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

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

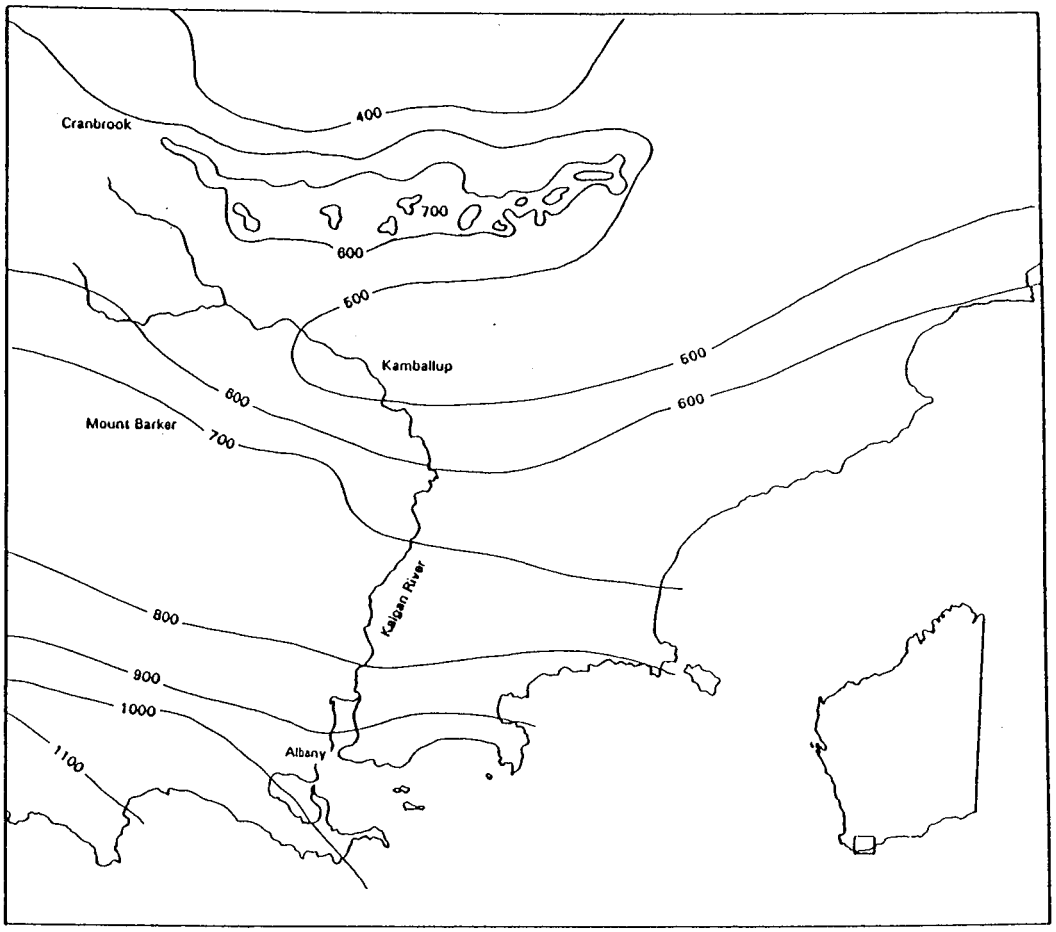

Figure 1.2.1 Annual rainfall isohyets in the Albany region.

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).

