Mick E. Hanley · Byron B. Lamont

Herbivory, serotiny and seedling defence in Western Australian Proteaceae

Abstract We examined how acceptability characteristics displayed by 28-day-old seedlings of 12 species of Western Australian Proteaceae affect the likelihood of seedling herbivory in the field. The seedling attributes quantified were cotyledon phenolic, cyanide and nitrogen concentrations, and cotyledon area, thickness and specific leaf area. Only phenolic content was significantly correlated (negatively) with field rates of herbivore attack. This finding shows that the phenomenon of selective herbivore attack on seedlings may be influenced by a specific plant life-history trait, (in this case cotyledon phenolic concentration). In addition, we also studied the interaction between fire, serotiny and herbivory in matched burned and unburned plots. Although herbivore activity was greater in unburned plots, weakly serotinous species were as prone to defoliation as congeneric, strongly serotinous species, even though their seedlings recruit successfully in the absence of fire. This result suggests that seedlings of species able to establish between fires are not better defended against the higher levels of herbivory normally associated with unburned vegetation.

Keywords Fire · Phenolics · Seedling herbivory · Serotiny · Specific leaf area

Introduction

Despite the key role seedling regeneration success plays as a factor controlling plant ecological processes (Harper 1977), and the likelihood that the greatest effects of her-

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bivory on plant survival are manifest at the seedling stage (Watkinson 1997), we still know little about which particular species are attacked as seedlings and why. Furthermore, while it has been suggested that selection for chemical and/or physical defence should be comparatively strong in the juvenile stages (Bryant et al. 1983; Kearsley and Whitham 1989), in comparison to what is known about the defence of mature plants, we know very little about the early development and expression of seedling defences (Karban and Thaler 1999).

Variations in seedling acceptability have been attributed to differences in chemical or physical properties and/or seedling size (Hanley et al. 1995a; Hanley 1998; Fenner et al. 1999). Other aspects of seedling biology, such as nutritional quality and size as well as physical characteristics such as leaf thickness and specific leaf area (SLA), may also influence herbivore-feeding behaviour (Waller and Jones 1989; Groom and Lamont 1999). Yet like chemical defence, the influence of these characteristics on herbivore feeding behaviour is largely unknown.

The main aim of the experiments described here was to determine whether seedling attributes displayed by 12 species of fire-following Western Australian Proteaceae affected the likelihood of herbivore attack in their native plant communities. However, a second aspect of the study involved an examination of the interaction between seedling herbivory and fire.

In many fire-prone ecosystems, significant seedling establishment occurs only in the first few years following a fire. As a consequence, certain plant life-history traits are thought to have evolved in order to exploit the improved post-fire germination and growing conditions (Whelan 1995). One such trait, the retention by plants of seeds in persistent cones or fruits (serotiny), is particularly well represented in the fire-prone vegetation of Western Australia (Lamont et al. 1991). Not only are canopy-stored seeds better insulated from fire than non-stored seeds, fire-stimulated seed release is believed to maximise seedling establishment by restricting germination to the more favourable establishment conditions occurring after fire (Lamont et al. 1991).

A variety of factors may encourage post-fire germination and seedling establishment (Lamont et al. 1991; Hanley and Fenner 1997). Significant amongst these is the elimination, or at least significant reduction, of herbivore populations. A number of studies have highlighted the interaction between herbivory and fire (Leigh and Holgate 1979; Whelan and Main 1979; Bond 1984; Mills 1986; Tyler 1995). However, while these experiments have identified the overall impact of herbivores on post-fire and inter-fire seedling populations using herbivore-exclusion techniques, this kind of approach sheds little light on the actual mechanisms involved (Hulme 1996). For instance, we know relatively little about how fire-adapted life history traits may affect the likelihood of attack by herbivores. Despite the advantages that immediate post-fire germination has for many plant species, some (weakly serotinous) plants release seeds periodically throughout the intervals between fires (Cowling et al. 1987; Witkowski et al. 1991). Where conditions are suitable for inter-fire establishment (such as the appearance of small gaps, exceptionally wet years), some spontaneous seed release may be advantageous (Whelan et al. 1996). However, the environmental conditions these seeds and seedlings face are very different from those encountered by plants emerging immediately after fire. Among these differences is the relative importance of herbivory.

