents were either empty or hard and yellow. Conidioma were assessed as active if they contained white to pale pink spore masses or inactive if they were empty or decomposed. The contents of four perithecia or conidioma per stem (if available) were removed, mixed with a drop of water and streaked over the surface of a petri plate containing 2% water agar (WA). Plates were incubated at 20°C for 12 hrs. After incubation, the percentage of germinated ascospores and conidia in each sample was determined.

Experiment 2

Inoculum survival was assessed at seven temperatures (50, 75, 100, 125, 150, 175, 200°C) and four durations of temperature (5, 10, 15, 20 min), and an unheated control was also included. Five approx. 50 mm stem pieces of *B. coccinea* containing perithecia and five similar stem pieces containing conidioma were used for each temperature by duration combination. Stem pieces were placed on aluminium foil dishes and heated in a controlled temperature oven. On withdrawal from the oven stems were allowed to cool under ambient conditions. Stem pieces were then moistened and spores were sampled from three fruiting bodies per stem and streaked onto agar plates containing 2% WA. Plates were incubated at 20°C for 12 hr before determining the percentage spore germination from a count of 100 spores per sample. Percentages were transformed to angles (arcsin √%).

RESULTS

Experiment 1

The proportion of stems containing perithecia and conidioma were the same in both the unburnt and burnt parts of the stand (Table 6.1.2.1), however there were significantly more active perithecia (ie. perithecia containing mucous) in the unburnt stand ($P<0.001$, Table 6.1.2.1). There were significantly less stems which contained viable ascospores in the burnt stand ($P<0.001$). Ascospore viability was significantly less in the burnt stand ($t_{34}=4.97$, $P<0.001$), being only 2.8% compared with 26.0% in the unburnt stand.

Experiment 2

Germination of ascospore and conidia of *C. melanocraspeda* was reduced by exposure to 100°C for 15 or more minutes and 125 - 200°C for 5 or more minutes (Fig. 6.1.2.1). Germination of ascospores and conidia at 50 and 75°C was similar to the unheated controls.

Table 6.1.2.1 Contingency table comparing the occurrence of (A) perithecia and (B) conidioma; activity of (C) perithecia and (D) conidioma and viability of ascospores (D) in samples from burnt and unburnt areas parts of RPN1.

	Unburnt		Burnt			
	Observed	Expected	Observed	Expected	$\chi 2$	*P*
(A) Perithecia						
Absent	6	5	3	4	0.81	N.S.
Present	19	20	19	18		
(B) Conidioma						
Absent	6	7	8	6	0.85	N.S.
Present	19	18	14	15		
(C) Perithecial activity						
Absent	2	8	15	8	15.5	<0.001
Present	17	11	5	11		
(D) Conidiomal activity						
Absent	15	16	13	12	1.21	N.S.
Present	4	3	1	2		
(E) Viable ascospores						
Absent	11	16	20	14	11.4	<0.001
Present	14	14	2	7		

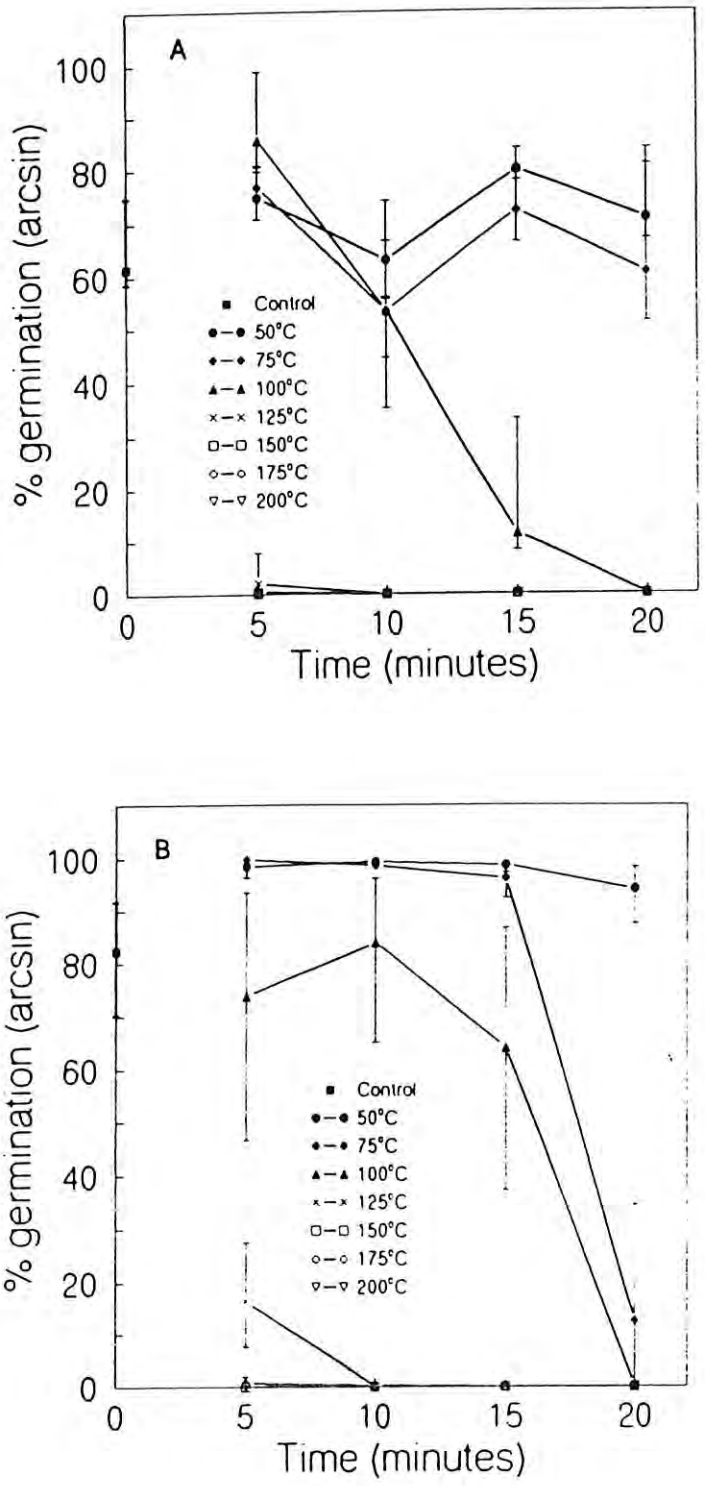

Fig 6.1.2.1 Germination of (A). ascospores and (B). conidia of *Cryptodiaporthe melanocraspeda* from stems heated at seven temperatures: 50, 75, 100, 125, 150, 175 and 200°C and four temperature durations: 5, 10, 15 and 20 min. Germination of spores from untreated stems is shown on the Y-axis. Bars represent ± 1SE.

DISCUSSION

The low post-fire viability of ascospores indicates that complete combustion of cankers is not a requirement for inoculum reduction in cankered stands. Hot gases rising above the flames were sufficiently hot to kill both ascospores and conidia of *C. melaocraspeda*. The lethal temperature for plant cells is around 60°C for 60 secs (Kayll, 1966; cited Bradstock *et al.*, 1994). Fruiting bodies of *C. melanocraspeda* are located within the outer 0.5 - 1 mm of bark. This thin covering of bark would provide a little insulation from the heat of the fire, and the low moisture content of dead stems would further add to this. Studies of heat transfer through living bark indicate cambial death would occur in less than 60 secs in stems with < 2 mm thick bark after exposure to a heat source of around 750°C (Vines, 1968). Temperatures above the flaming zone are unlikely to exceed 400°C (Bradstock *et al.*, 1994, Hobbs and Atkins, 1988). The scorch heights that were recorded in the burnt area exceeded the height of the *B. coccinea* canopy, indicating that temperatures in the canopy exceeded the minimum for scorching leaf tissue (ie approx. 60°C for 60 secs). Lethal temperatures for conidia and ascospores of *C. melanocraspeda* have not been determined. Further work is required to assess survival of inoculum at temperature above 200°C and for shorter heating times.