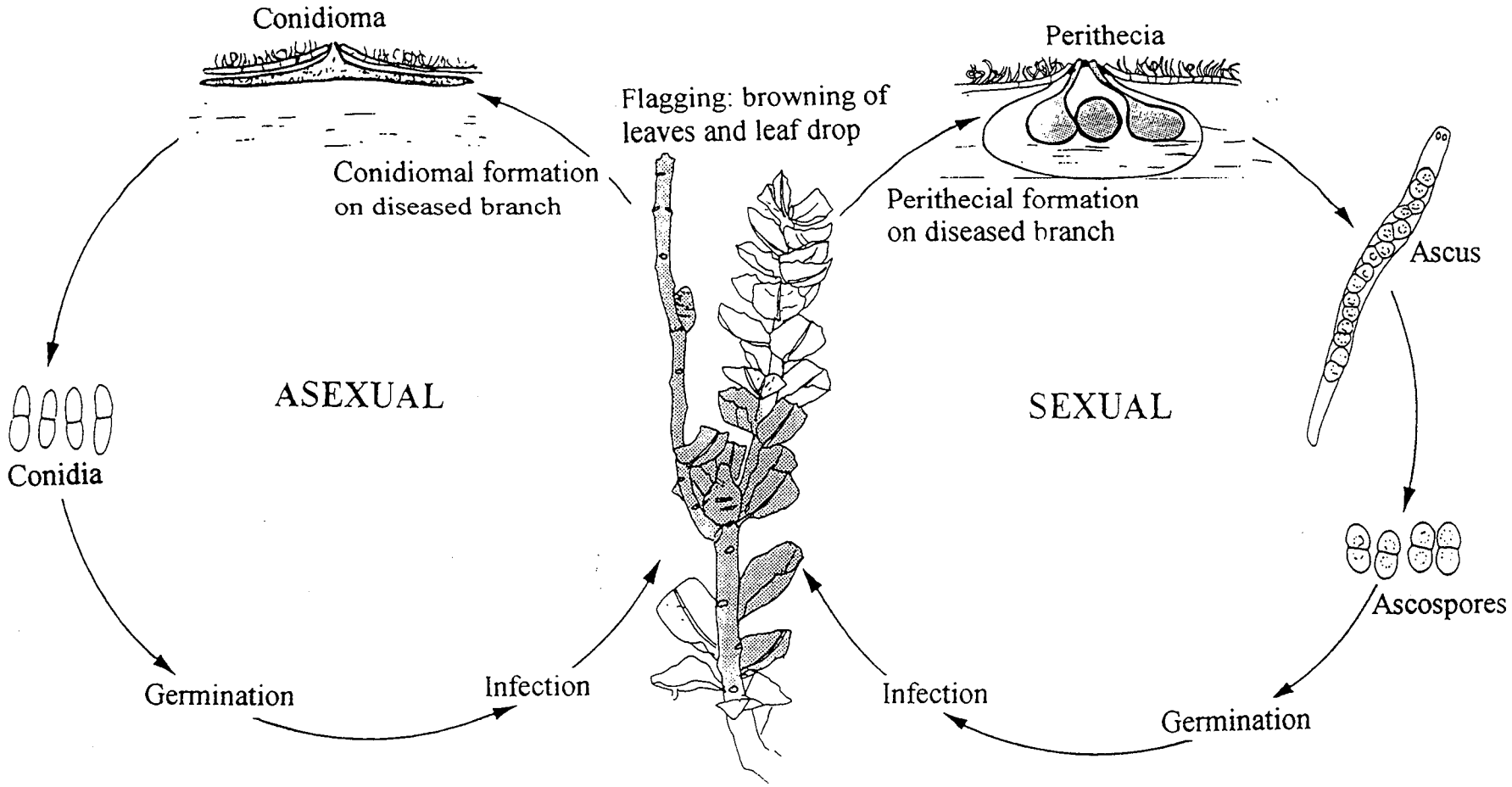

Figure 2.1 The lifecycle of *Cryptodiaporthe melanocraspeda* on *Banksia coccinea*.

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).

