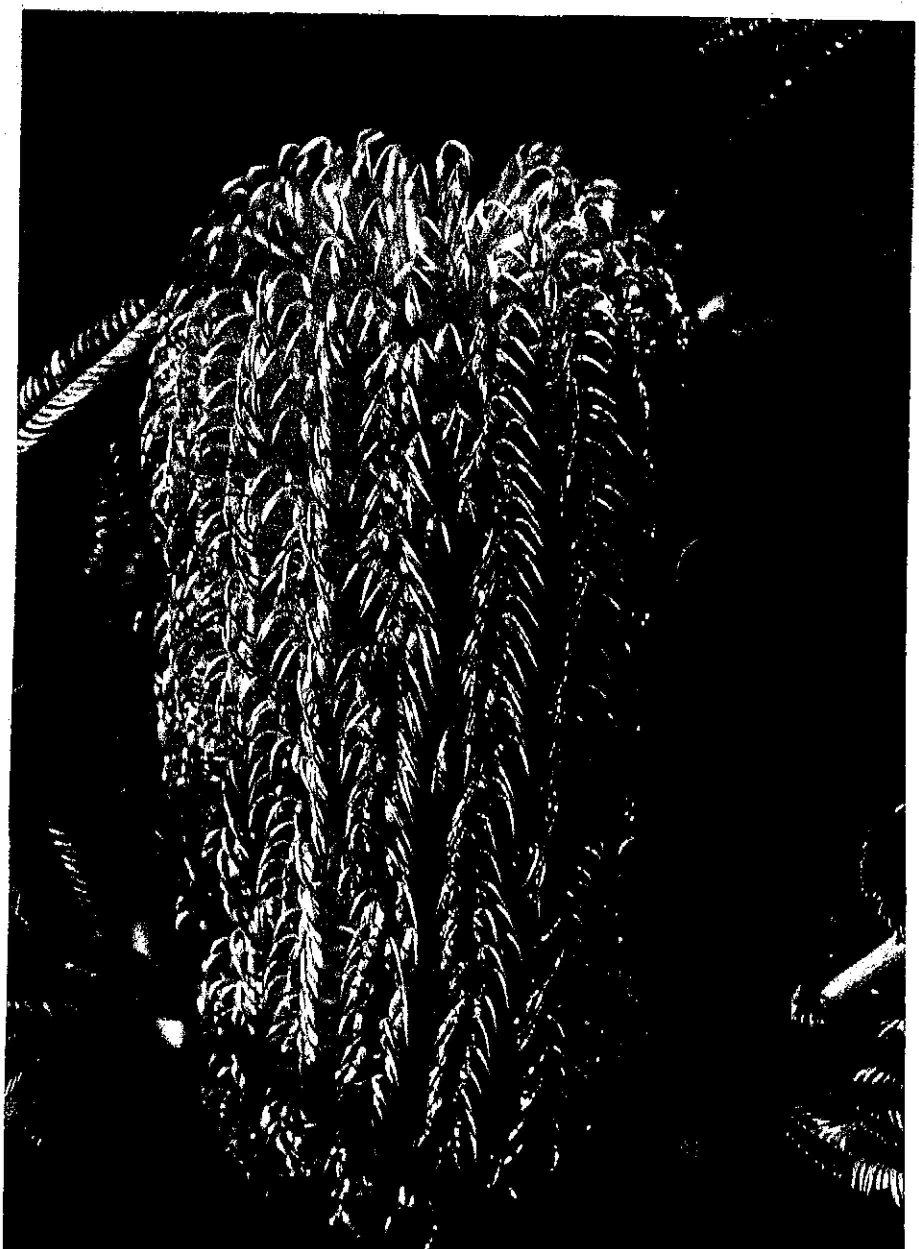
POPULATION ECOLOGY of the RARE and ENDANGERED *Banksia brownii*



by Helen Galea and Byron Lamont

Abstract

Alternative habitats for the possible establishment of *Banksia brownii* in *Phytophthora cinnamomi* free areas were investigated. The habitats included casuarina woodland, wet and dry sclerophyll woodlands, calcareous soil types and scrub-heath sites. This investigation is ongoing but the results to date indicate that the level of herbivory overrides other site factors.

Seed viability was studied in three populations: Millbrook Reserve, South Sister Reserve and Mount Hassell in the Stirling Range National Park. The three populations differed in average number of seeds available on living and dead plants, but in all cases the living plants stored far more seeds.

An intense fire was set on the south side of Millbrook Road on 14 April 1993. The numbers of seed released from the living plants was much greater than the dead plants, because they stored fewer seeds and their cones were more likely to be incinerated. Many seeds were removed after dispersal, apparently by granivorous parrots. About 4% of seeds produced were converted into seedlings by October, representing about six seedlings per adult plant.

Acknowledgments

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General Introduction

Introduction

Banksia brownii Baxter ex R.Brown (1830) is a large shrub native to the south-western part of Western Australia. It is rare: there are about 3000 individuals confined to 15 populations (pers. comm. Dr. Ken Atkins). It has been chosen by State Government authorities for management action. The populations occur on road verges, small nature reserves and other Government Land within the Albany Shire, about 320-350 km south-east of Perth. Although most populations occur in conservation areas, the species is threatened by the dieback disease *Phytophthora cinnamomi* Rands.

Banksia brownii plants grow to about 1-2 m, with some occurrences up to 4 m. The bark is thin and smooth. The leaves are featherlike and are used commercially for the flower trade. The red coloured flowers open from the top of the flower spike (Wrigley and Fagg 1989). The flowers appear in autumn and winter.

In the Stirling Ranges the species grows on mountain tops and slopes at altitudes between 500 and 1100 m. The Stirling Range habitat is heath and open mallee on rocky skeletal soils. The southern populations occur in heath and woodland on gradual slopes in gravelly lateritic sands. The climate is mediterranean signifying hot, dry summers and cool, wet winters.

Phytophthora cinnamomi

Phytophthora cinnamomi Rands (Oomycetes, Peronosporales) is a soil borne pathogenic fungus. In *Eucalyptus*, the fungus invades the small fibrous roots (Zentmyer 1980). The symptoms of crown deterioration, chlorosis and dieback of small branches may take some time to develop (Zentmyer 1980; Wills 1993) or the plant may suddenly die, without any apparent symptoms. *Banksia* spp. usually experience stem girdling, with lesions to the stems and roots (Plate 1).

The symptoms of *P. cinnamomi* infection of dieback, wilting and chlorosis are similar to water stress and phosphorous toxicity (Grose 1991). Although Proteaceae cannot take up large amounts of phosphorous, they have been known to increase growth when given small amounts (Grose 1991). Phosphorous uptake in infected *B. hookeriana* increased. *P. cinnamomi* altered the allocation of phosphorous in the new and old leaves. Healthy plants usually allocate most phosphorous to the youngest leaves as they are areas were most phosphorous activity occurs. However, in infected plants the reverse is true.

In tests carried out by Cho in Hawaii (in McCredie *et al.*, 1985), *B. brownii* had 80% plant death after 96 days after *P. cinnamomi* inoculation. In this investigation it was the second highest susceptible species of *Banksia* studied. The most susceptible species was *B. cuneata*. *Banksia brownii* was categorised with response A, which implied that the highest death rate occurred at 35-40 days after inoculation. Some deaths occur through summer (60-90 days), increased in autumn (140-180 days) and continued through the winter months. All individuals of *B. brownii* which survived root inoculation died 20 days after stem inoculation with *P. cinnamomi*. This death may indicate that most surviving plants had escaped infection.

Plate 1. *Banksia brownii* showing stem lesions.