The importance of the 9% of stems with viable inoculum in the epidemiology of the disease is unknown but would be expected to be low. The small size of seedlings (< 30 mm high in August when the study as conducted) would make them inefficient interceptors of spores compared to large leafy plants (Ingold, 1971). There was no evidence of production of new fruiting bodies on old cankers in the burnt stand. Consequently survival of the pathogen would probably decline as the remains of the stand are recolonised by saprophytes (Gibbs, 1980). Sections 3.3 and 6.1.3 discuss other sources of inoculum which may be important in regenerating stands.

6.1.3 UNBURNT STAND REMNANTS AS INFECTION FOCI

The aim of this study was to determine the importance of unburnt remnants of *B. coccinea* stands in the formation of infection foci in regenerating stands.

METHODS

Four sites where patches of *B. coccinea* were left unburnt by the most recent fire were selected. The age of the old and new stand were determined either from fire

history information or stem increment counts (Lamont, 1985). Incidence of limb dieback was assessed in the old stand and in the young stand at i) 0-25 m, ii) 25-100m and iii) 100+ m from the old stand. Disease was assessed in four replicates of 20 plants in each of the four areas. At RPN2 and RPS2, disease was assessed at 25-50 m and 50-100 m and data were combined for analysis to give 8 replicates at the 25-100 m distance category. Average incidence of limb dieback in the young stand were analysed as a two factor ANOVA with four sites and three distances. Data were transformed to angles (arcsin √%) prior to analysis

Table 6.1.3.1. Locations of stands, dates of last fire and disease incidence in the old stands at RPN2, RPS2, CB2 and SET2.

		Old stand		Young stand	
Location	Site No (Fig. 6.2.1)	Last fire	Incidence[A] (%)	Last fire	Stand age (years)
RPN2	42	1969	63	1983	9-10
RPS2	34	1969	51	1983	9-10
CB2	30	1981	62	1987	6
SET2	28	1969	70	1984	9

[A] estimate from Section 6.2.

RESULTS AND DISCUSSION

Site, distance and the site × distance interaction had a significant effect on incidence of limb dieback (Table 6.1.3.2). Disease intensity was greatest at all sites 0-25 m from the old vegetation and least 100 m or more away it (Fig. 6.1.3.1). There were differences between sites in disease intensity at 0-25 m, with means varying from 13 to 55%, however at 100+ m, all sites had low incidence of disease (0 - 6.5%). Disease incidence and severity in the old stands was high, with 51 to 70 % of the plants having limb dieback.

Table 6.1.3.2 Analysis of variance results of mean disease incidence at 4 sites at three distance ranges from old vegetation.

Source	Sum of squares	d.f.	Mean squares	*F*	*P*
Sites	3174	3	1058.112	15.674	<0.001
Distance	7557	2	3778.527	55.972	<0.001
Interaction	1478	6	246.420	3.650	0.005
Error	2970	44	67.508		

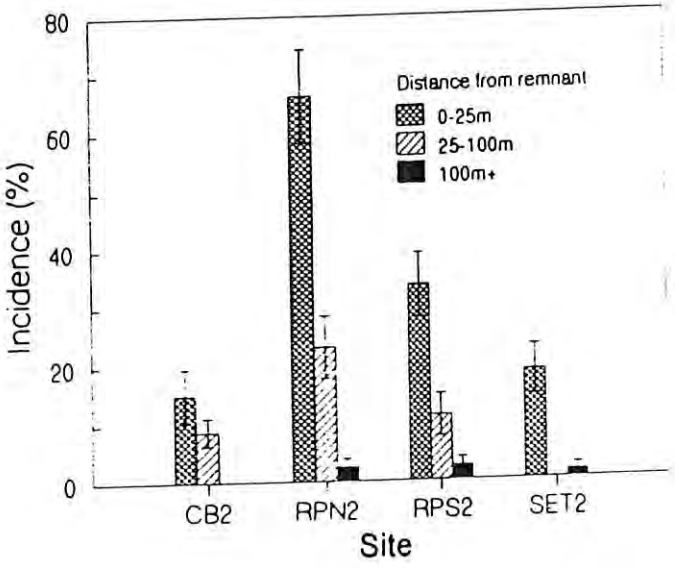

Figure 6.1.3.1 Average incidence of limb dieback of *Banksia coccinea* caused by *Cryptodiaporthe melanocraspeda* at three distance ranges from old stand remnants at four sites: CB2, RPN2, RPS2 and SET2. Bars indicate ± 1SE.

The close association between the incidence of limb dieback and distance from the old stand indicates that old unburnt vegetation is acting as an infection foci. Levels of disease were high in the older stands, so there would be many dead limbs to act as sources of ascospores and conidia. The age of the regenerating stands was between 7 and 11 years. CB2 was the youngest stand and also had least infection. This pattern of disease development early in the epidemic is termed a focal epidemic (Zadoks and Schein, 1979) and contrasts with general epidemics where disease develops from well dispersed initial inoculum.

The degree of patchiness resulting from a fire is the product of many site factors including variation in fuel load and chance and can vary in scale from meters to tens of meters (Williams *et al.*, 1994). Patchiness can also vary in terms of the amount of vegetation consumed (Williams *et al.*, 1994). In 6.1.2., it was shown that large reductions in inoculum level occur following scorching of the canopy, and complete consumption of cankers is not required to significantly reduce levels of viable inoculum. Fire regimes, however, which result in patches of vegetation remaining unburnt, will create infection sources for the regenerating stand. The scale of distance of spread of the disease (Vanderplank, 1963) is relatively small, with disease incidence dropping to between 0 and 6.4% within 100m of the infection source even 9-10 years after the stand was burnt.

Three outcomes may arise from burning a *B. coccinea* stand. 1. The entire stand is burnt, destroying the inoculum. 2. Part of the stand is burnt, leaving a block of burnt vegetation adjacent to a block of unburnt vegetation. 3. Burning results in a mosaic of regenerating plants and living remnants of the old stands. In the first situation,. disease development would be dependant upon long distance dispersal of inoculum into the stand from nearby *B. coccinea* stands or alternative hosts. In situation 2, a dispersal gradient would develop along the boundary between the old stand and the regenerating stand, which, by 7-11 years, would take the disease about 100m into the younger stand. In situation 3, dispersal gradients would develop around the patches of older vegetation. If the distance between patches is < 200m, then by 7 to 11 years, disease foci would have commenced to merge. Clearly in order to minimise losses where management objectives require mosaics of young and old vegetation, patch sizes should be in the order of hectares if the whole stand cannot be burnt, and fire intensity should at least cause complete scorching of the *B. coccinea* shrub layer.

6.2 SEED BANK DYNAMICS OF *B. COCCINEA* AND THE IMPACT OF DISEASE ON SEED PRODUCTION AND STORAGE.

INTRODUCTION

Frequent fires are a common feature of the sclerophyllous shrublands of Western Australia. Results presented in Section 6.1 indicate that burning offers a means of reducing levels of infectious plant material in *B. coccinea* stands. *B. coccinea* is killed by fire, regeneration after fire is by seed stored in fire-resistant cones in the canopy (George, 1981; Witkowski *et al.*, 1991). The success of regeneration following fire will depend on sufficient numbers of viable seeds being available for stand replacement. Canker has the potential to reduce seed storage, firstly by reducing cone accumulation through branch death and secondly by reducing the longevity of seed in cones on dead branches and plants.

Witkowski *et al.* (1991) determined canopy seed storage on the eastern edge of the geographic range of *B. coccinea*, and found that *B. coccinea* accumulated seed rapidly in its first 10 years. Seed production tended to peak at 16 years and then declined as the plants senesced and died. The authors suggested canker may have been the cause of senescence and death and that a period of 16 years between fires was necessary for stand replacement of *B. coccinea*. Similar studies have not been conducted in the major area of occurrence of *B. coccinea*, between Albany and Bremer Bay and north to the Stirling Range. There is also no information on the effects of disease on yearly seed production and total seed storage.

The objectives of this study were (1) to determine the relationship between stand age and disease intensity through a survey of major stands of *B. coccinea* and (2) to determine the rate of cone and branch production in stands of varying age; and to evaluate the effect of *Cryptodiaporthe* canker on seed production, seed storage and branch production.