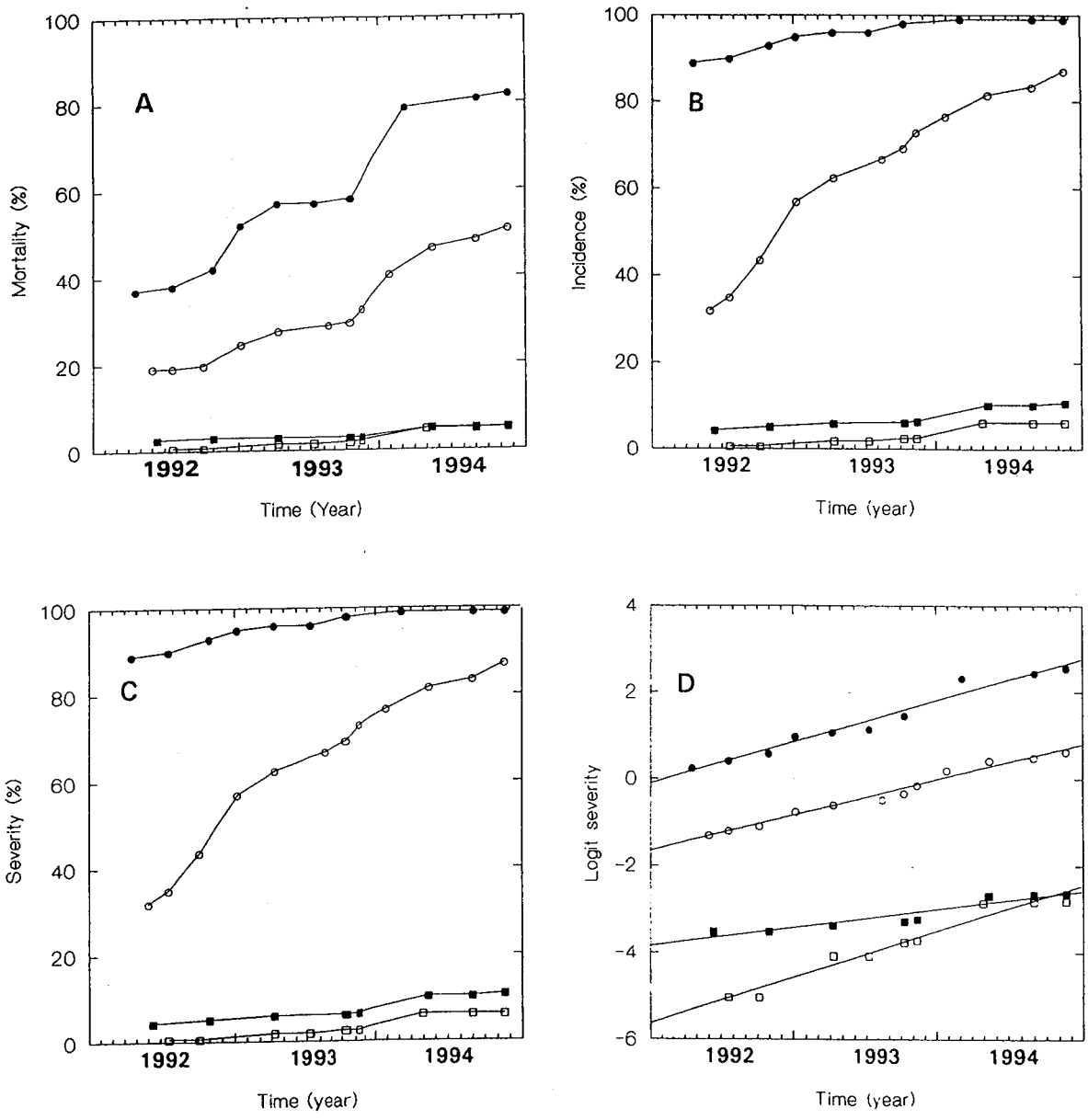

Figure 5.1 Progress in (A). mortality, (B). incidence of limb dieback, (C). severity of limb dieback and (D). Logit transformed severity in four *Banksia coccinea* stands. ● = SET1, O = CB1, ■ = HH and □ = CB2

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.

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.