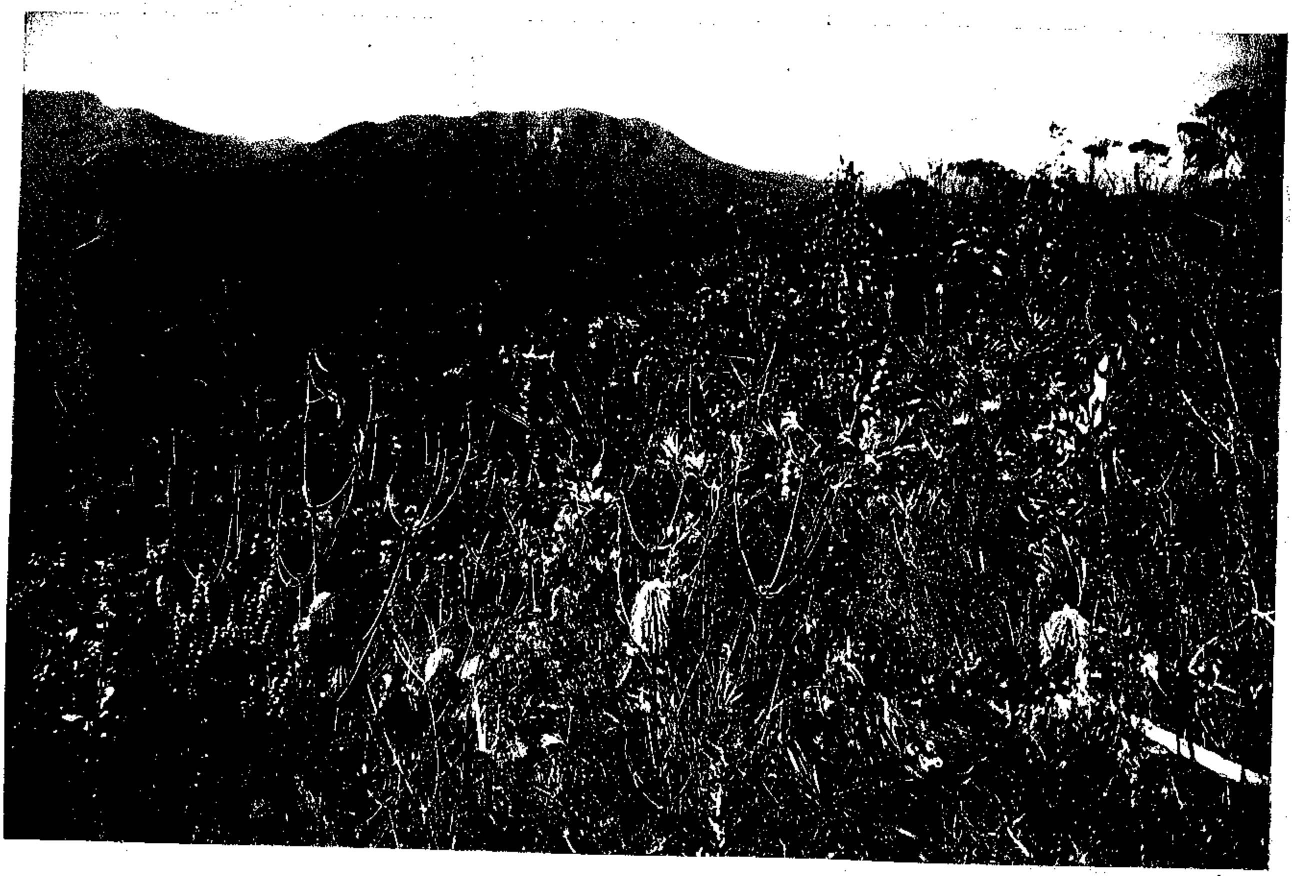

Plate 2. Dead *B. brownii,* Mount Hassell, Stirling Range National Park. Plants about 1.5 m high.

Fire ecology

Mediterranean-type ecosystems of the world share a group of relatively long-lived, often dominant, trees and shrubs which are particularly sensitive to fire (Bond 1984). These species accumulate seed reserves in the canopy of the plant, usually in woody cones which protect the seeds from damage, and release them *en masse* when the parent is killed. This delayed seed release is known as serotiny. Serotiny is derived from the Latin adjective serotinous meaning late in occurrence (Lamont *et al.*, 1991). *B. brownii* is serotinous.

Serotiny appears to have evolved in response to fire. However, only a small range of fire regimes is tolerated (Lamont *et al.*, 1991). All seed reserves are released after fire and none is stored in the soil until the next fire, so that if seedlings fail to establish, the population will become locally extinct. Major declines in population size have been observed where the vegetation has been burnt before the populations have reached reproductive maturity. Also, when they are left to senesce, populations decline because seed reserves do not persist after the death of the plants. Testing of the contribution of dead plants as a viable seed source was undertaken on *B. brownii.*

Seed bank dynamics have been studied for the rare *Banksia burdettii.* The amount of viable and stored seeds on *B. burdettii* increases with plant age (Lamont and Barker 1988). No soil-stored seeds were observed. No seedlings were sighted in unburnt stands. Insect damage was negligible. Losses due to seed senescence and spontaneous follicle opening were negligible in the first 2 years. Although some seeds were released each year, a total of 96% of viable seeds remained on the plant (Lamont and Barker 1988).

While old and dead plants of *B. burdettii* release some viable seeds spontaneously, this is insignificant compared with that released by burnt cones, as in other species (Lamont and Cowling 1984). Rapid seed release from burnt cones relies on frequent wet-dry cycles (Cowling and Lamont 1985). Rapid seed release when conditions first become suitable for germination and growth (May-July) ensures satiation of post-dispersal predators and maximises the opportunity for root growth before the onset of the hot dry summer. Non-autumn fires could result in premature seed release in this species and lead to poor recruitment, although seed release is not complete in the absence of rain.

The fire regime if therefore important in the perpetuation of populations of serotinous species. These species survive only as seeds; therefore, their population dynamics is dependent on their seed biology (Bond 1984).

The major factors are:

1. size of the seed reserves before fire,
2. rates of seed loss after dispersal and before germination,
3. extent of germination, and
4. early seedling mortality.

The number of seeds stored on the plant determine the potential population after a burn. The age of a plant affects the size of the seed bank (Enright and Lamont 1989). As the seed-bearing structures age, then there is an increase in the proportion of seeds released spontaneously (Lamont *et al.*, 1991). These seeds are less likely to germinate because of competition with mature plants and the thickness of leaf litter, which may not allow the seeds to be in contact with the soil (Lamont *et al.*, 1993).

Seeds released from a spring fire could result in premature seed release and lead to poor recruitment, although seed release will not occur in the absence of rain (Lamont and Barker 1988). Premature seed release could result in the loss of seed viability over the hot, dry summer period. Seeds released from an autumn burn are subject to a short time to predator pressure (Enright and Lamont 1989), so germination is high compared with spring burns.

Extended seed exposure on the soil surface increases the probability of predation (birds and rodents) and decay. Seed predation may have been important in the evolution of serotiny. The recruitment of *Eucalyptus delagatensis* between fires is limited by predation. After fires, seed-eating ants are satiated by massive seed release from the serotinous fruit allowing dense seedling recruitment. Seed predators (birds and rodents) are also important in depleting seed reserves in North American serotinous pines.

Some seed destruction may occur inadvertently from birds seeking insects contained within the flowers. The flower head of *B. tricuspis* is invaded by moth larvae (Lamont and van Leeuwen 1988). Cockatoos destroy the flower heads while looking for the larvae. It was found that 42% of flower heads were destroyed in error. Ants and wasps controlled the moth larvae before they entered the rachis. Beetle larvae had a minor impact on seed destruction. The amount of mature seeds would be four times greater if it were not for feeding by these animals.

The number of seeds germinating after a burn depend on the number of microsites available (Lamont *et al.*, 1993). Large numbers of seeds are usually found in litter microsites (Enright and Lamont 1989). Litter-covered areas and the location of seedlings were strongly associated in spring and autumn burn sites. This indicated that seed dispersal into microsites was important for germination and establishment. Once buried in the litter, the seeds remained viable for about 6 months after the fire, until conditions became suitable for germination (Enright and Lamont 1989).