In this study we examined differences in the intensity of seedling herbivory between matched burned and unburned plots. Subsequently, we attempted to relate potentially important aspects of seedling defence, as well as rates of herbivore attack in burned and unburned plots, to the serotiny level displayed by each of our 12 experimental species. Our experimental species were chosen in matched congeneric pairs, such that each species had a closely related, weakly or strongly serotinous partner. By examining the physical and chemical characteristics displayed by seedlings of plants from different life-history groups, we hoped to relate any observed differences in patterns of seedling defence to changes in the likelihood of seedling herbivory following fire.

Materials and methods

Collection of plant material

Seeds were collected from two locations in Western Australia in April 1999. Fruits (cones or follicles) of *Banksia prionotes* (nomenclature follows McCarthy (1999), *Banksia leptophylla*, *Hakea trifurcata*, *Hakea psilorrhyncha*, *Dryandra sessilis* ssp. *cygnorum* and *Dryandra carlinoides* were collected from sites near Eneabba (29°32′S, 115 19′E), 300 km north of Perth. Fruits of *Banksia baxteri*, *Banksia coccinia*, *Hakea lasiantha*, *Hakea cucullata*, *Dryandra sessilis* ssp. *sessilis* and *Dryandra mucronulata* were collected from sites near the Waychinicup National Park, 50 km east of Albany (34 38′S, 118°45′E). In order to extract the seeds, follicles were burned for approximately 30 s until they split open. Fruits were then immersed in water before being placed in direct sunlight and dried until the seeds fell out.

Serotiny and establishment success

In order to quantify differences in the ability of our matched congeners to germinate and establish in the absence of fire, we attempted to age the populations from which seeds were collected. These populations were aged in vegetation unburned for at least 15 years. All individuals along a 5-m-wide transect were aged until 100 plants were encountered. Plant age was estimated by counting the number of branch junctions from the highest point of the plant to its base, one junction representing a single year (Lamont 1985).

Seedling characteristics and herbivore attack

Those seedling characteristics thought to significantly influence the likelihood of herbivore attack under field conditions (Hanley 1998) were quantified in 28-day-old seedlings of each of the 12 experimental species. These characteristics included aspects of chemical defence (cotyledon phenolic and cyanide content), cotyledon nutrient content (percentage nitrogen), sclerophylly (cotyledon thickness and SLA), and seedling size (cotyledon area).

Chemical defence

Three hundred seeds of each of the 12 experimental species were germinated in Petri dishes in a dark incubator set at 15°C. Immediately following germination (10–14 days), seedlings were transferred to 7×7-cm square pots containing a 4:1 sand:peat compost mixture. Nine seedlings of the same species were planted into each pot in a 3×3 array, such that each seedling was 2 cm from its nearest neighbours. There were ten pot replicates for each species.

When the seedlings were exactly 28 days old, all shoots within each pot were harvested and dried in an oven for 48 h at 50°C. The relatively low drying temperature reduces the risk of denaturing carbon-based secondary metabolites (Hagerman 1988). Following drying, the plant material was ground using a pestle and mortar. Each pot of nine seedlings represented a single replicate (individual seedlings being too small to accurately test for phenolics). Total phenols were extracted from 50 mg ground leaf material added to 7.5 ml distilled water boiled for 2 h. The extract was centrifuged at 3,000 r.p.m. for 15 min and a 1-ml sample removed and added to 7.5 ml distilled water. To this, 1 ml Folin-Denis reagent and 0.5 ml sodium carbonate were added and samples left to stand for 15 min before a final centrifuge treatment at 3,000 r.p.m. for 15 min. The absorbance of each sample was read at 760 nm and plotted against a standard curve produced from a similar analysis of tannic acid.

Cyanogenic glycosides have also been recorded in the Proteaceae (Lamont 1993). In order to determine whether cyanide was important in our experimental species, we conducted a simple qualitative test (Lamont 1993). One 28-day-old seedling (about 0.1 g freshly harvested plant material) was placed in a 10-ml screw-top vial and crushed with a metal rod. Three drops of de-ionised water and three drops of 0.1% β-glucosidase (Emulsin, Sigma) were then added. Feigl-Anger test paper was cut into strips 60 mm long and 5 mm wide and suspended just above the mixture in each vial via the cap. Samples were left for 2 h at room temperature, the presence of cyanide indicated by the test paper turning blue. Cyanide tests were replicated 10 times for each species.