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		

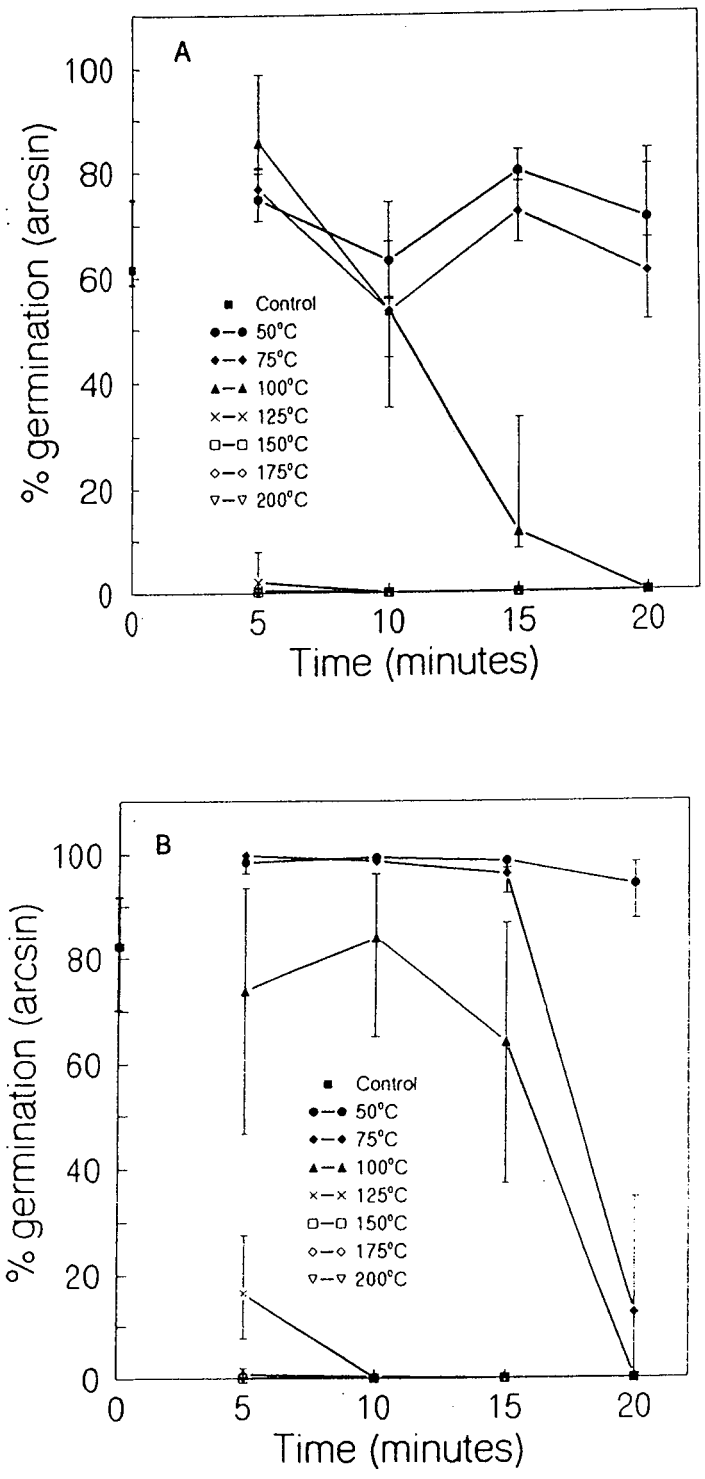

Fig 6.1.2.1 Germination of (A). ascospores and (B). conidia of *Cryptodiaporthe melanocraspeda* from stems heated at seven temperatures: 50, 75, 100, 125, 150, 175 and 200°C and four temperature durations: 5, 10, 15 and 20 min. Germination of spores from untreated stems is shown on the Y-axis. Bars represent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