The germination of seedlings in the litter microsites did not always appear advantageous. After a summer wildfire and a backburn in the sclerophyll shrubland of Western Australia germination of seedlings was compared. Germination was high over the winter period. However, seedlings were less likely to survive in the litter area than in the sand areas due to increased competition. The seedlings were smaller than those in the sand by the end of the first summer (Lamont *et al.*, 1993). Pre-summer thinning increased the survival of the litter seedlings. The high mortality of litter seedlings was probably due to the early depletion of surface soil water due to the number of seedlings. The lack of water was confirmed as the limiting factor by the negligible death in the area that was thinned. Nutrients can be dismissed as a limiting factor as the levels in the litter were much higher than in the other microsites.

Objectives

This project involved three objectives. The first was to compare seed storage and release from living and dead (*P. cinnamomi* killed) plants of *B. browniii* in the same population. The second was to assess the fate of seeds stored on plants as a result of an experimental fire. The third was to assess survival of *B. brownii* seedlings in a range of alternative *P. cinnamomi* free habitats and in the presence of the fungicide, phosphorous acid. At the time of this report, the latter two objectives are ongoing.

Seed Bank dynamics of *Banksia brownii*

Introduction

The aim of this section was to compare live and dead plants of *Banksia brownii* as a source of seeds for the next generation. There is no literature available on this topic. The only relevant information is that for *B. cuneata* where Lamont *et al.*, (1991) showed that dead branches shed more of their seeds from living branches.

Methods

Three populations of *B. brownii* in the Albany region were studied. Condition of stored seeds was assessed on all populations studied at Mount Hassell, Millbrook Reserve and South Sister Reserve. On the 13th and 14th January, 1993 the plants in the Millbrook Reserve and Mount Hassell were aged by Byron Lamont, according to their stem growth pattern (Lamont 1985). Growth is strictly terminal and each count terminates the previous season's growth increment.

At the same time, 20 cones, one for each of 20 plants, from the dead and live parts of the populations were harvested. The cones were ignited to expose and rupture the follicles, and then submitted to wet-dry cycles to release the seeds (Lamont and Cowling 1984). The numbers of follicles which were intact, open prior to burning (seed released) or damaged by insects, were determined. Numbers of aborted, eaten, rotted and firm seeds still present were counted. All firm seeds in each population were germinated at the probable optimum (15°C) temperature (Cowling and Lamont 1986) and percentage germination recorded over 20 days, after which no more germination occurred. Seeds which germinated under these conditions were regarded as the total viable seeds (Lamont and van Leeuwen 1988) (Figure 1).

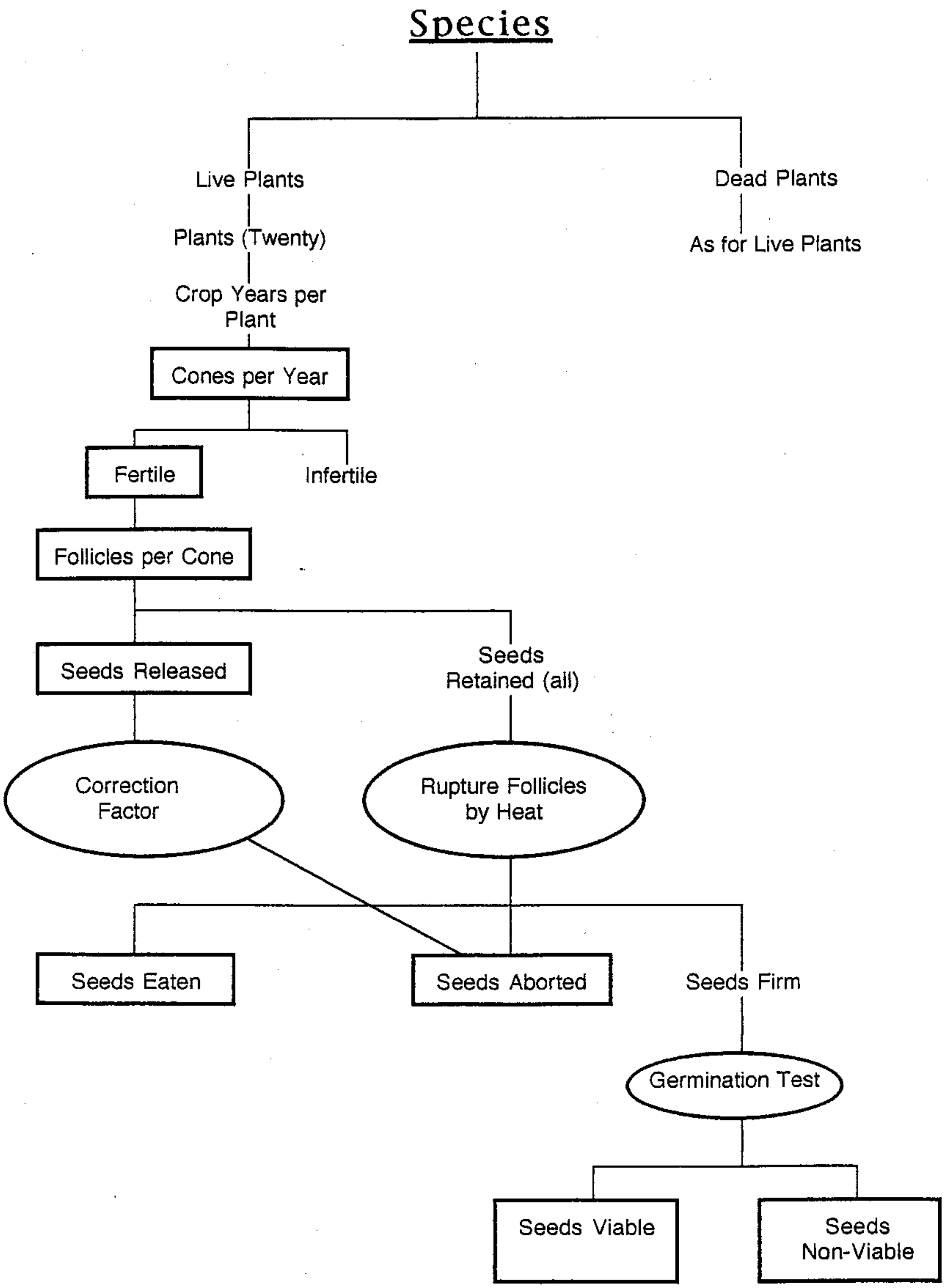

Figure 1. Flow diagram used to determine seed bank dynamics of *B. brownii.* A box represents a component used for further numerical analysis, while an oval represents a process.

Results

The ages of the plants at the three populations studied differed (Table 1). The oldest plants were present in South Sister Reserve, with Millbrook Reserve and Mount Hassell plants similar in age. The South Sister population had a number of dead plants that were lying prone to the ground (20%). The cones from these plants were mostly rotten. A total of 94 follicles were present on the 20 representative cones taken from these plants. Only 12 seeds (13%) were extracted from these cones and 10 seeds (11%) were viable. This is comparable to the other cones collected from upright, dead plants which had 22% of their seeds firm and 12% viable.

Table 1. Modal age of a sample of twenty plants from the three populations studied. The percentage of plants which had this age are in parenthesis.

	Millbrook Reserve	South Sister Reserve	Mount Hassell
Live	12 (45%)	25 (20%)	11 (55%)
Dead	11 (35%)	21 (30%)	8 (45%)

The two populations at Millbrook Reserve and South Sister Reserve were compared for the number of seeds released and the amount of insect damage. The cones taken from the live plants at Millbrook had the lowest level of seed loss. The Millbrook Reserve sample of cones from dead and live plants did not have any follicles/seeds that had rotted.

The South Sister Reserve sample had a high proportion of pre-fire seed dispersal in cones taken from live and dead plants (8.8% and 34.55 respectively). Total follicles rotted was 17%.