MATERIALS AND METHODS

Stand survey

Fifty stands within the main area of occurrence of *B. coccinea* were selected and surveyed between March and October 1993 (Figure 6.2.1; stands 1 to 50). The sites were selected to include a range of stand ages from 2 to 25 years of age and covered

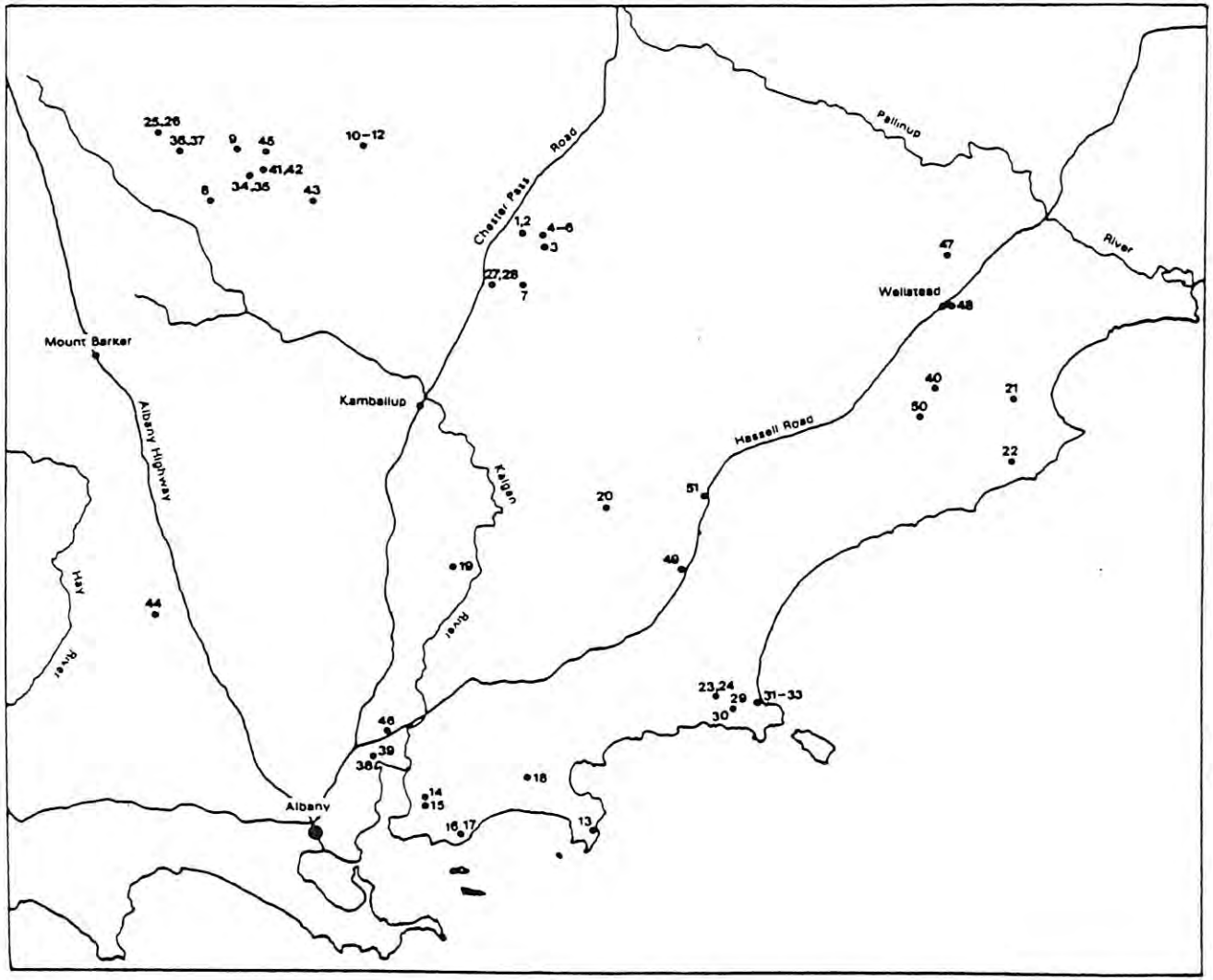

Figure 6.2.1 Location of *Banksia coccinea* stands surveyed in 6.2 and referred to elsewhere in this report. Numbers refer to stand numbers in Appendix 1.

0.5 hectares or more, with over 200 plants/ha. Sites included both roadside stands and stands located well within the bush.

Incidence of canker, severity of limb dieback, plant age, number of fertile cones per plant, plant density and height were recorded in each site. Canker incidence was assessed as the percentage of plants with cankers per stand. Branch dieback was assessed as the percentage of dead limb area per plant. Plant age was assessed by node counts (Lamont, 1985). Fertile cones were considered to be cones with developed follicles (Witkowski *et al.*, 1991); cones which had shed all their seed were not counted. Height was estimated visually and stands were rated by their maximum height according to the scale, 1 = <1m; 2 = 1-2 m; 3 = 2-3 m; 4 = 3-4 m and 5 = >4 m.

The effects of stand age on disease incidence, severity and mortality was examined by analysis of variance. Stands were grouped by age for analysis: 1 = < 5 yrs; 2 = 6-9 yrs; 3 = 10-13 yrs; 4 = 14-17 yrs and 5 = 18 + yrs. Percentages were arcsine transformed prior to analysis. The effect of six variables: stand age, height rating, density, disease incidence, severity and mortality on the number of cones per plant and the number of cones per hectare (both log transformed) was assessed through stepwise regression analysis.

Cone and follicle production and canopy stored seed.

Three sites containing two stand ages were selected for the study (Table 6.2.1). Cankers were present in the stands in the Stirling Range National Park. Sampling was

Table 6.2.1 Location of *Banksia coccinea* stands and date of last fire and stand age.

Location	Stand No. (Fig 6.2.1)	Stand name	Date of last fire	Stand age (yr)
Stirling Range National Park	28	SET2	1983	9
Stirling Range National Park	27	SET1	1969	21
Gull Rock National Park	15	GR2	?	11
Gull Rock National Park	52	GR1	?	36
Stirling Range National Park	42	RPN2	1983	11
Stirling Range National Park	41	RGN1M	1969	20
Stirling Range National Park	41	RPN1H	1969	20

? = no records available, age estimate from node counts (Lamont, 1985)

conducted in the moderate and high disease parts of RPN1 which were discussed in Section 6.1.2. GR1 and GR2 were canker free.

Stand density was estimated by counting the number of *B. coccinea* individuals in four 5 x 5 m plots in each stand. In the RPN1 stand, density was estimated in seven 3 x 3 m plots due to higher stand densities. The height and number of branches was measured of 20 plants in each stand. The incidence (percentage of cankered plants) and severity (average percent limb dieback) of disease was also assessed in each stand.

Thirty plants were sampled from SET2, GR2, RPN2 and RPN1 for seed bank assessment. Fewer plants were sampled from SET1 (17 plants) and GR1 (5 plants) due to the larger, bushier form of these plants. Sampling was carried out as outlined by Witkowski *et al.* (1991). Cones were removed from each years growth and the number of fertile and infertile cones from each year was determined. At RPN1H cones were not separated by age due to the difficulty of aging cones on dead plants.

Ten cones (or all the cones if the total was < 10) from each years growth were burnt to rupture the follicles. Cones were soaked overnight and dried for 24 hrs at 50°C to assist seed extraction after burning. The number of empty follicles and follicles containing healthy, predated or aborted seed was recorded. Seed viability was assessed in two replicates of 30 seeds from each year's cones. Seeds were surface sterilised in chlorox (5% w/v sodium hypochlorite, 70% ethanol) for 5 mins, rinsed in sterile water, and placed on filter papers over vermiculite in petri plates. The filter papers were moistened with sterile water and the plates were incubated at 15°C. Germination was assessed over 50 days.

Differences between stands was compared by one way analysis of variance, and means were compared by Tukey's HSD test. Where necessary, data were normalised prior to statistical analysis by log transformation. The mean number of seeds produced was regressed against plant age.

Assessments of cone and branch production over 3 years

Permanent strip transects were set up in four stands of different age in 1992 (Table 6.2.2). Transects were 50 m long and 2 to 3 m wide, and were divided into five 10m long plots.