Leaf nitrogen

Percentage leaf nitrogen was determined on a three-channel auto-analyser system, using a modified Kjeldahl digestion method (Anonymous 1977). Samples were taken from the same plants used in the phenolic test and were prepared in a similar way. About 0.1 g dried and milled shoot material was digested in a 6 ml mixture of sulphuric acid and 6% salicylic acid overnight (Ekpete and Cornfield 1964). This mixture was boiled at 350°C for

20 min, allowed to cool and 2 ml hydrogen peroxide added. The process was repeated 3 times until the samples became clear, after which a final heat treatment at 350°C for 30 min was applied (Yuen and Pollard 1954). Nitrogen was colorimetrically determined by indophenol blue (Searle 1984).

Cotyledon area, thickness and SLA

Cotyledon thickness was quantified using vernier callipers placed mid-way across the long axis of a single cotyledon. Cotyledon area was determined by measuring the area of a single cotyledon using an image analyser (Delta-T Devices, Cambridge). The same cotyledons were oven dried at 80°C overnight and dry weight biomass determined. SLA was calculated by dividing cotyledon area by dry weight. The process was repeated for ten individuals of each species.

Determination of seedling herbivory in the field

Field experiments were conducted in July 1999 near the seed collection sites. At Eneabba, the most recent burn had occurred in March 1998 at a site 15 km north of the town. This site (area ≈40 km²) however, still displayed markedly different vegetation to an adjacent unburned area protected from the 1998 fire by a road. The unburned site, which had not experienced fire for 14 years, was dominated by a dense, 1-m-high layer of *Scholtzia* sp. and also contained large numbers of *B. leptophylla* plants. *B. prionotes*, *H. trifurcata*, *H. psilorrhyncha* and *D. sessilis* ssp. *cygnorum* were also present within the experimental site, as were infrequent *B. leptophylla* and *H. trifurcata* seedlings (≈1 seedling 5 m⁻²). The burned site was more open, the sparse vegetation dominated by *Stirlingia latifolia* and *Conospermum* spp. The site also contained juvenile plants of all of the experimental species except the two Dryandras.

Since there had been no recent fires near the Waychinicup NP area, we selected an area adjacent to the Hassell highway near Wellstead, 40 km east of Waychinicup (34 20′S, 119°20′E). Roadside verges here are wide (>100 m) and run continuously for over 70 km, separating (fenced) sheep pasture from the highway. Prescribed fires are regularly conducted along these verges, the experimental site having been burned in April 1999. The unburned site was situated a further 500 m east along the Hassell highway, in an area of dense *B. baxteri* scrub unburned for over 20 years. There were also various other Proteaceae species present, including *B. coccinea*, *H. cucullata*, *D. mucronulata* and *D. sessilis* ssp. *sessilis*. Few seedlings were observed. Vegetation within the burned site was sparse, although the charred remains of several *Banksia*, *Hakea* and *Dryandra* species were noted. There were also relatively large populations of 1- to 2-month-old Proteaceae seedlings (approximately 10 seedlings m⁻²). Other plants in the area included *Lomandra* spp. and resprouts of *Eucalyptus* spp.

Experimental plots were located within a 25×25-m area. At Eneabba, these plots were situated 100 m into burned/unburned vegetation. At Wellstead, the 25×25-m areas were located at a position equidistant from the road and the adjacent sheep pasture. The 25×25-m areas were subdivided into twenty-five, 5×5-m plots, and six selected at random with the provision that none were adjoining. These plots were then allocated at random to one of three treatments: seedlings (three plots), invertebrates (two plots) or small mammal (one plot). The same relative arrangement of sub-plots was used at each site.

In each seedling plot, thirty-six, 50-mm-diameter pots containing 28-day-old seedlings, were randomly arranged within a 6×6-m grid, such that each pot was 1 m away from its nearest neighbour. The same relative arrangement of individual plant species was maintained within sub-plots in both burned and unburned vegetation. Each pot contained four seedlings of the same species planted 2 cm apart. There were six replicate pots of each species in each seedling plot. The pots were planted flush with the ground and left in the field for 7 days. Damage to seedlings by herbivores

at the end of this period was classified as: (1) attacked (including killed), or (2) killed.

Invertebrate plots consisted of nine pitfall traps arranged 2.5 m apart in a 3×3-m grid. Individual pitfall traps were 9-cm-diameter, 7.5-cm-deep plastic cups, filled to 2 cm with a detergent solution, buried flush with the soil surface. These traps remained uncovered during the entire 7-day course of the seedling herbivory trial before being removed. Millipedes (Class: Diplopoda) were removed from the samples and the remaining invertebrates sorted to taxonomic order. Only invertebrate groups containing herbivorous species were considered in the statistical analysis.