The percentage of aborted seeds was high amongst all populations (in the range of 44 to 49% in the live sample). In the sample taken from the dead plants at South Sister Reserve, other factors such as rotted cones prevented precise counting of the aborted seeds. The Millbrook Reserve sample of dead plants had a high proportion (71%) that were aborted (Table 2).

Table 2. The mean contributions to the seed bank stored in the canopy of live and dead *B. brownii* in three populations in 1993. C = condition of plant (live/dead); S = site; C*S = interaction. Significance by two-way ANOVA. * P<0.05; ** P<0.01; *** P<0.001; NS not significant.

	Millbrook Reserve	South Sister Reserve	Mount Hassell	C	S	C*S
Fertile cones per plant						
Live plants	7.4 (7.3)	46.2 (26.8)	2.3 (1.5)	*	**	NS
Dead plants	5.2 (3.3)	29.2 (29.4)	0.5 (0.7)			
Follicles per cone						
Live plants	23.7 (6.0)	27.0 (8.3)	20.0 (14.2)	NS	*	NS
Dead plants	27.5 (9.6)	27.7 (6.4)	17.6 (8.9)			
Non-aborted seeds per cone						
Live plants	10.8 (4.2)	8.4 (5.8)	2.0 (2.8)	***	***	**
Dead plants	3.3 (2.3)	7.6 (5.8)	1.2 (1.3)			
Viable seeds per cone						
Live plants	9.7 (4.4)	3.4 (5.2)	1.9 (2.6)	***	***	***
Dead plants	1.5 (1.6)	5.1 (4.5)	0.5 (0.6)			
Viable seeds per plant						
Live plants	66.9 (60.2)	232.9 (558.6)	5.8 (8.6)	***	***	***
Dead plants	7.7 (13.6)	87.7 (98.1)	2.9 (4.5)			

Discussion

B. brownii is one of the few *Banksia* species that produces only one seed per follicle. This factor may have contributed to the relatively low seed set by most standards (Stephenson 1981), but is comparable to *B. coccinea* (Witkowski *et al.*, 1991), which also occurs in the Albany region. The percentage of fertile cones per tree did not differ from the results for *B. grandis* (Abbott 1985) in the jarrah forest. The abortion rate compared with two other species in the region, *B. baxteri* and *B. speciosa*, was high but it was similar to *B. coccinea* (Witkowski *et al.*, 1991).

Other *Banksia* species have been shown to have a large number of infertile cones (Abbott 1985; Cowling *et al.*, 1987; Witkowski *et al.*, 1991). Seed destruction by insect larvae did not have a major impact on the reduction in viable seeds compared with other *Banksia* species (Abbott 1985; Lamont and van Leeuwen 1988; Witkowski *et al.*, 1991).

B. brownii has a mixed mating system (Sampson *et al.*, unpublished). Most inbreeding is the result of self-fertilisation occurring when there is no pollen carried from other plants by birds and mammals within the population. This inbreeding may relate to the high rate of abortions observed. Therefore, abortion may occur when the genotype is of low quality.

Seed destruction by the wet conditions at South Sister Reserve appeared to be a significant loss to the seed bank in dead plants. The population at this reserve is present on the south-east side of the reserve in a dense thicket of vegetation. The prolonged exposure for up to four years has resulted in high levels of dehiscence and rotting (34.5% and 17% respectively).

Despite the fact that follicle numbers per cone were not significantly different between live and dead plants, there were far fewer viable seeds per cone on the dead plants. There is thus a strong case for harvesting cones as plants die from *P. cinnamomi* in order to save the seeds for replanting.

Fire and Seedling Recruitment

Introduction

Most *Banksia* species accumulate seed reserves in woody cones which protect the seeds from damage, and release them *en masse* when the parent is burnt. This delayed seed release is known as serotiny. Some banksias may take at least 10 years to accumulate sufficient seeds to ensure population replacement after fire (Lamont & Barker 1988). Most seed reserves are released after fire and none is stored in the soil until the next fire. If seedlings fail to establish after fire, the population may become locally extinct if the parents are also killed (Burgman & Lamont 1992). Plants which die through disease, drought or senescence release more seeds than living plants of the same age but these are usually wasted (Lamont & Barker 1988). Any seeds still stored on dead plants are usually incinerated when fire does occur. The fire regime is therefore important in the perpetuation of serotinous species. As fire-sensitive species exist only as seeds after fire, population dynamics are controlled by their seed and seedling biology (Bond 1984).

The number of seeds stored on the plant determines the potential population size after a fire. The age of a plant affects the size of the seed bank (Lamont & Barker 1988; Lamont *et al.*, 1991). As the seed-bearing structures age, then there is an increase in the proportion of seeds released spontaneously (Lamont *et al.*, 1991). These seeds are unlikely to produce juvenile plants because of competition with mature plants and the thickness of leaf litter, which may not allow the seeds to be in contact with the soil (Lamont *et al.*, 1993).

Therefore, we set out to determine the extent of seed dispersal and seedling recruitment after fire. Another objective to determine if there was a difference in the number of incinerated cones between live and upright and prostrate dead plants.

Methods

A fire trial was undertaken on Millbrook Road 3.4 to 3.7 km from the Albany Highway turnoff on 14 April 1993. The trees were aged at a modal age of 15 years, representing the period since the last fire. There were three treatments, each made up of 13 plants. One set consisted of the live plants on the site and these were matched with dead plants that were either laid on the ground or placed upright. The plants were placed upright to simulate recently dead plants (< 2 years ago) and the other treatment was to simulate plants that had died over two years ago and had fallen to the ground, as we had observed in both South Sister and Millbrook Nature

Reserves. For each plant, five of its representative cones were tagged with aluminium labels of dimensions 50 mm x 50 mm and 0.5 mm thickness. This gave a total of 65 cones per treatment. Every cone that was numbered had a recording of the treatment type it belonged to, whether it was on a living or dead plant, which was either standing or lying prone on the ground.

The day was windless and the fire removed all leaves from all plants except one living plant on the edge. The cones and stems of most of the dead plants on the ground were incinerated. The Albany Fire Control staff considered the fire to be very intense.

The next day, 15 April 1993, each cone was identified and the number of total and open follicles and the number of seeds released was recorded. During subsequent trips to the sites at 28 (12 May, 1993), 76 (1 July, 1993) and 97 days (21 July, 1993) after the fire numbers of seeds released per cone were recorded.

As few seeds were seen on the ground while bird droppings were conspicuous, it was hypothesised that granivores were removing many of the seeds. Three experimental plots, therefore, were set up within the fire area on 14 May 1993 (26 days after the fire). These plots contained 30 seeds that were broadcast on the surface of the soil and 30 seeds that were buried at 5 to 10 mm below the surface. The numbers of seed germinated was recorded on a monthly basis.

Data analyses

The differences between treatments was compared by one-way analysis of variance with Tukey's multiple comparison of means. Where necessary data was normalised using arcsin transformations (Zar 1984). Contingency tables were used to analyse whether there was any difference between the number of incinerated cones between treatments. Contingency table analysis, with Yates correction for factor continuity (Zar 1984), was used to compare seedling establishment of scattered and buried seeds.

Results

A chi square analysis showed that the number of cones that were totally or partly incinerated was dependent on whether the plant was alive or dead prior to the fire. Whether the cones were incinerated was independent of the dead plants' position before the fire. The live plants had 4.6% of their cones that were partially incinerated (Table 3). The dead plants had 20 to 30% of their cones totally incinerated and 15 to 17% partially incinerated.

The intense fire left only 55.4% and 63.1% of the dead upright and ground level cones intact. A total of seven plants out of the thirteen used in the upright, dead treatment were leafy plants. The leafy plants would have burnt at a greater temperature and therefore incineration of cones was more likely.