The average age and number of *B. coccinea* plants per plot was assessed. The number of fertile cones (cones with at least 1 closed, fully developed follicle) from the current year, the total fertile cone store, number of blooms and number of living and dead branches per plant was measured in each plot in 1992, 1993 and 1994. Cones counted in each year were those produced from flower heads in the previous year. Cones produced from 1994 flower heads were not mature at the time of the study. Numbers produced in 1994 in SET1 and CB1 were estimated using mean values for percent flower heads setting fruit from the previous two years.

Table 6.2.2 Location of stands and dates of last fires.

Location	Stand No. (Fig 6.2.1)	Stand Name	Stand Age
Waychinicup National Park	30	CB2	6
Hassell National Park	51	HH	8
Waychinicup National Park	29	CB1	15
Stirling Range National Park	27	SET1	23

Data from years and sites were combined and analysis tested whether relative cone production, RCP (RCP = new cones / total cones stored), was related to stand age, number of alive branches per plant, percentage alive branches and stand density variables. Quantitative relationships between cone production and independent variables were examined with correlation and regression analysis.

RESULTS

Stand survey

The stands surveyed varied in age from 2 to 25 years. Aerial canker was widely distributed, being present in over 70% of the stands. The disease was most frequently observed in older stands, and cankers were present in all stands over 14 years of age. Only 22% of stands less than 5 years old contained cankers (Table 6.2.3). The percentage of highly infected stands (severity >25%) increased from nil in 0-9 year old stands to 63% in stands over 14 years old (Figure 6.2.2). Large variations in disease intensity occurred between sites of similar age. This was most apparent in stands aged 10-13 years in which 27% of the sites had no disease and 36% had >25% severity (Figure 6.2.2).

Analysis of the disease data grouped by stand age revealed a significant effect of stand age group on percent mortality and disease incidence and severity ($P<0.01$; Table 6.2.4). Three sites (one in each of age group 3, 4 and 5) had very high levels of disease (>80% mortality) and appeared as outliers in the analysis. The outcome of the analysis was not affected by removal of the outliers so they have been retained in the analysis presented here. Mortality in age groups 1 and 2 averaged 3% and 6% respectively, compared with over 25% in groups 4 and 5. Disease incidence and severity showed a similar pattern with stand age. Low average incidence and severity, less than 15%, was found in age groups 1 and 2, and high values, over 40 and 30% respectively, in age groups 4 and 5.

Table 6.2.3 Mean, maximum, minimum and standard error of (A) mortality, (B) canker incidence and (C) severity of limb dieback in 50 *B. coccinea* stands.

Age Group	No. sites	Mean	SE	Range
(A) Mortality				
1 (2-5 yrs)	9	2.9	2.1	0.0-18.4
2 (6-9 yrs)	11	6.0	2.6	0.0-22.8
3 (10-13 yrs)	11	15.9	5.2	0.0-56.8
4 (14-17 yrs)	10	33.1	8.0	0.0-90.0
5 (18 + yrs)	9	26.8	7.8	0.0-79.4
All stands	50	16.8	2.9	0.0-90.0
(B) Incidence				
1 (2-5 yrs)	9	2.0	1.4	0.0-10.0
2 (6-9 yrs)	11	12.3	3.9	0.0-37.8
3 (10-13 yrs)	11	27.6	7.2	0.0-70.6
4 (14-17 yrs)	10	54.8	6.2	20.2-90.0
5 (18 + yrs)	9	45.7	7.8	8.1-90.0
All stands	50	27.7	3.6	0.0-90.0
(C) Severity				
1 (2-5 yrs)	9	1.7	1.2	0.0-10.0
2 (6-9 yrs)	11	9.9	3.1	0.0-27.3
3 (10-13 yrs)	11	20.5	5.8	0.0-62.0
4 (14-17 yrs)	10	39.2	6.7	10.0-90.0
5 (18 + yrs)	9	33.9	7.6	5.7-84.2
All stands	50	20.9	3.0	0.0-90.0

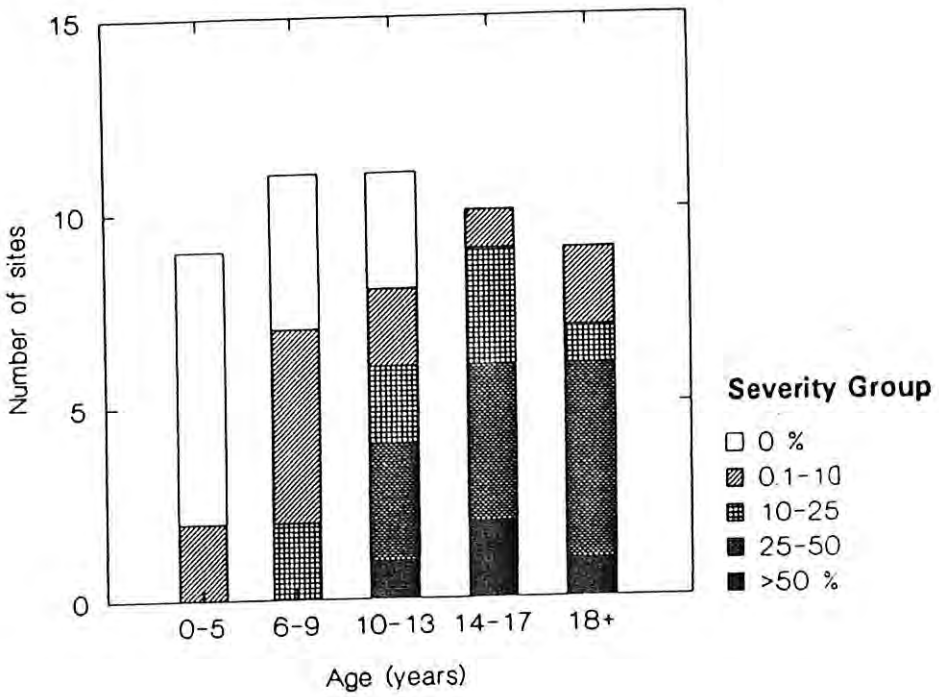

Figure 6.2.2 Frequency of *Banksia coccinea* stands grouped by age in five severity of limb dieback classes: 0, 0.1-10, 10-25, 25-50 and >50 % disease severity.

Table 6.2.4 Analysis of variance of the effect of stand age group on (A) mortality, (B) canker incidence and (C) severity of limb dieback on age.

Source	Sum of squares	d.f.	Mean squares	*F*	*P*
(A) Mortality (%)					
Age group	6603.1	4	1650.8	5.2	0.002
Error	14263.9	45	317.0		
(B) Incidence (%)					
Age group	17244.1	4	4311.0	12.7	0.000
Error	15256.3	45	339.0		
(C) Severity (%)					
Age group	9506.0	4	2376.5	8.2	0.000
Error	13108.0	45	291.3		

The number of cones stored per plant was highly variable (Figure 6.2.3). Stands with the lowest numbers of cones (less than one cone per plant) ranged in age from 2 to 18 years (Figure 6.2.3). Stands over 17 years of age had most cones and four of 11 stands had 5 or more cones per plant. Cones per plant averaged 1.9 in stands which had passed the juvenile stage (ie. were over 5 years old) and 2.3 in stands over 9 years of age (Table 6.2.5). Results from stepwise regression analysis of six independent variables on log cones per plant resulted in 53% of the total variation in cones per plant being explained by two variables, stand age and density (Table 6.2.6). Stand age had the greatest effect on cones per plant.