Small mammal plots were used to assess the activity of small marsupials and rodents in field plots, both of which may attack seedlings. Traps consisted of five 300-mm-diameter, 400-mm-deep plastic containers buried flush with the ground. Similar pitfall trap methods for catching small mammals have been used successfully in Western Australia previously (Wooller et al. 1993). After burying one container in the centre of the 5×5-m plot, the four remaining containers were buried 5 m away, north, south, east or west of the central container. A length of 0.5-m high, 2-mm² mesh, aluminium wire fence ran from the central container to each of the peripheral containers for a distance of 5 m. These traps were checked daily during the 7-day seedling herbivory trial and the identity and number of any mammals caught in the traps noted.

The presence and activity of larger animals in our field sites were estimated using a spoor assessment technique (Tiver and Andrew 1997). Introduced European rabbits (*Oryctolagus cuniculus*), in particular, are present in large numbers in Western Australia and are important predators of small seedlings in temperate grasslands (Edwards and Crawley 1999). Western grey kangaroo (*Macropus fuliginosus*) and emu (*Dromaius novaehollandiae*) are unlikely to attack small seedlings, but both were present in our field sites. All existing spoor was removed from within the 25×25-m experimental sites at the start of the field trials. Any new spoor was recovered at the end of the 7-day grazing trial.

Statistical analysis

In order to determine the effects of fire on invertebrate populations, the numbers of individuals of each herbivorous invertebrate group (millipedes, Coleoptera, Hymenoptera and Orthoptera) collected in individual pitfall traps in sub-plots were first pooled. Following square root and arcsine transformation (these data, and all subsequent transformations, were checked for homogeneity of variances using the Bartlett test), the average number of individuals of each invertebrate group in burned and unburned plots at both Eneabba and Wellstead were compared using three-way ANOVA. Specific differences between mean numbers of individuals in burned and unburned plots at both sites, were identified using S-N-K tests (Underwood 1997).

The effect of fire on seedling herbivory was examined in two ways. The total number of seedlings (all species combined) attacked in sub-plots in burned and unburned vegetation at both field sites was calculated and divided by the total number of seedlings (144) in each sub-plot. Following arcsine transformation, the mean proportions of seedlings attacked within burned and unburned vegetation at Eneabba and Wellstead were compared using two-way ANOVA. Having demonstrated a significant overall effect, we attempted to identify the individual species responsible for this effect using a Wilcoxon matched pairs' test (since the relative layout of both sub-plots and the pots within them was identical, this was done by pairing individual pots in adjoining burned and unburned vegetation).

In order to examine possible interactions between herbivory and seedling acceptability attributes, we had to standardise seedling herbivory data for each Proteaceae species at Wellstead and Eneabba. This was done by calculating an "attack index", determined by dividing the number of seedlings of a given species attacked in field plots (unburned sites only) by the total number of seedlings attacked at that site.

Those seedling attributes (SLA, cotyledon thickness and area, phenolics, and nitrogen contents) most closely correlated to the

field-derived attack index ($\log_{10}$ transformed), were determined using a standard principal components analysis (PCA). Following ordination, we constructed a Pearson's correlation matrix to compare all possible pair-wise interactions between seedling attributes and attack index. A sequential Bonferroni correction was applied to reduce the possibility of making type-I errors (Rice 1989).

The ability of weakly and strongly serotinous species to establish between fires was determined by comparing the age structure of matched congeners using χ^2 tests. The effect of serotiny on seedling defence (mean percentage phenolics) was examined by subjecting the individual species means to nested ANOVA, following arcsine transformation. Specific differences between paired species were located using S-N-K tests. In order to determine the effect of serotiny on the likelihood of seedling herbivory in the field, we first calculated the proportion of seedlings (of a possible 24) of each species attacked in individual sub-plots in unburned vegetation. The mean proportion of weakly- and strongly-serotinous seedlings attacked was compared using nested ANOVA.

Results

Effects of fire on herbivore activity and seedling attack

Three-way ANOVA revealed a significant "site"×"fire treatment"×"invertebrate group" interaction (Table 1). More invertebrates were caught at Wellstead than Eneabba, although in both cases, the only significant effect of fire on invertebrate numbers was a reduction in recently burned vegetation. Of the invertebrates caught in pitfall traps at Eneabba and Wellstead, Orthoptera at Wellstead (S-N-K test, $P<0.01$), and millipedes at both field sites ($P<0.01$), showed a significant response to burning treatment (Fig. 1). The only small mammals caught during the two herbivory trials were six honey possums (*Tarsipes rostratus*) in unburned plots at Wellstead. This species is largely nectivorous (Cronin 1991), but the fact that it was only encountered in unburned plots may indicate that activity of small marsupials and rodents, in general, is greater in unburned vegetation. No new spoor was recorded during the duration of the field trials, suggesting that larger vertebrate herbivores (including rabbits) had not visited the experimental sites.