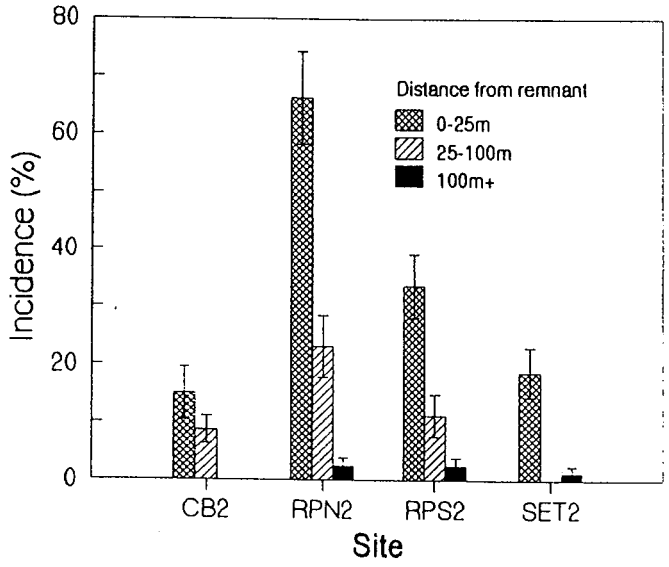

Figure 6.1.3.1 Average incidence of limb dieback of *Banksia coccinea* caused by *Cryptodiaporthe melanocraspeda* at three distance ranges from old stand remnants at four sites: CB2, RPN2, RPS2 and SET2. Bars indicate ± 1SE.