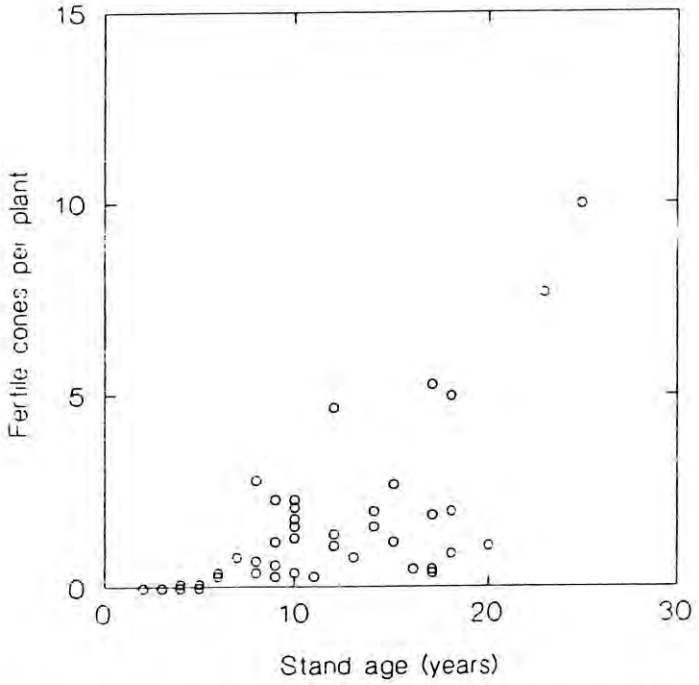

Figure 6.2.3 Average number of fertile cones stored per plant in 50 *Banksia coccinea* stands of varying age.

The data was also analysed on an area basis. Variations in stand density bought further variability into the data when cone numbers were converted to numbers per hectare. The number of cones per hectare varied from 0 to 128 000, and averaged 18 000 in stands 5 years and over, and 20 000 in stands 9 years and over. Stepwise regression analysis resulted in three variables: stand height, stand density, and stand age, accounting for 48% of the variation in log cones per ha (Table 6.2.7), with stand height having the strongest relationship to log cones per ha. Plant height and stand age were strongly correlated (r=0.76).

Table 6.2.5 Mean, standard error, maximum and minimum and of (A) cones per plant and (B) cones per ha. in 50 *Banksia coccinea* stands.

	No. sites	Mean	SE	Min.	Max.
(A) Cones/plant					
All stands	48	1.6	0.29	0.0	10.0
Age > 5 yrs	41	1.9	0.3	0.3	10.0
Age > 9 yrs	30	2.3	0.4	0.3	10.0
(B) Cones/ha (x 1000)					
All stands	47	14.4	3.0	0.0	128.
Age > 5 yrs	38	17.8	3.5	0.5	128.4
Age > 9 yrs	28	20.2	4.5	1.0	128.4

Table 6.2.6 Linear regression model of the effect of stand age and stand density on log cones per plant.

Variable	Parameter estimate	Standard Error	Partial R^2	Model R^2
Constant	-0.046	0.15	-	-
Age	0.076	0.01	0.50	0.50
Density	-0.005	0.00	0.03	0.53

Table 6.2.7 Linear regression model of the effect of stand age and stand density on log cones per hectare.

Variable	Parameter estimate	Standard Error	Partial R^2	Model R^2
Constant	-0.21	0.42	-	-
Height	0.06	0.04	0.25	0.25
Density	0.46	0.23	0.20	0.45
Age	0.03	0.01	0.03	0.48

Cone and follicle production and canopy stored seed.

There was a large difference between stand density in RPN1 and that of the other stands (Table 6.2.8). Plant density was five times greater than GR2, and 15 times greater than SET1. There was no relationship between stand age and stand density. Plants at RPN1 had fewer branches and were shorter than plants in SET1 and GR1. The Gull Rock stands were taller, had greater height to age ratios and were more branched than the comparable aged stands at the other sites.

Table 6.2.8 Stand age, density, branching and disease severity and intensity and reproductive attributes of *Banksia coccinea* in six stands.

	SET2	SET1	GR2	GR1	RPN2	RPN1M	RPN1H
Stand Age	9	21	11	36	11	20	18
Density (no. per ha.)	7 400	7 200	21 100	8 900	16 700	103 809	103 809
Branches	3.2	11.3	7.4	86.4	1.3	3.0	2.6
Mean Height (m)	1.8	2.6	2.8	6.2	2.2	2.5	nm
Severity Limb Dieback (%)	13	58.5	0	0	27	37.5	100
Incidence Limb Dieback (%)	26.7	94.1	0	0	33.3	52.5	100
Age to first flowering	4	-	3	-	6	-	-
Age to first fert. cone	4	-	4	-	9	-	-
Fruiting Plants (%)	58	96	87	100	32	52	46
Flower heads / plt [A]	1.1 ab	1.9 c	2.0 c	19.7 d	1.5 bc	0.9 a	-
Fertile cones / plant [B]	1.8 cd	2.7 d	7.4 d	50.7 e	0.85 a	1.5 bc	1.1 ab
Cones per ha. (1 000's)	7.7 b	18.0 c	43.7 d	451.3 f	4.5 a	76.7 e	52.4 de
Follicles per fertile cone	14.5 b	16.7 c	11.9 ab	9.8 a	10.6 ab	12.4 ab	10.8 ab
Firm seed per plant [B]	25.7 c	73.0 d	14.0 b	457.1 e	8.0 a	11.0 ab	8.9 ab
Seed Released (%)	1.6	5.3	31.7	11.4	8.0	30.7	31.2
Firm seed per ha. (x 1000)	110.5 c	483.1 e	256.8 d	4069.4 f	42.7 a	591.2 e	21.2 d
Contrib. to yr-1 firm seed to tot seed bank (%)	70.1	18.9	39.3	63.5	85.2	68.6	0.0
Viable Seed (%)	46.8	43.5	46.3	68.3	53.1	48.3	21.1
Viable Seed / plt [B]	11.2c	34.9d	6.7b	312.6e	4.2b	5.3b	1.9a
Viable seed / ha (x 1000)	48.1b	231.4d	122.5c	2780.1e	22.7a	285.5d	88.8 c

[A] Values in rows followed by the same letter are not significantly different by Tukey's HSD test.

[B] values for fruiting plants only.

nm = not measured

The incidence of canker was greatest in RPN1 and SET1. At RPN1H, disease incidence was 100% and mortality was approaching 100% in the severely infected part of the stand (Table 6.2.8). Disease incidence averaged 52% in RPN1M. Disease incidence was above 90% at SET1. Cankers were also present in the younger stands at Red Gum Pass and South East Track, however their incidence was less than 40%. No cankers were recorded in the Gull Rock stands.

The age to first flowering ranged from four to six years, and age to first cone from 4 to 9 years. The proportion of plants with fertile cones in the young stands ranged from 32 to 87% in the 9 to 11 year old stands. Fruiting was variable in the older stands, with 100% of the plants at GR1 fruiting compared with only 52% at RPN1M.

There was a large degree of variability between stands in the number of flowers, cones and seeds produced. GR1 stand had the highest reproductive output, producing more cones and seeds both on a per plant and per hectare basis. The total seed store per hectare in the two old stands in the Stirling Range was similar, although there were more cones per hectare at RPN1M. On a per plant basis, SET2 and SET1 produced significantly higher numbers of cones and seeds than RPN2 and RPN1M. RPN2 had similar numbers of seeds stored per plant and had produced 60% more flowers than RPN1M.

Seed storage was adequately explained by an exponential function in both young and old stands at all sites (Figure 6.2.4). At SET1 seed storage initially increased exponentially, then declined after plants reached 19 years of age. The contribution of the current year's cones and seeds to the total cone and seed store was smallest in SET1, accounting for only 21% of stored seed and 19% of fertile cones (Table 6.2.8). The current year's seeds and cones in the other stands accounted for 40 - 85 % of cones stored and 35 - 70 % of seed stored.

The percentage of seed released from cones increased from 2.5% in year 1 to 92% in year 9. Regression of percentage seed released per cone on cone age indicated the degree of serotiny was 11 (Cowling and Lamont, 1985). Over 50% of stored seed was estimated to be lost from dead plants 3 years after death of the plant (Table 6.2.9). By 5 years, over 90% of stored seed would be lost.