In comparison with seedling mortality due to total consumption at Wellstead (a total of 47 seedlings of all six species combined in unburned plots and two in burned plots), mortality at Eneabba (seven in unburned, zero in burned), was very low. This probably reflects, in part, the generally lower level of herbivory recorded at Eneabba (Fig. 2). Since we were therefore unable to directly compare mortality rates at the two locations, subsequent analyses were based on seedling attack rates only.

Seedling herbivory was significantly reduced in burned plots (two-way ANOVA $F=9.49_{1,8}$, $P=0.015$). However, there was no significant difference between seedling herbivory rates at the two field sites ($F=0.24_{1,8}$, $P=0.64$) and no interaction with fire treatment ($F=0.59_{1,8}$, $P=0.46$). At the individual species level, two species at Eneabba (Wilcoxon matched pairs test: *B. leptophylla*, $Z=-2.032$, $P<0.05$; and *D. carlinoides*, $Z=-2.226$, $P<0.05$) and three at Wellstead (*B. baxteri*, $Z=-2.200$, $P<0.05$; *D. mucronulata*, $Z=-2.032$,

Table 1 Results of a three-way ANOVA model (site×fire treatment×invertebrate group). The model was based on comparisons of the square root, arcsine-transformed mean numbers of individuals of four invertebrate groups [Class Diplopoda (millipedes); Class Insecta, Coleoptera, Hymenoptera and Orthoptera] caught in pitfall traps located in sub-plots within burned and unburned vegetation at Eneabba and Wellstead, Western Australia

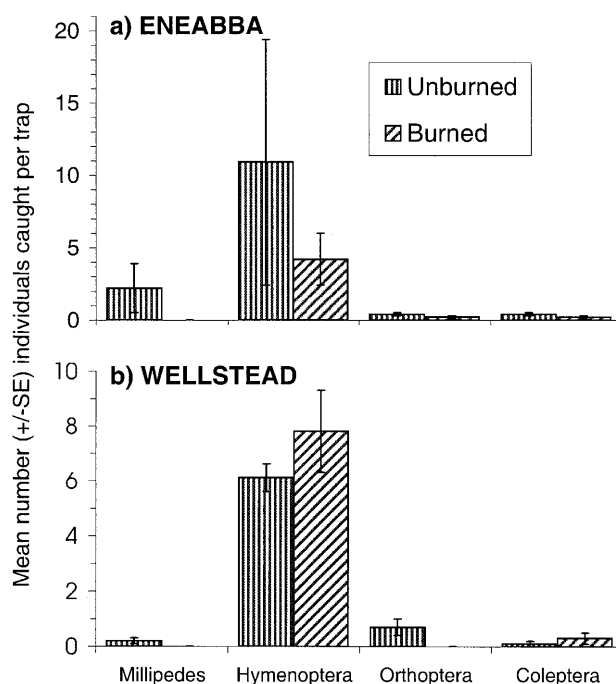| Factor	df	Mean square	F	P
Site	1	8.131	6.93	0.018
Invertebrate group	3	75.455	64.32	<0.001
Fire treatment	1	32.421	27.64	<0.001
Site×group	3	1.354	1.15	0.358
Site×fire	1	2.201	1.88	0.189
Group×fire	3	14.121	12.04	<0.001
Site×group×fire	3	6.163	5.25	0.010
Error	16	1.173		
Total	31			

Fig. 1 The mean number of individuals (±SE) of four invertebrate groups [Class Diplopoda (millipedes), Class Insecta: Orders Hymenoptera, Orthoptera and Coleoptera] caught in pitfall traps in sub-plots within burned and unburned vegetation at **a** Eneabba, and **b** Wellstead, Western Australia

$P<0.05$; and *D. sessilis*. $Z=-2.214$, $P<0.05$) exhibited greater rates of herbivore attack in unburned vegetation.