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

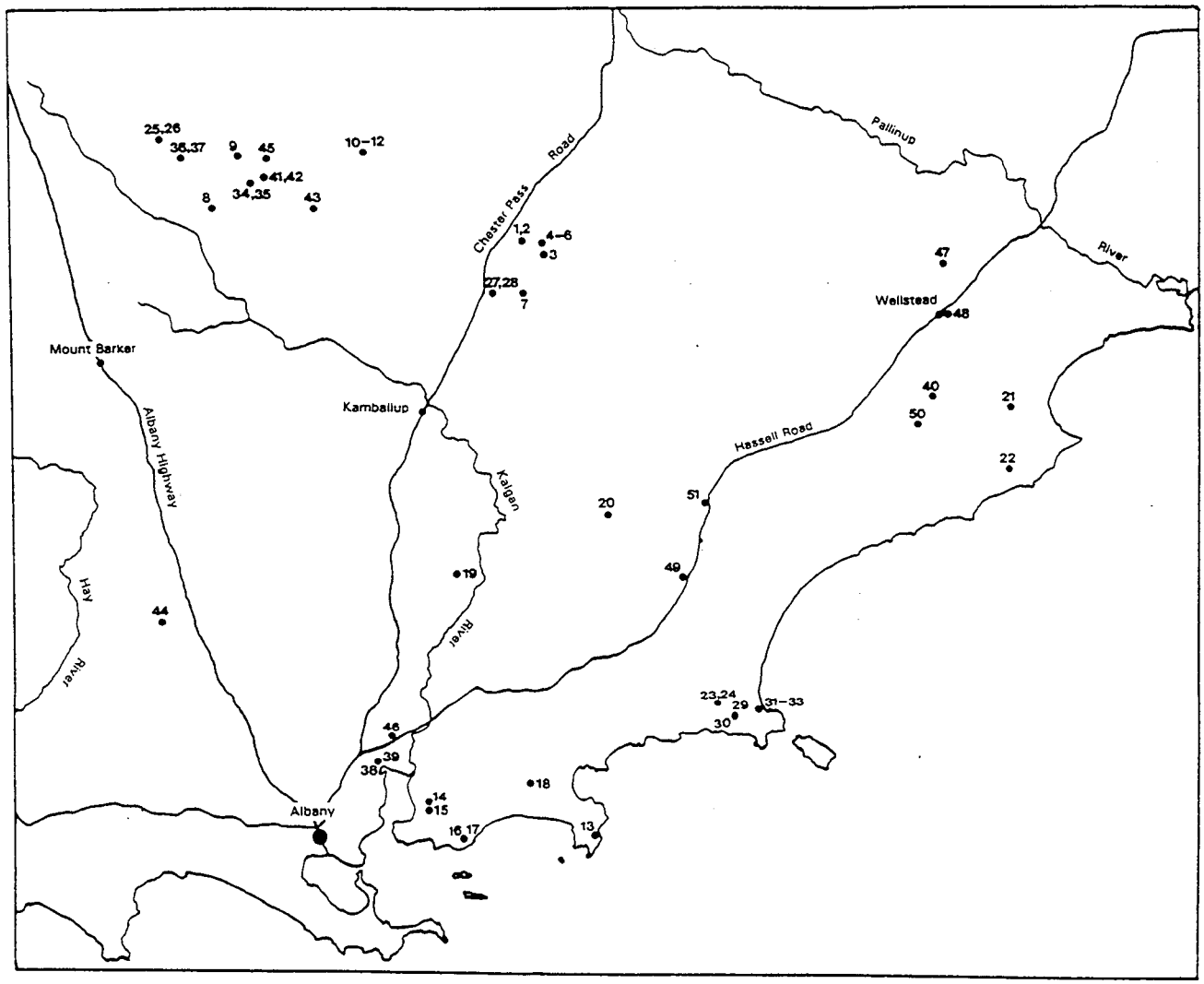

Figure 6.2.1 Location of *Banksia coccinea* stands surveyed in 6.2 and referred to elsewhere in this report. Numbers refer to stand numbers in Appendix 1.

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 5×5 m plots in each stand. In the RPN1 stand, density was estimated in seven 3×3 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

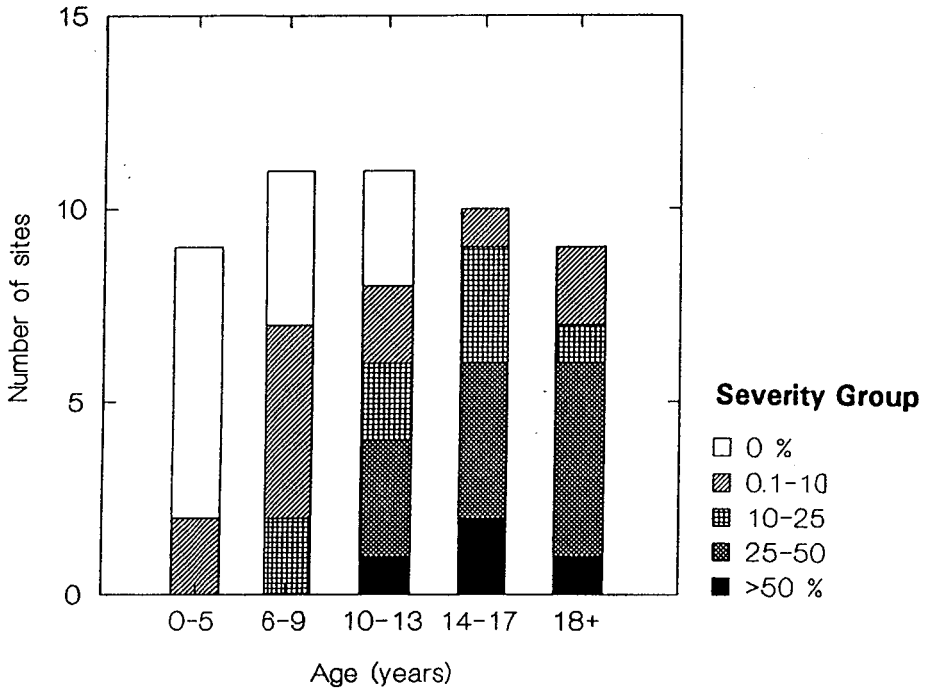

Figure 6.2.2 Frequency of *Banksia coccinea* stands grouped by age in five severity of limb dieback classes: 0, 0.1-10, 10-25, 25-50 and >50 % disease severity.

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.