Most of the intact follicles opened during the fire, with only a small percentage (1%) opening since that time (Figure 2) to the present. There was a significant difference ($P < 0.001$) between the number of follicles open before and 97 days after the fire, when the number of follicles open had reached it maximum. A comparison of means using Tukey's pairwise comparison established that there was a significant difference ($P < 0.05$) between the treatments. The same significance testing on the post-fire number of follicles resulted in no significant difference ($P = 0.114$) in the treatments for an ANOVA, and the Tukey's pairwise comparison at $P = 0.05$ confirmed that the means were similar.

Table 3. The total number of intact, incinerated and partially incinerated cones (percentage of total in parentheses) of a random sample of 65 cones of *B. brownii* cones after fire on 14 April, 1993 on the southern side of Millbrook Road.

Significance by chi-square analysis: * *P*<0.05; ** *P*<0.01; *** *P*<0.001; NS not significant.

Plant	Number of cones			Chi-square	*P*
	Intact	Partially Incinerated	Completely		
Live, upright	62 (95.4)	3 (4.6)	0 (0)		
				29.67	***
Dead, upright	36 (55.4)	10 (15.4)	19 (29.2)		
				1.50	NS
Dead, ground	41 (63.1)	11 (16.9)	13 (20.0)		

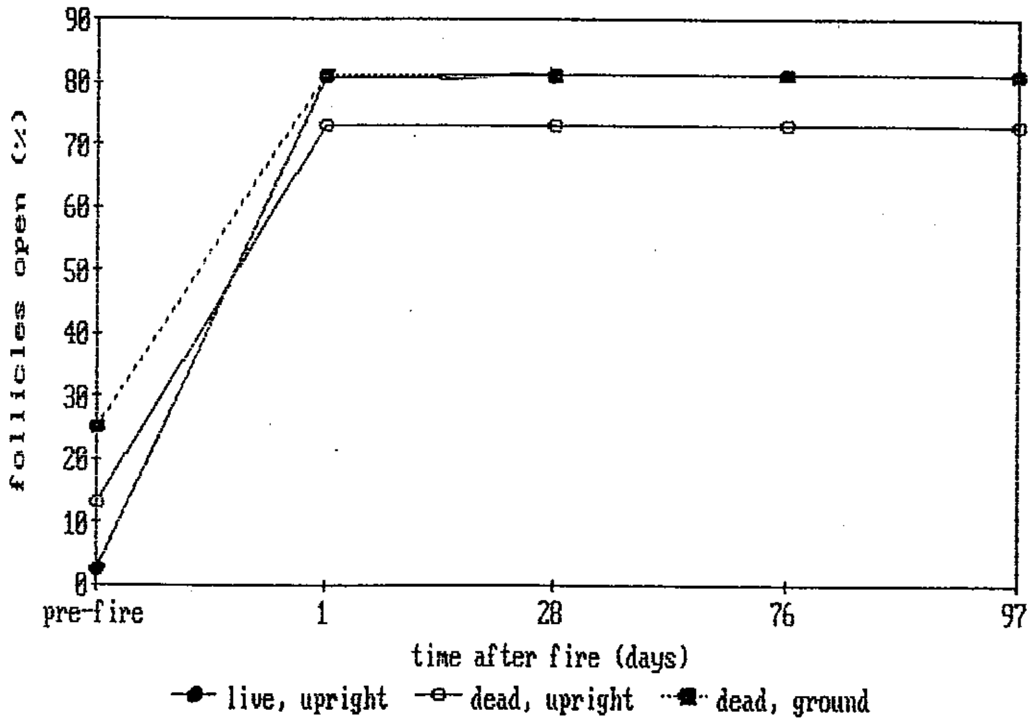

Figure 2. Percentage follicles open in *Banksia brownii* at the burned site on the southern side of Millbrook Road.

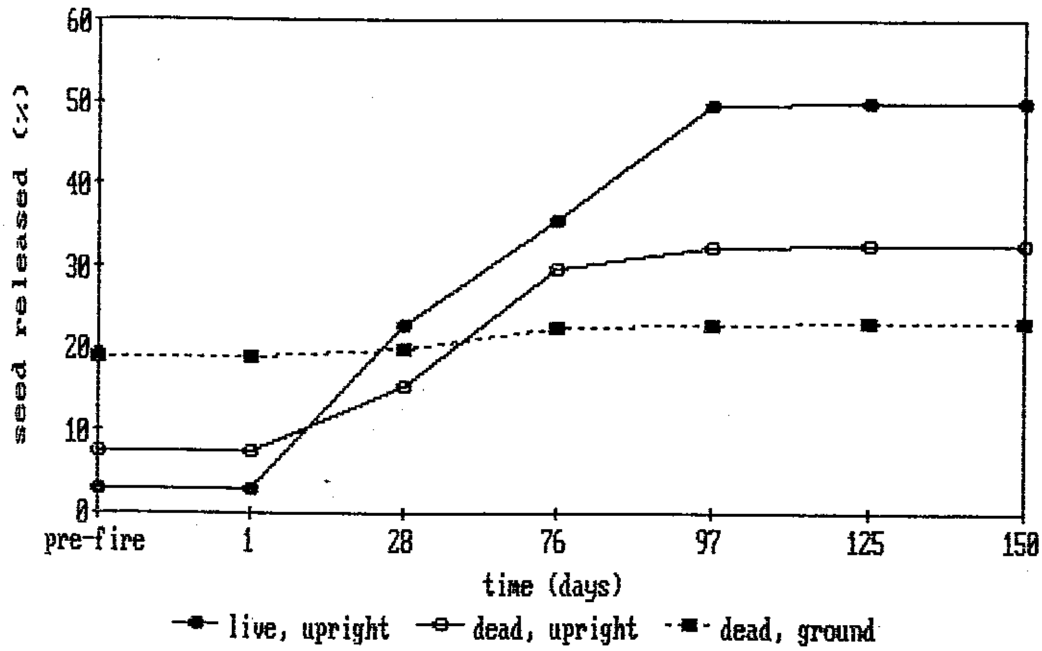

Figure 3. Percentage seed release in *Banksia brownii* at the burned site on Southern side of Millbrook Road.

Table 4. Percentage germination of *Banksia brownii* seeds sown on 14 May, 1993. Three replicates of 30 seeds were scattered on the ground or buried to a depth of 10 mm on the south side of Millbrook Road after an intense fire on 14 April, 1993. Standard deviations are in parentheses.

	Germination (%)	
Days after sowing	Seeds buried	Seeds scattered
48	46.7 (8.8)	4.4 (4.8)
76	46.7 (8.8)	4.4 (4.8)
97	46.7 (8.8)	4.4 (4.8)
125	46.7 (8.8)	4.4 (4.8)

The number of seeds released from the plants alive before the fire, 97 days after the fire, was 45.8%. The majority of closed follicles contained aborted seeds. Only 24.7% of the seeds were released from the dead trees that were upright and 3.8% from the plants that were on the ground. The amount of seed released was significantly different ($P < 0.01$) between the treatments.

All seeds that were scattered on the ground in the plots, within the burned area, were missing by the next visit (48 days) except for two seeds, present as seedlings. Of the seeds that were buried, 46.7% (4.8 sd) were observed as seedlings and the rest assumed to be still buried, as there was no soil disturbance by possible granivores. There was no actual granivory observed, but five western rosellas (*Platycercus icterotis*) appeared resident at the site and were seen feeding on the ground a few metres from the burnt area on 20 July 1993.

The total recruitment of seedlings was 238 (4.4% of unopened follicles on all plants at the study site), 200 days after the intense fire on 14 April, 1993. This corresponded to 6.1 seedlings per parent. Weeds originating from the adjacent farmland are now prevalent. There was little mortality amongst the seedlings as only 6.3% had died up to 2 November 1993. There was no evidence as to the cause of death. A small proportion (7.8%) had their cotelydons chewed, but the new leaves were untouched. This was probably caused by grasshoppers from the adjacent farm.