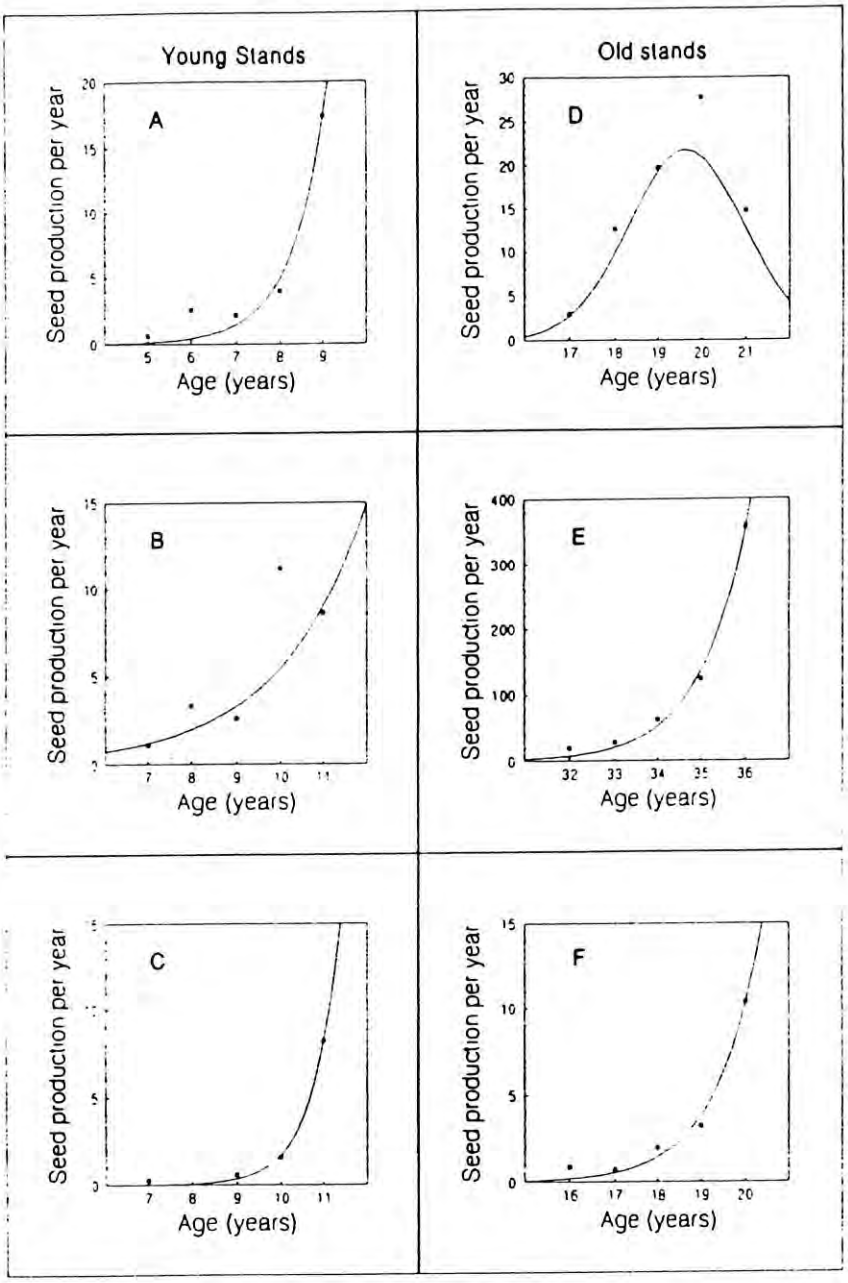

Figure 6.2.4 Number of seeds produced per crop year, P, in relation to plant age, A, for three young stands (A) SET2, (B) GR2 and (C) RPN2 and three old stands (D) SET1, (E) GR1 and (F) RPN1. For the young stands (A) $R=e^{(-8.329+1.242A)}$; (B) $R=e^{(-3.322+0.502A)}$; (C) $R=e^{(-15.122+1.566A)}$ (r^2= 0.9, 0.8 and .1.0 respectively). For the old stands, (D) $R=e^{(-110\ 357+11.550A-0.2994A^2)}$; (E) $R=e^{(-28.187+0.946A)}$; and (F) $R=e^{(-17.232+0.978A)}$ (r^2= 1.00, 1.00 and 0.99 respectively).

Table 6.2.9 . Mean percentage of seeds per cone age that were released, mean percentage of total firm stored seed per cone age; and estimates of percentage seed loss with time after plant death.

Seed Age	Released seed (%)[A]	Firm Seed (% of total)	Seeds remaining after plant death (%)					
			Year 1	Year 2	Year 3	Year 4	Year 5	Year 6
1	2.5	57.8	0	0	0	0	0	0
2	13.6	24.8	51.2	0	0	0	0	0
3	24.8	10.6	21.6	38.5	0	0	0	0
4	36.0	4.6	9.1	13.8	24.6	0	0	0
5	47.1	2.0	3.8	4.8	7.3	13.0	0	0
6-9	58.3	0.2	2.1	2.2	2.6	3.7	6.4	1.9
TOTAL		100.0	87.8	59.3	34.5	16.7	6.4	1.9

A Calculated from the equation RS = -8.701 + 11.169A, where RS is percentage of seeds released per cone and A is cone age. Equation derived from combined data for all stands by regression, r^2=0.92.

B Calculated from the equation FS = $134.724e^{-0.846A}$, where FS is percentage of total firm seed and A is cone age. Equation derived from combined data for all stands by regression, r^2=0.98.

Assessments of cone and branch production over 3 years.

Cone storage, yearly cone production and total branches increased with time in the two younger stands, CB2 and HH. Dead branches also increased during this time, however they composed <3 % of the total branches in the stands (Figure 6.2.5). The rate of increase in total cones reached a plateau at CB1 and declined at SET1 after the percentage of dead branches exceeded 50% (Figure 6.2.5 (B) & (C)). Annual cone production declined when the percentage of dead branches was lower (30% at CB1 and prior to the commencement of the study at SET1).

Relative cone production (RCP) was strongly correlated with percent branches alive, however the trend was non-linear (Table 6.2.10). There was also a weak, though significant correlation between stand age and relative cone production. Stand density, live branches per plant and total branches per plant were not correlated with RCP. Results from stepwise multiple regression analysis on log RCP indicated that 87% of variation in log RCP was explained by percent branches alive (Table 6.2.11).

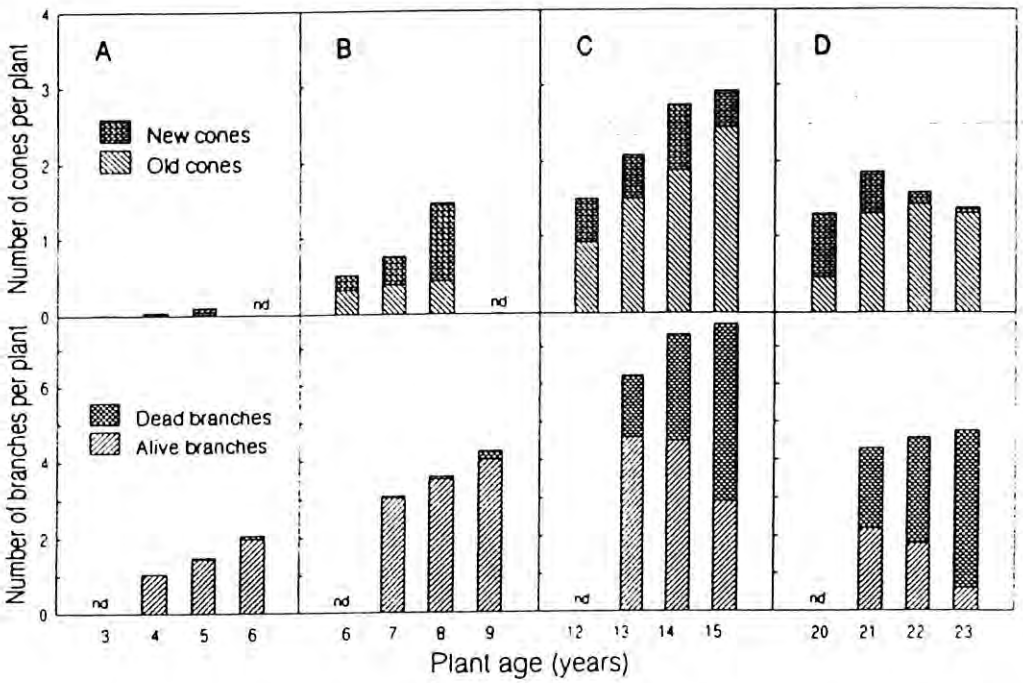

Figure 6.2.5 Number of new and old cones and number of alive and dead branches per plant in relation to plant age for four stands, (A) CB2, (B) HH, (C) CB1 and (D) SET2.
nd=no data

Table 6.2.10 Pearson correlation coefficients for variables likely to affect relative cone production (RCP).