Seedling acceptability attributes and herbivory in the field

Since SLA exhibited a strong inverse relationship with cotyledon thickness ($r^2=-0.833$, $F=49.89_{1,10}$, $P<0.001$), SLA was used as the sole measure of leaf hardness in

Fig. 2 The mean percentage of seedlings (±SE) of twelve 28-day-old Proteaceae species attacked by herbivores in sub-plots within burned and unburned vegetation at **a** Eneabba, and **b** Wellstead, Western Australia
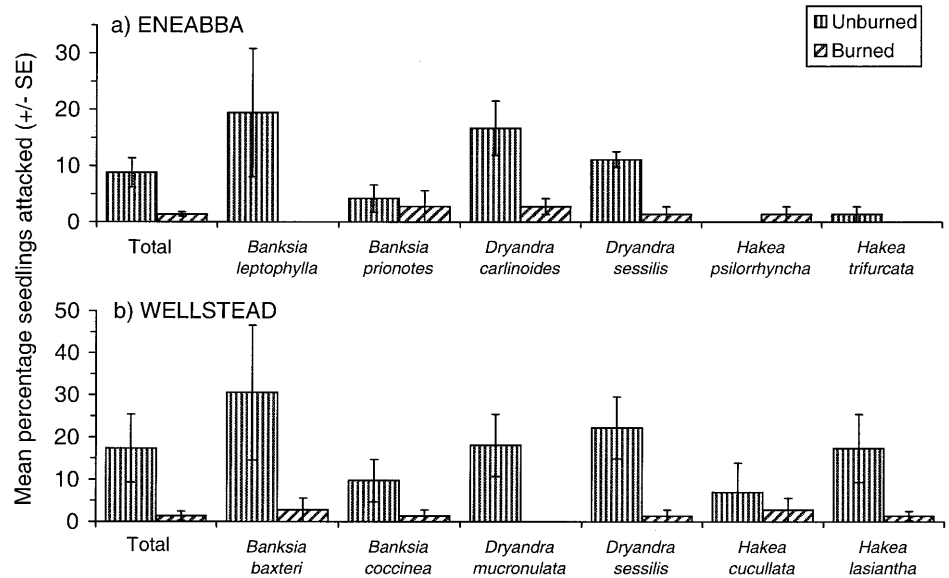

Table 2 A summary of mean values (±SE) of five seedling attributes thought to influence the likelihood of herbivore attack in 28-day-old seedlings of 12 Western Australian Proteaceae species. Standardised, field-derived herbivory rates for each species (attack index) determined in field trials at two locations (Eneabba and Wellstead) in Western Australia are also given. *SLA* Specific leaf area

Location and species	% Phenolics (±SE)	% Nitrogen (±SE)	Cotyledon area (±SE) (mm^2)	SLA (±SE) (mm^2 mg^{-1})	Leaf thickness (±SE) (mm)	Attack index
Eneabba						
Banksia leptophylla	1.29 (0.04)	12.48 (0.18)	160.8 (2.2)	9.6 (0.4)	0.71 (0.03)	0.37
Banksia prionotes	1.69 (0.04)	9.96 (0.14)	264.2 (11.4)	8.1 (0.2)	0.97 (0.03)	0.08
Dryandra carlinodes	1.56 (0.07)	9.41 (0.60)	157.8 (6.0)	12.9 (0.5)	0.72 (0.02)	0.32
Dryandra sessilis	1.59 (0.05)	5.24 (0.14)	131.2 (3.8)	15.6 (0.6)	0.59 (0.02)	0.21
Hakea psilorryncha	3.23 (0.12)	8.46 (0.17)	191.2 (9.0)	7.1 (0.3)	1.10 (0.01)	0.00
Hakea trifurcata	2.62 (0.06)	8.50 (0.15)	199.6 (11.0)	9.5 (0.2)	0.79 (0.02)	0.03
Wellstead						
Banksia baxteri	1.63 (0.04)	11.91 (0.11)	317.6 (20.2)	6.2 (0.1)	1.32 (0.04)	0.28
Banksia coccinea	1.52 (0.07)	13.10 (0.34)	108.0 (3.8)	7.0 (0.3)	1.17 (0.03)	0.09
Dryandra mucronulata	1.58 (0.05)	11.97 (0.26)	104.2 (6.4)	9.7 (0.4)	0.86 (0.04)	0.21
Dryandra sessilis	1.88 (0.06)	9.88 (0.22)	146.2 (6.0)	11.2 (0.3)	0.78 (0.04)	0.21
Hakea cucullata	3.56 (0.16)	10.89 (0.21)	156.4 (4.4)	8.2 (0.2)	0.97 (0.03)	0.06
Hakea lasiantha	2.34 (0.06)	9.83 (0.07)	132.0 (6.2)	9.9 (0.7)	0.91 (0.04)	0.15