Discussion

Seed release from *B. brownii* occurred gradually over a 97 day period which is characteristic of the genus (Zammit 1984). All current seedlings produced could be accounted for by only 4% of unopened follicles before the fire. In the other studies, 5-20% of follicles yielded germinants in the laboratory. Seed predation following dispersal is the significant difference between these two sets of results. Other species of *Banksia* (*B. attenuata* and *B. menziesii*) are affected by post-dispersal predators after fire (Cowling & Lamont 1987). Although a number of western rosellas (*Platycercus icterotis*) were observed in the area, there was no sighting of them eating the seeds. Seed scattered on the surface in designated sites compared with the seeds that were buried had ten times fewer seedlings. Although the identity of the animals responsible for seed removal is unknown they were probably parrots, and perhaps pigeons, since similar large seeds are seldom taken by ants (Abbott & Van Heurck 1985).

Dead plants held on to 85.4% of their seeds. This is comparable with *B. burdettii*, which retained 82% (Lamont & Barker 1988). *B. brownii* was able to release same seeds (4-25% of that produced) from the dead plants, whereas *B. burdettii* had all seeds incinerated by fire. The best source of seeds is clearly from the live plants (46% of that produced). Clearly, the dead plants are a poor source of seeds.

Alternative habitats for *B. brownii*

Introduction

Population locations

All populations occur within a range of 150 km. They are all located between 117°30'E and 118°15'E longitude and 34°30'S and 35°30'S latitude.

The populations within Millbrook Reserve, South Sister Nature Reserve and along Millbrook Road were studied. The populations within the reserves had a number of dead plants (pers. obs.). The Millbrook Reserve and Millbrook Road populations did not have any young plants. However, at the South Sister population young plants were present (Denise Day pers. comm.). All these populations have dieback disease (*Phytophthora cinnamomi*) (D. Coates pers. comm.).

Millbrook Reserve

Millbrook Reserve 18739 is located at 117°52'E longitude and 34°52'S. This reserve is located at the end of an unnamed road 6 km from the junction of Settlement Road and Albany Highway. This population is one of the larger populations with about 100-150 adult plants (pers. obs.). The Reserve occupies a remnant of bushland of 1479.3 hectares.

This Reserve has a variety of vegetation types. The areas that were chosen for alternative sites for the introduction of *Banksia brownii* consisted of woodlands of *Eucalyptus marginata* (jarrah), *Casuarina frasierana* (sheoak) and *Eucalyptus staeri*. The jarrah and sheoak woodlands had very little understorey, whereas the *E. staeri* had an understorey of either low scrub or thickets of various proteaceous species.

Soil

Millbrook Reserve has a relief of about 40 m (Griffin 1985). The valleys have steep slopes and narrow valley floors. The uplands are usually flat with steep slopes and a marked change in slope at the boundaries. The soils of the uplands are usually fine sands and gravels with small areas of duricrust. Most soils of the uplands are poorly drained and therefore small drainage lines have appeared. The soils on the valley floors are sandy, and shallow in places, overlying lower soil horizons of the weathered Pallinup siltstone. Lateritic gravels cover a small proportion of the slopes, nearby to lateritic duricrust formed on some uplands.

Vegetation

When mapping the Albany-Mt Barker areas, Beard (1979) drew his boundary through Millbrook Reserve to divide the Albany and Narrikup Systems. These systems are in the Menzies subdistrict of the Darling Botanical Province. Low forests of *Eucalyptus marginata, Casuarina fraseriana* and *Eucalyptus staeri* occur on the uplands with sedges and shrubs dominating the lowlands of the valleys.

South Sister Reserve

South Sister Reserve is located on Mt Pleasant Road approximately 35 km north-east of Albany. The reserve covers 338.32 ha. It is bordered by farmland to the west, east and south. To the north it is bordered by Mt Pleasant Road and North Sister Reserve No. 26385.

The Reserve is dominated by the granite outcrop of South Sister which rises to 240 m above sea level. The slope on all sides of the granite outcrop is fairly even. The soil varies from lateritic to pisolitic gravel on the ridges to grey sand on the low lying areas. Firebreaks have been constructed around the perimeter of the Reserve, with a deep gully to the south. South Sister Reserve has a varied flora ranging from low open heath to jarrah/marri forest. The most important floristic component is the large population of *B. brownii.*

Climate

According to Beard (1979) the climate of this region is warm mediterranean, which is dry for 5 to 6 months of the year.

The rainfall varies from 611.6 mm to 966.7 mm pa. The majority of rainfall occurs between May and August, with the maximum rainfall occurring in July. The rain is the result of fronts from the south, moving north-east across Western Australia.

Although the mean temperatures for January and February are 25.2^{O}C and 25.1^{O}C respectively, the temperature may rise to 45.5^{O}C in January. This extremely high temperature over the summer period coupled with low rainfall would constrain seedling establishment (Lamont *et al.*, 1991). The low temperatures experienced in the winter months, with temperatures for June and July falling to -0.2^{O}C and 0.0^{O}C on same days would also restrict seedling establishment and growth due to frosts.

Transplant sites

Dieback-free alternative areas for establishing *Banksia brownii* populations were sought because this species is very prone to *P. cinnamomi.* Differences in the water holding capacity of some soils is not conducive to this soil fungus (Shea *et al.* 1983). Associations with certain plants, such as *Acacia pulchella,* can also reduce the incidence of *P. cinnamomi* (Shea *et al.*, 1983). Therefore, the establishment of *B. brownii* in different soils and vegetation types may be an option in the management of this rare species.

Methods

The transplant sites were chosen to represent the range of vegetation and soil types within the South Sister and Millbrook Reserves. In addition, calcareous sites were selected as *P. cinnamomi* may be inhibited by high pH. Zentmyer (1980) found that *P. cinnamomi* growth varied in relation to the nitrogen source. Hepting *et al.*, (in Zentmyer 1980) showed that high levels of nitrogen retarded the development of *P. cinnamomi.* Later studies by Weste *et al.*, (1976) in the Brisbane Ranges in Victoria, showed a significant difference in the nutrient levels in the soil of which *P. cinnamomi* had killed most of the flora. They found that soils that lacked the pathogen had a high organic matter content (8.03 vs. 2.29%), higher total phosphorous (537.3 vs. 149.0 ppm), and higher total nitrogen (1450.7 vs. 431.32 ppm) than the soil that contained the fungus (Weste *et al.* 1976).

Paired sites were selected at Millbrook (woodland) and South Sisters Reserves (scrub-heath), matched for habitat-type but apparently varying in the presence of *P. cinnamomi* (Table 5). *B. brownii* was present in the Millbrook pair but absent from the South Sisters pair. Additional matched sites at the two reserves included a casuarina woodland and jarrah (dry sclerophyll) woodland. A wet sclerophyll woodland and a scrub-heath site were positioned in the Waychinnicup area to match those in South Sister as there was no equivalent at Millbrook. Two calcareous sites were situated near the coast in the vicinity of the Waychinnicup and Cheyne Beach areas. This provided 13 transplant sites in cell. Soil samples were taken from each site and tested for *P. cinnamomi* using the lupin-baiting technique (Blowes 1980).

B. brownii seeds were placed on moist paper towels in large open trays and left to germinate in the 15^{o}C cabinet. On the 13-15th April 1993 thirty seeds were planted at each treatment site. These sites were then assessed on the following dates (at approximately thirty day intervals): 12th May, 30th June, 21st July, 4th September, 2nd October and 2nd November 1993.

Table 5. Descriptions for transplant sites.