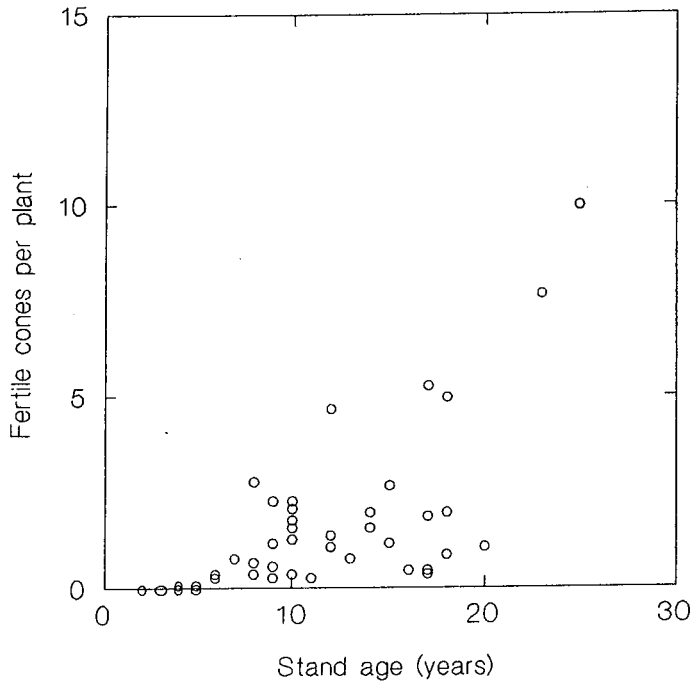

Figure 6.2.3 Average number of fertile cones stored per plant in 50 *Banksia coccinea* stands of varying age.

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.