Variable	RCP	Age	PBA	Density
log (RCP)	1.00	-0.85*	0.93*	0.74
Age	-0.78	1.00	-	-
Branches alive (PBA)	0.85*	-0.90*	1.00	-
Density	0.73	-0.78	0.87*	1.00

* indicates $P<0.05$.

Table 6.2.11 Linear regression model of the dependency of relative cone production (RCP) on alive branches per plant (%).

Variable	Parameter estimate	Standard Error	Model R^2	Adjusted R^2
Intercept	-3.031	0.257	-	-
Percent Branches Alive	0.026	0.003	0.871	0.857

DISCUSSION

Stand survey

The survey confirmed the widespread occurrence of *Cryptodiaporthe* canker in *B. coccinea* stands. Disease intensity and mortality was greatest in older stands, which may have been due to inoculum levels gradually building up in stands as they age. Cankers were uncommon in juvenile stands, however low disease severity in 20% of juvenile stands indicates that early build up of disease in young stands can occur. One explanation for infections in these stands is there were large sources of inoculum either within, or near to, the stands. Sources of inoculum are discussed in Chapter 3 and Section 6.1.3. Unburnt patches of the old stand are likely to be most important as local sources of inoculum. It is unlikely that the best burning management practices could keep stands disease free for longer than 13 years considering all stands over this age contained cankers. The use of sanitation measures for disease control rarely provide complete control of disease, but can be effective in delaying the start of epidemics or reducing the infection rate (Vanderplank, 1963; Zadoks and Schein, 1979).

The major question for management of stands is how will varying levels of disease intensity affect cone production and seed storage in stands, and how will this affect the ability of the stand to regenerate following fire? Stands showed a trend of increasing cones per plant and per hectare with stand age, however there was so much inter-stand variability any effects of canker on cone storage which may have occurred could not be determined. Studies which try to interpret age related phenomena by substituting space for time are useful alternatives to long term studies (Pickett, 1989) and they allow a large number of sites to be sampled. There are drawbacks with this approach, for example the difficulty of adequately matching sites (Gill and Mahon, 1986), and the assumption that different aged sites have been subjected to the same environmental conditions and stresses (Pickett, 1989). Studies of this nature should therefore be interpreted with caution. One-off studies are useful as tools for generating hypotheses about long term trends. These hypotheses can then be tested through longer term monitoring, such as the approach in the 3 year study of cone accumulation and branch death (Chapter 5).

Seed banks

The pattern of cone production and seed accumulation in young *B. coccinea* stands was similar to that reported for other *Banksia* species (eg Cowling *et al.*, 1987; Cowling *et al.*, 1990; Witkowski *et al.*, 1991). After a juvenile period of 4 to 6 years,

numbers of cones produced increased with time. Cone production and seed storage in two of the older stands showed similar exponential increases with age. The exponential increase in cone production in old stands contrasts with Witkowski *et al.* (1991) who reported declining seed storage in *B. coccinea* after 16 years. Their findings are similar to those in the highly diseased stand, SET1, and do not indicate the pattern of seed accumulation which would occur in stands with no disease or low levels of disease. Witkowski *et al.* (1991) also concluded that *B. coccinea* should be burnt on a < 20 year cycle, which agrees with our recommendation for diseased stands. This, however, is not necessary for stands with low levels of disease as seed storage can continue to increase once the stand passes 20 years of age. It is possible that errors could be made if burning recommendations for disease affected stands are based only on seed bank dynamics. Consideration of the epidemiology of the disease and additional requirements for disease control is essential to avoid the implementation of burning regimes which could exacerbate the disease (Section 6.2). Yield loss and early stand senescence are a product of disease and need to be managed as such.

Differences between stands were large, especially in stand density, both within nearby stands of different age and between stands of similar age. Large variability in average numbers of cones stored per plant, height and number of branches per plant also occurred. The lack of a relationship between stand age and density suggests variations in seed stored at the time of the last fire, or post fire environmental conditions may have a larger role in determining differences between stands than age alone (Gill and McMahon, 1986). Stand density usually declines as stands age (Gill and McMahon, 1986), however information is sparse on the extent of fluctuations in stand density which occur within sites after successive fires. Large fluctuations in stand density would be expected in the sites studied for seed bank dynamics were they burnt. A reduction in stand density would be expected at RPN1 after burning, given there are only two viable seeds per adult plant. Using an establishing fraction of 0.2 (Gill and McMahon, 1986; Lamont, pers. com.), the expected stand density after fire would be 57 700 plants per hectare, about half the pre-fire density but still high density when compared to the other stands. At SET1 a six fold increase in stand density to 46 280 plants per hectare would result from burning, using the same establishing factor, however there is strong evidence that seed resources in SET1 are being rapidly degraded.

B. coccinea forms a tall narrow shrub due to its acute branch angle and low frequency of branching (Witkowski *et al.*, 1991). The low level of fruiting plants and branching

at RPN1 compared to the other stands suggests plants respond to high density by producing fewer branches and cones rather than self thinning to reduce stand density.

Reductions in cone production, and therefore seed storage, occurred in stands where disease was severe (>50%). Low or nil production of seed from the current year, combined with yearly losses in stored seed through spontaneous release, suggests seed reserves may be rapidly lost from these stands. Estimates of the rate of seed loss from a single dead tree (Table 6.2.9) indicate 50% of the stored seed could be lost within 3 years. Cones on dead plants are also prone to incineration when burnt (Lamont and Barker, 1988), therefore fire after plant death may cause further losses in the available seed store.

The seed store continued to increase in stands with disease severity less than 40%, indicating the rate of production of flower heads and cones exceeded the rate of branch death at these sites. Reductions in the annual cone production were the first signs that stands were nearing peak levels of stored cones, and cone storage peaked once 50% of the branches were dead. The relationship between percent alive branches and relative cone production provides a simple means of estimating the impact of disease on cone production. The number of flower heads and cones which will be produced in a particular year is related to the number of living shoots because *B. coccinea* produces terminal flower spikes. Long term monitoring of disease and seed production is required to determine the pattern of disease development and its effect on seed production and storage in young stands such as HH and CB2. The rate of branch death in these stands was low compared to SET1 and CB1, but once the exponential stage of the epidemic is reached, branch death will increase rapidly. The time interval between the first appearance of the disease in stands and the commencement of the exponential phase of disease development is yet to be determined. This study indicates it occurs more than two years after the initial infestation.

Seed loss is predicted to be relatively rapid from dead plants due to the low degree of serotiny of *B. coccinea*. *B. coccinea* is able to establish between fire (Witkowski *et al.*, 1991), however the low numbers of seedlings found in diseased stands indicates inter-fire establishment occurs at a very low level and would only successfully re-establish a stand during years of highly favourable environmental conditions (Lamont *et al.*, 1991). Fire sensitive species, such as *B. coccinea*, generally occur in even aged stands, indicating their dependence upon fire for regeneration.

Diseased stands should be burnt when living branches have declined to about 50% of total branches. Lower limits for burning diseased stands are flexible, however they depend on sufficient seed having been accumulated for stand replacement. Upper limits for burning are inflexible and should not exceed 50% branch death to avoid seed loss from the stand by spontaneous release of seeds and cone incineration at the time of the burn.

7 GENERAL DISCUSSION

C. melanocraspeda is an aggressive pathogen capable of infecting unwounded stems and achieving rapid rates of disease progress. The ability to infect through non-wounded tissue is uncommon in canker pathogens, although it has been reported for several diseases. The infection process of *C. melanocraspeda* is unclear, although it is apparent that tissues are extensively colonised prior to canker development. This may account for the long incubation period of the disease. Stands may be infected several years prior to the appearance of obvious symptoms of the disease.