Table 3 Pearson's correlations between all possible pairs of four seedling acceptability attributes and field-derived herbivory rates (attack index), for 28-day-old seedlings of 12 Western Australian Proteaceae species

Trait	Attack index$_{(log10)}$	% Phenolics	% Nitrogen	SLA
% Phenolics	−0.77*			
% Nitrogen	0.26	−0.23		
SLA	0.42	−0.30	−0.67	
Area	−0.11	0.26	0.28	−0.46

*$P<0.05$ (after Bonferroni correction, based on 10 *df*)

our ordination. None of the 12 experimental species yielded a positive response to the test for cyanide, hence cotyledon phenolic concentration was the sole measure of chemical defence relevant to the PCA. Mean values for phenolics and nitrogen content, cotyledon thickness, area, and SLA are given in Table 2.

Table 3 is a correlation matrix between all possible pairings of seedling herbivory (attack index) and the four seedling acceptability attributes used in the PCA. Only the correlation between percentage phenolics and attack index$_{(log\ 10)}$ proved to be significant after Bonferroni correction (Fig. 3). This relationship is further illustrated in the PCA ordination (Fig. 4), which highlights the strong negative relationship between phenolic content and attack index. There was also a tendency for nitrogen content and SLA to be associated with high levels of seed-

Fig. 3 The effect of mean percentage phenolic concentration in the cotyledons of twelve 28-day-old Proteaceae species on the likelihood of herbivore attack (attack index) on seedlings planted out in field plots at two locations (Eneabba and Wellstead) in Western Australia. The equation and statistics for the exponential relationship of the dependent variable (attack index) on percentage phenolics are given
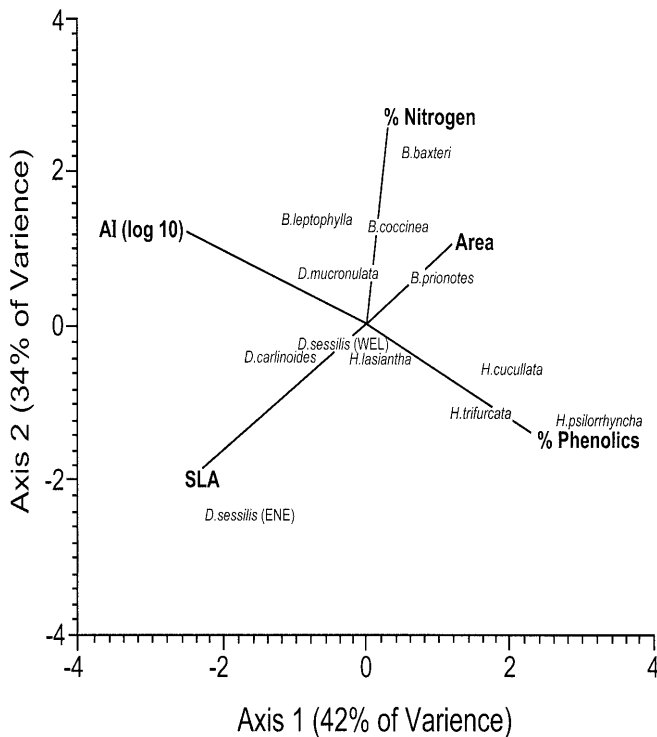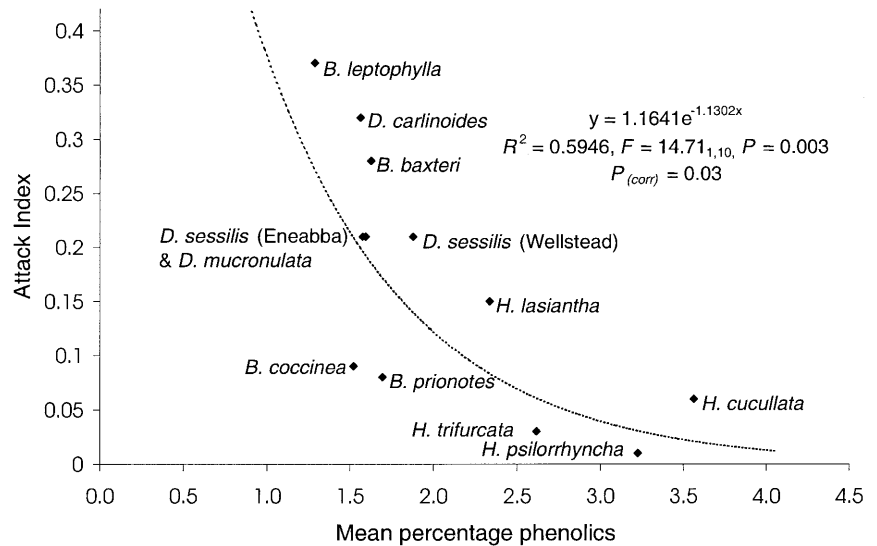