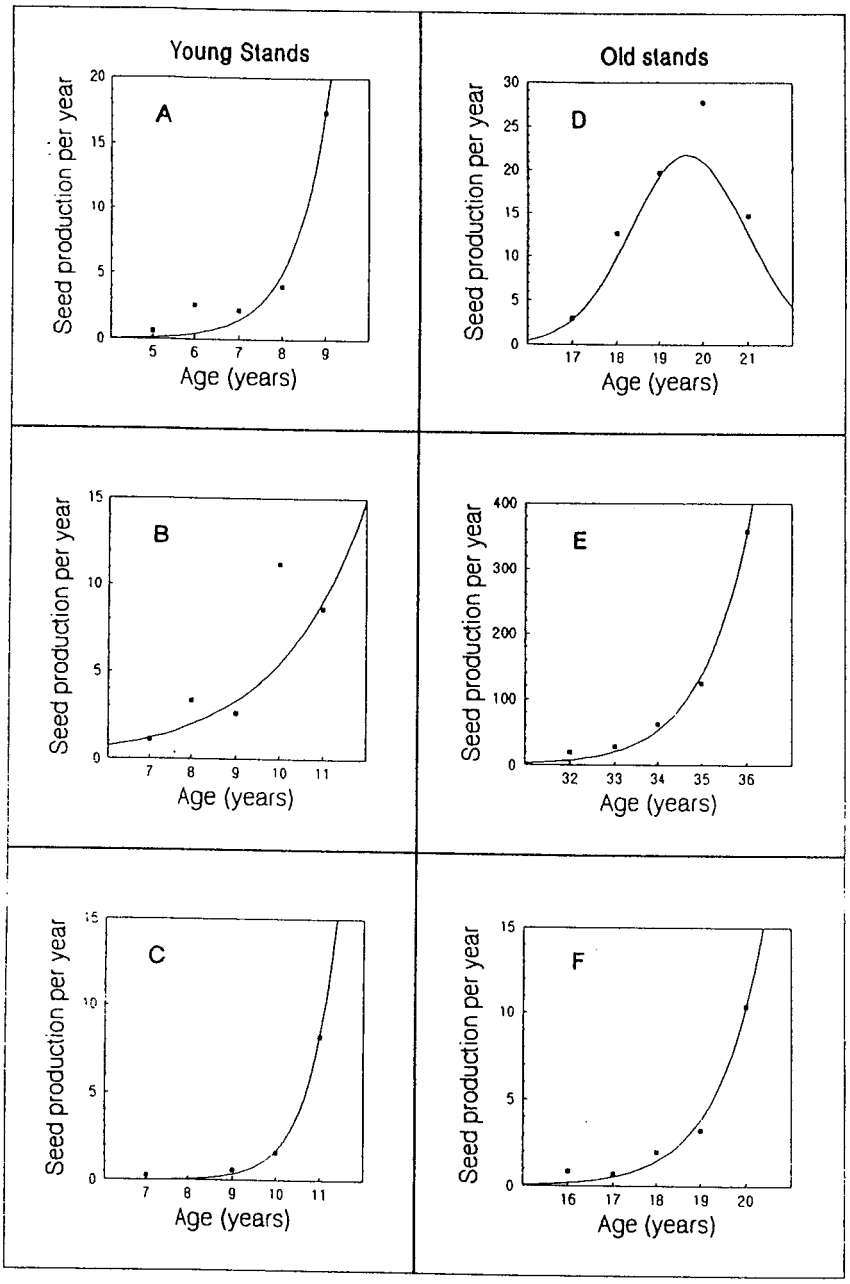

Figure 6.2.4 Number of seeds produced per crop year, P, in relation to plant age, A, for three young stands (A) SET2, (B) GR2 and (C) RPN2 and three old stands (D) SET1, (E) GR1 and (F) RPN1. For the young stands (A) $R=e^{(-8.329+1.242A)}$; (B) $R=e^{(-3.322+0.502A)}$; (C) $R=e^{(-15.122+1.566A)}$ (r^2= 0.9, 0.8 and .1.0 respectively). For the old stands, (D) $R=e^{(-110.357+11.550A-0.2994A2)}$; (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).

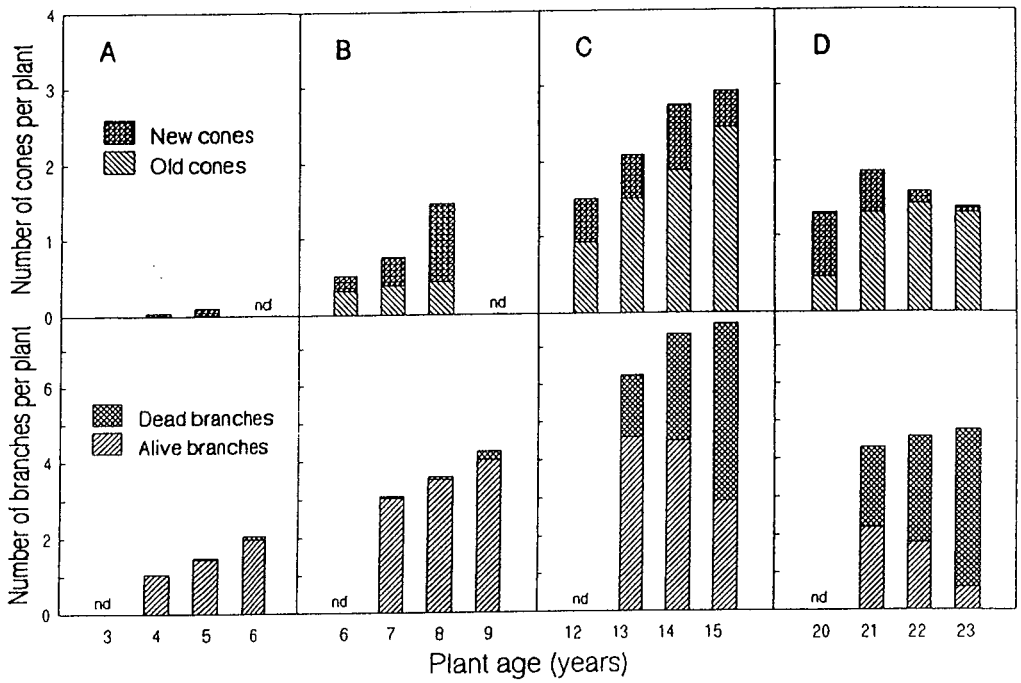

Figure 6.2.5 Number of new and old cones and number of alive and dead branches per plant in relation to plant age for four stands, (A) CB2, (B) HH, (C) CB1 and (D) SET2.
nd=no data

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 I. 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