It is unknown whether the pathogen is indigenous or introduced, however it is unlikely that such widespread destruction of stands could result from an indigenous pathogen. Indigenous diseases in natural ecosystems are not uncommon but are generally limited in time and space (Schmidt, 1978). At present, there is no indication of a balance developing between host and pathogen or of the rate of spread declining in the stands studied. The pattern of disease development is therefore consistent with that of an introduced pathogen or an ecosystem which has been disturbed. The growth of *B. coccinea* in dense even aged sands, plus the ability of the fungus to produce abundant inoculum and infect unwounded tissue are probably major determinants of the pathogen's success.

Fire may offer a potential means of breaking the disease cycle through the regeneration of healthy seedlings in an environment with little inoculum. However, disease carry-over into regenerating stands by seedborne infections and inoculum entry into stands from alternative hosts is poorly understood and may be important for reinfection of stands. Remnants of the old stand which survived burning may be a major source of inoculum and act as infection foci in regenerating stands. An improved knowledge of inoculum dynamics, in terms of dispersal gradients from inoculum sources is required to determine optimum fire regimes in terms of the scale and patchiness of the fire.

Canker has both chronic, in terms of yield loss, and acute, in terms of longevity, affects on the *B. coccinea* host. Species which rely on canopy stored seed for regeneration are vulnerable because their ability to recover after fire depends upon sufficient stored seed. Relatively high proportions of seed are lost from *B. coccinea* stands in the absence of fire through spontaneous seed release. Consequently, once a plant dies, seed reserves are rapidly diminished. Maximising regeneration through burning when seed production has reached its peak must be an objective of management.

Decisions of when to burn would be better based on the proportion of live branches in the stand than on stand age alone. Cone production and therefore seed storage was influenced by the proportion of living branches in the stand and there was some evidence that cone numbers began to decline once 50% of the branches had died. Stand age was also a major determinant of cone numbers and disease intensity, however there were several older stands which had low levels of disease.

Disease levels could be reduced in *B. coccinea* stands if either the infection rate is reduced or the period before the commencement of the epidemic is lengthened. This objective is unlikely to be achieved unless fire regimes that reduce sources of inoculum within regenerating stands are implemented. A program of long term disease monitoring in stands is necessary to assess the effects of burning on stand health. The reliance upon assessments of spatial trends of disease for interpreting age related phenomena is an inadequate substitute for the long term study of disease in stands.

Successful stand management also depends upon the feasibility of burning. The number of days suitable for burning is often very few in the south coast region Fire may be difficult to control when high mortality has created large fuel loads. The presence of mountains and the inaccessibility of many areas will also add to the difficulty of disease management. The Stirling Range National Park and national parks and reserves between Gull Rock and Cape Riche contain many large stands of *B. coccinea*. Given that 8% of the stands surveyed in this report had disease severity greater than 50%, and almost 30% had disease severity over 25%, the problem requires urgent attention, to avoid local extinctions of *B. coccinea*.

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Appendix L Location of stands referred to in the report, stand age and disease severity (in 1993). Map numbers refer to Figure 6.2.1.

Map No	Location	Text Ref	Latitude/Longitude	Age	Disease Severity
1	Stirling Range N.P. - East Pillenorup Track # 1		34°26'S/118°06'E	15	43
2	Stirling Range N.P. - East Pillenorup Track # 1		34°26'S/118°06'E	2	0
3	Stirling Range N.P. - East Pillenorup Track # 2		34°27'S/118°08'E	4	0
4	Stirling Range N.P. - Yungermere track # 1		34°26'S/118°08'E	15	60
5	Stirling Range N.P. - Yungermere Track # 1		34°26'S/118°08'E	4	1
6	Stirling Range N.P. - Yungermere Track # 2		34°26'S/118°08'E	3	0
7	Stirling Range N.P. - Yungermere Track # 3		34°29'S/118°07'E	8	0
8	Stirling Range N.P. - Donnelly Track		34°24'S/117°44'E	17	3
9	Stirling Range N.P. - Madyerip Track		34°21'S/117°46'E	9	7
10	Stirling Range N.P. - Yetemerup Track # 1		34°21'S/117°55'E	14	21
11	Stirling Range N.P. - Yetemerup Track # 1		34°21'S/117°55'E	4	0
12	Stirling Range N.P. - Yetemerup Track # 2		34°21'S/117°55'E	7	1
13	Two Peoples Bay N.R.-Mt Gardner		35°00'S/118°11'E	23	39
14	Gull Rock N.P. - Gull Rock Road # 1		34°59'S/118°00'E	25	1
15	Gull Rock N.P. - Gull Rock Road # 2	GR1	34°59'S/117°59'E	10	0
16	Gull Rock N.P. - Herald Point # 1		35°01'S/118°03'E	10	0
17	Gull Rock N.P. - Herald Point # 2		35°01'S/118°02'E	18	28
18	Two Peoples Bay - Pipeline Road		34°57'S/118°07'E	25	2
19	Mindijup Road		34°45'S/118°02'E	8	18
20	Pfieffer / Johnson Rd		34°42'S/118°12'E	16	22
21	Reserve 31240 - Sandalwood Rd		34°34'S/118°41'E	10	13
22	Reserve 31240 - Turner Road		34°39'S/118°40'E	10	1
23	Waychinicup N.P. - Waychinicup Rd - # 2		34°52'S/118°21'E	12	18
24	Waychinicup N.P. - Waychinicup Rd # 1		34°52'S/118°21'E	4	0
25	Stirling Range N.P. - West Boundary Road # 1		34°20'S/117°41'E	11	6
26	Stirling Range N.P. - West Boundary Road # 2		34°20'S/117°41'E	8	1
27	Stirling Range N.P. - 0.5 km north of S E Track # 1	SET1	34°28'S/118°06'E	9	7
28	Stirling Range N.P. - 0.5 km north of S E Track # 2	SET2	34°28'S118°06'E	18	49
29	Waychinicup N.P. - Cheyne Beach Road # 1	CB1	34°53'S/118°21'E	10	30
30	Waychinicup N.P. - Cheyne Beach Road # 2	CB2	34°53'S/118°21'E	5	3
31	Cheyne Beach - Lookout # 2		34°53'S/118°24'E	12	78
32	Cheyne Beach - Lookout # 1		34°53'S/118°24'E	17	100
33	Cheyne Beach - Lookout # 1		34°53'S/118°24'E	5	0
34	Stirling Range N.P. - Red Gum Pass South #2	RPS2	34°22'S/117°47'E	10	26

35	Stirling Range N.P. - Red Gum Pass South #1	RPS1	34°22'S/117°47'E	17	39
36	Stirling Range N.P. - Quarry Track		34°21'S/117°42'E	18	99
37	Stirling Range N.P. - Quarry Track		34°21'S/117°42'E	6	6
38	Bon Accord N.R. - # 1		34°56'S/117°57'E	25	27
39	Bon Accord N.R. - # 2		34°56'S/117°57'E	12	0
40	Mettler - Mettler Road		34°34'S/118°34'E	13	31
41	Stirling Range N.P. - Red Gum Pass North #1	RPN1	34°22'S/117°48'E	14	47
42	Stirling Range N.P. - Red Gum Pass North #2	RPN2	34°22'S/117°48'E	9	21
43	Stirling Range N.P. - Stirling Drive		34°24'S/117°52'E	6	0
44	Reserve 25965 - Chokerup-Narricup Rd		34°49'S/117°42'E	5	0
45	Stirling Range N.P. - Red Gum Pass		34°19'S/117°47'E	8	0
46	Bakers Junction N.R.		34°55'S/117°57'E	9	0
47	Wellstead - Old Boundary Rd		34°26'S/118°36'E	20	20
48	Wellstead Town, East of Tip		34°30'S/118°36'E	18	32
49	Hassell N.P. - # 1		34°44'S/118°19'E	14	17
50	Mettler - Tilbrook Farm		34°35'S/118°37'E	15	39
51	Hassell N.P. - # 2	HH	34°38'S/118°22'E	8	7
52	Gull Rock N.P. - Gull Rock Rd	GR1	34°59'S/117°59'E	36	0