Millbrook Reserve

	P. cinnamomi present	absent	Jarrah woodland	Sheoak woodland
Soil colour	grey	grey/brown	dark brown	grey
Soil type	fine sand	fine sand over laterite at 5-10 cm	50% gravel and loamy sand	deep sand
Litter depth	3 cm eucalypt leaves /moss	2 cm eucalypt leaves	2 cm eucalypt leaves	2 cm sheoak needles
Slope	2^{o} NS	3^{o} NS	3-4^{o}NS	5^{o}NS
Vegetation	woodland scrub-heath thicket	woodland scrub-heath	woodland	low forest

Dominants

P. cinnamomi present

Banksia brownii
Eucalyptus staeri
Hakea ferruginea
Hakea lasiantha
Dryandra baxteri

P. cinnamomi absent

Banksia brownii
Eucalyptus staeri
Agonis parviceps
Beaufortia anisandra

Jarrah woodland

E. marginata
Banksia grandis
Persoonia longifolia

Sheoak woodland

Casuarina fraseriana
Anarthria prolifer
Agonis parviceps
Dasypogon bromeliaefolius

South sister Reserve

	P. cinnamomi present	absent	Jarrah woodland	Sheoak woodland
Soil colour	dark brown	grey/brown	orange/yellow	grey
Soil type	gneissic rock and silty loam	2 cm sand over laterite	pisolithic laterite	deep sand
Litter depth	1 cm sedge	1 cm hakea	2 cm hakea/ eucalypt leaves	2 cm casuarina eucalypt leaves
Slope	6° NS	6° NS	8-4°NS	4°NS
Vegetation	scrub-heath	scrub-heath sedge sword	woodland to 20 m	low forest with open scrub-heath

Dominants

P. cinnamomi present

Hakea cucullata
Hakea lasiantha
Kingia australis
Agonis parviceps
Lepidosperma spp.

P. cinnamomi absent

Hakea cucullata
Hakea lasiantha
Lepidosperma spp.

Jarrah woodland

Eucalyptus marginata
Eucalyptus calophylla
Hakea amplexicanis
Banksia grandis
Bossiaea linophylla

Sheoak woodland

Casuarina fraseriana
Agonis parviceps
Kingia australis

Other sites

	South Sister wet sclerophyll	Waychinnicup wet sclerophyll	Bluff Creek calcareous	Cheyne Beach calcereous	Waychinnicup scrub-heath
Soil colour	yellow/brown	grey	grey	white	grey
Soil type	sandy clay pisolithic gravel	sand and humus	fine deep sand	fine deep sand	coarse sand
Litter depth	1-2 cm eucalypt leaves	4 cm eucalypt leaves	1 cm moss and lichen	1 cm sedge	negligible
Slope	10^{o} NS	0^{o} NS	7^{o}NS	2^{o}NS	3^{o}W
Vegetation	forest to 20 m closed heath	woodland	woodland tree clumps	low wind-pruned scrub-heath	scrub-heath

Dominants

South Sister wet sclerophyll

E. marginata
E. calophylla
Agonis hypericifolia
Bossiaea linophylla
Acacia sp.

Waychinnicup wet sclerophyll

E. megacarpa
Agonis linearifolia
Hypocalymma cardifolium
Acacia drummondii

Bluff Creek calcareous

Agonis flexuosa
E. calophylla
Adenanthos cuneatus
Casuarina humilis

Cheyne Beach calcareous

Agonis flexuosa
Leucopogon parviflorus
Adenanthos sericeus
Olearia axillaris

Waychinnicup scrub-heath

Banksia coccinea
Banksia baxteri
Hakea cucullata
Lepidosperma spp.

Results

Seedlings at the sheoak site at South Sister Reserve was apparently attacked by red-legged earth mite (*Halotydeus destructor* Tucker). Almost all seedlings were missing from both *Banksia brownii* sites at Millbrook Reserve, apparently eaten by invertebrate granivores. The stumps of hypocotyls were sometimes observed inmoist sites such as the South Sister jarrah site. The Wet Sclerophyll site at Waychinnicup were apparently killed by damping off fungi, as the stems were rotted. The scrub-heath alongside Cheyne Beach Road did not have a single seedling remaining by 48 days and no evidence could be found to determine the death of the seedlings. Presumably this was the result of herbivorous insects since death from other causes would have left remnants visible on the surface.

Lupin-baiting showed that the Millbrook *P. cinnamomi* site and the South Sister +/- *P. cinnamomi* sites all had *P. cinnamomi* in the surface soil.

A chi-square showed that the number of seedlings was dependent on site ($P < 0.001$). Further analysis showed that number of seedlings at the sites within the South Sister Nature Reserve were independent of site location ($P < 0.20$). In the Millbrook Reserve the results of the chi-square analysis showed that the number of seedlings per site were dependent on the location of the site within the reserve ($P < 0.001$).

Analysis between matched areas, such as the calcareous sites, showed that the amount of growth (height) was independent of site location ($P < 0.98$). Results for the jarrah sites in both reserves were independent of location ($P < 0.20$). Location of the sheoak sites greatly effected the number of seedlings still alive ($P < 0.01$), even though both sites were located within 20 m of pasture.

Discussion

The site at Millbrook Reserve with the greatest survival of *B. brownii* seedlings (90%) was located in the sheoak woodlands. There is some possibility that this site may be grazed by kangaroos, as a small number had the main shoot damaged. At South Sister Reserve, the non-*P. cinnamomi* scrub-heath site had 90% survival. The calcareous soil site at Cheyne Beach and Bluff Creek had 80% survival. The other sites had survival rates of 7-47%. This reduction was apparently for establishing *B. brownii* caused by invertebrate predation. Establishment of alternative sites, will require the isolation of the seedlings from invertebrate predators, until they are of a suitable size to escape the attention of herbivores.

These preliminary findings do not take into account survival over the summer period, which is crucial to the overall survival of the species. Lamont *et al.*, (1991) studied the effect of water availability required in seedling establishment and found that the numbers of seedlings decreased as monthly rainfall decreased and the spring and summer temperatures rose. Seedlings planted in lower areas or watered artificially were more likely to survive the summer drought.

There is no apparent effect of *P. cinnamomi* at present on any of the sites at South Sister, that have been identified as having the fungus. However, Wills (1993) suggests that even though *P. cinnamomi* may be present in the soil, the symptoms of it will not become apparent until a critical biomass of the host species is reached. The other sites were tested as *P. cinnamomi* free and therefore could be used in the future to establish alternative stands of *B. brownii*. To date the best sites are the calcareous sites at Cheyne Beach and Bluff Creek and the sheoak site at Millbrook Reserve.

Phosphorous acid application

Introduction

Phosphorous acid, H_3PO_3, is hydrolysed from fosetyl-Al. These chemicals have good phloem mobility and can be applied to foliage or by trunk injection (Coffey & Joseph 1985). The mycelial growth of *Phytophthora* species is sensitive to phosphorous acid, with EC50 values of 5-90 ug/mL, for in vitro inhibition (Coffey & Joseph 1985). *P. cinnamomi* was inhibited by 50% (EC50) by concentrations between 4.1 and 6.2 ug/mL (Coffey & Joseph 1985). Sporangium formation, zoospore release, oospore production and chlamydospore production were inhibited for EC50 ranging in concentration from 1.8 ug/mL to 44 ug/mL (Coffey & Joseph 1985).

The Department of Conservation and Land Management (CALM) have been studying the effects of phosphorous acid on *P. cinnamomi* susceptible species. It has been found that 2-10% phosphorous acid injected into *B. grandis* inhibits lesion development (B. Shearer pers. comm.). However, *B. coccinea* has a phytotoxic effect at 10% with the most effective dose at a concentration of 2% (B. Shearer pers. comm.). This injection is effective for four years (B. Shearer pers. comm.). Foliar spraying has been studied on a number of *Banksia* species in the Albany area. An effective concentration of 0.5% gives adequate control over a period of 2 - 2.5 years (B. Shearer pers. comm.). Phytotoxic effects have occurred in *B. coccinea, Daviesia* spp., *Petrophile* spp. and *Xanthorrhoea* spp. at concentrations greater than 1%. Studies of broad scale spraying began at South Sister Reserve on the *B. brownii* population in autumn this year. The outcome of these tests are not yet available.

Methods

Thirty seedlings at the two scrub-heath sites in the South Sister Nature Reserve were sprayed with phosphorous acid (H_3PO_3) concentrations of 0.1 and 0.35% on a monthly basis (Plate 3). This solution was made up of 2.0% surfactant and 0.35% acid solution. These two sites were infected with *P. cinnamomi.* Each plant was assigned a treatment due to its position within the population. The treatment seedlings were chosen by random number selection. The treatment was modified if the random numbers of a treatment were grouped together (stratified sampling).

Height and leaf number were recorded to determine growth rates. A two-way ANOVA was used on these data to determine if there was a difference between sites and concentrations.

Plate 3. Phosphorous spraying, South Sister Reserve.

Results

There was a significant difference ($P < 0.001$) between the sites. There was no significant difference between treatments ($P < 0.05$) and no interaction between treatments and sites ($P < 0.05$), (Table 6).

Table 6. Leaf number and height of *B. brownii* seedlings used in the phosphorous acid trial at South Sister Reserve. Results are mean (s.d.) for x seedlings. Significance by 2-way ANOVA between treatments (T) and sites (S) * P<0.05; ** P<0.01; *** P<0.001.

		Treatments				
	Control	0.1%	0.35%	T	S	T*S
Leaf number / seedling						
Western site	10 (3)	11 (3)	11 (1)	NS	***	NS
Eastern site	7 (5)	9 (3)	8 (4)			
Height (mm)						
Western site	100 (26)	102 (18)	98 (15)	NS	***	NS
Eastern site	65 (27)	68 (22)	55 (19)			

Discussion

P. cinnamomi has had no effect on the seedlings so far. However, Wills (1993) suggests that even though *P. cinnamomi* may be present in the soil, the symptoms of it will not become apparent until a critical biomass of the host species is reached. There was no difference between the two concentrations and the control. Therefore, these concentrations of phosphorous acid have had no phytotoxic effect on the seedlings sprayed at monthly intervals.

The Western site had better growing conditions, probably due to the fact that the other site was waterlogged during the winter months. Monitoring of all transplant sites is continuing.

General Discussion

The number of open follicles was greater in the dead plants than on live plants. This means that after death the follicles open and release their seeds. The recruitment of these seedlings is negligible, probably because of an unsuitable seed bed and competition with the mature plants. The fire at Millbrook Road showed that the greatest proportion of incinerated cones came from the dead plants. This has also been shown with the restricted species *B. burdettii* in which all dead plants were incinerated and the seeds from the cones were lost (Lamont and Barker 1988). Therefore, it is recommended that cones are harvested immediately after a plant has died and the seeds stored for further replanting programs.

The numbers of *B. brownii* have declined over time due to clearing for farmland and the introduction of *P. cinnamomi.* Clearing for farmland has isolated populations from each other and reduced their size, and this isolation probably has had a detrimental effect on pollinator activity and viability of seeds. This disproportional effect on fertility with decreasing population size is known as the Allee effect (Lamont *et al.*, 1993). The small population studied at Mount Hassell showed all the signs of the Allee effect. The population was isolated and numbers are diminishing because of *P. cinnamomi* in the area. This population had on average two fertile cones per plant compared with over 7 and 46 at the Millbrook and South Sister reserves respectively. One would expect there to be no problem with pollinators as the area has an abundance of proteaceous species. However, *B. brownii* flowers in the winter period (D. Day per. comm.) when there is little else flowering in the vicinity.

After five months there appears to be no effect of *P. cinnamomi* on the seedlings at the alternative sites. This may be due to the presumed high concentrations of P in the cotelydons, which helps to establish the seedlings in the infertile soil environment of Australia (Abbott 1985; Lamont *et al.*, 1985) and reduces susceptibility to P. However, as the P levels in the cotelydons diminishes a difference may occur between the control and the treated plants. The phosphorous acid did not have any toxic effect on the seedlings sprayed at monthly intervals over the 4 months monitored, although toxicity levels have been found in adult plants at over 1% in *B. coccinea.*

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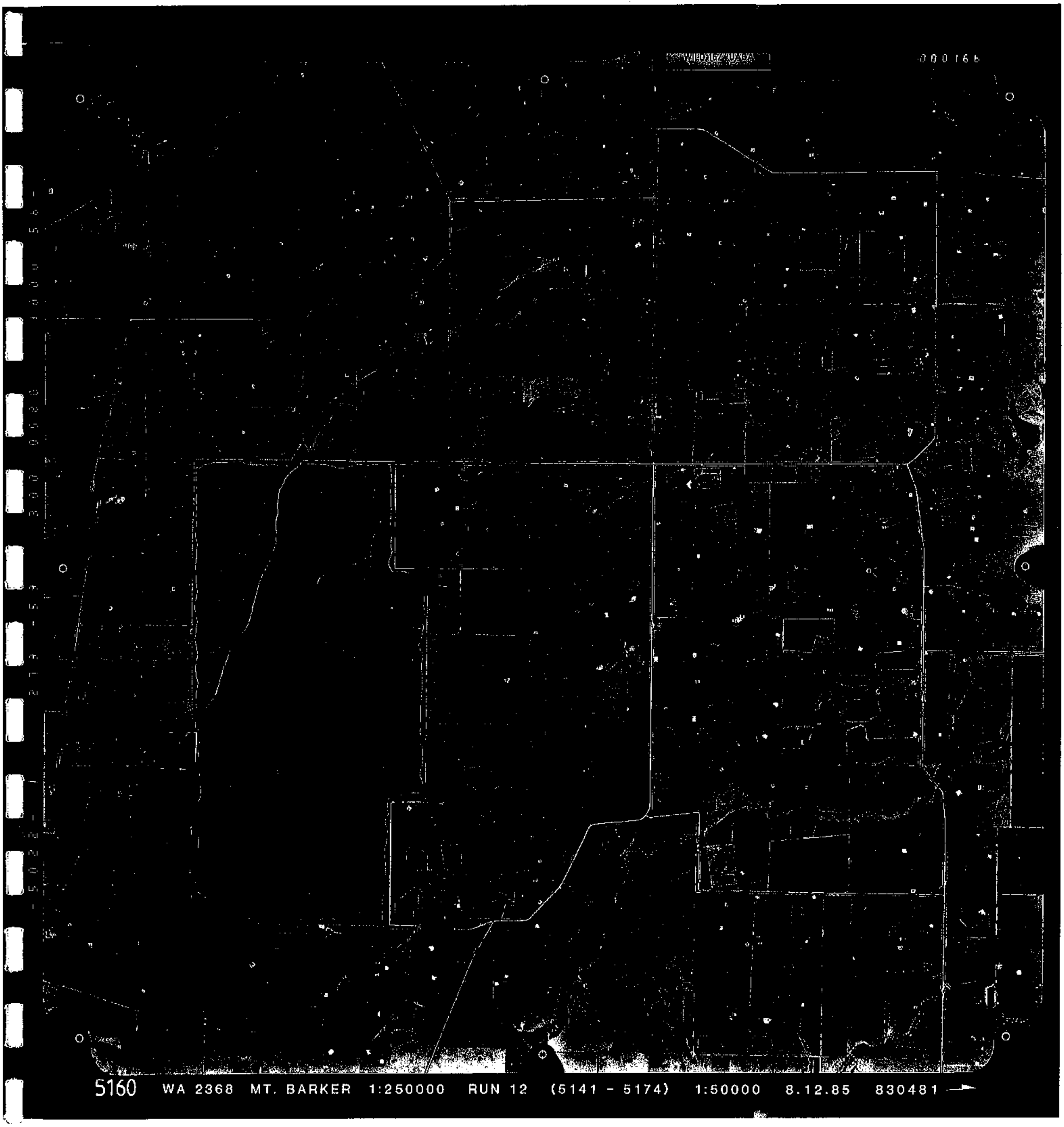
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