Fig. 4 A scatter plot of species position of 12 Western Australian Proteaceae species and their relationship with the direction and magnitude of four seedling acceptability attributes, and a field-derived, seedling attack index (*AI*), plotted on axes 1 and 2 of a principal components analysis. *SLA* Specific leaf area

ling attack and for larger cotyledons to have a higher phenolic content.

Serotiny, seedling defence and seedling attack

All six matched congeneric pairs (weakly vs. strongly serotinous) exhibited a significant difference between their population age-class structures (Table 4). In all cases, individuals of weakly serotinous species were generally younger and spread over a greater range of age classes than their strongly serotinous, congeneric partners.

However, we found no evidence for a relationship between serotiny level and the higher herbivore activity recorded in unburned vegetation. Although nested ANOVA highlighted a significant serotiny effect at both Eneabba ($F=17.19_{3,54}$, $P<0.001$) and Wellstead ($F=33.65_{3,54}$, $P<0.001$), the results of S-N-K tests showed no consistent relationship between the mean percentage phenolic content of cotyledons and serotiny in our 12 Proteaceae. Of the pair-wise comparisons, there were only two instances (*B. prionotes* vs. *B. leptophylla*, $P<0.01$; *D. sessilis* ssp. *sessilis* vs. *D. mucronulata*, $P<0.01$) where weakly serotinous species contained significantly greater concentrations of phenolics than their strongly serotinous, conspecific partner. There was no significant effect of serotiny on the rate of field herbivory observed at Eneabba ($F=2.01_{3,12}$, $P=0.17$), or Wellstead ($F=0.51_{3,12}$, $P=0.68$).

Discussion

Effects of fire on herbivore activity and seedling attack

Despite the lack of "adequate studies" (Whelan 1995), most evidence points to a reduction in invertebrate activity following fire (Whelan and Main 1979; Dolva 1993). Our results also suggest that for two groups of invertebrates at least, fire significantly reduces their activity in comparison with nearby unburned areas. This reduction is further emphasised by a parallel decrease in seedling herbivory in this study. This effect was even apparent at Eneabba, where the burned plots had not experienced fire for 18 months. Many authors have reported a similar reduction in herbivory following fire (Whelan and Main 1979; Bond 1984; Lamont et al. 1991; Tyler 1995), and Whelan

Table 4 The age-class frequency of 100 individuals of 12 Proteaceae species aged in unburned vegetation at Eneabba and Waychinicup, Western Australia. Species were selected in matched congeneric pairs, one partner of which was weakly serotinous, the other strongly serotinous. The results of χ^2 tests examining the significance of the difference in age-class frequency between matched weakly and strongly serotinous pairs are given

Species	Serotiny level	Age class (years)						Results of χ^2 test
		<1	2–5	6–10	11–15	16–20	>20	
Eneabba								
B. leptophylla	Strong	1	0	4	80	15	0	$\chi^2=106.664_4$, $P<0.001$
B. prionotes	Weak	32	22	22	18	6	0	
D. carlinoides	Strong	0	0	12	69	19	0	$\chi^2=133.352_4$, $P<0.001$
D. sessilis	Weak	41	26	24	9	1	0	
H. psilorrhyncha	Strong	0	0	2	23	62	13	$\chi^2=71.323_4$, $P<0.001$
H. trifurcata	Weak	19	8	22	29	16	6	
Waychinicup								
B. baxteri	Strong	0	0	0	0	16	84	$\chi^2=108.085_4$, $P<0.001$
B. coccinea	Weak	4	29	51	15	1	0	
D. mucronulata	Strong	0	0	0	8	45	47	$\chi^2=89.487_4$, $P<0.001$
D. sessilis	Weak	33	22	10	14	8	13	
H. cucullata	Strong	0	0	0	0	24	76	$\chi^2=20.517_4$, $P<0.001$
H. lasiantha	Weak	17	4	1	7	25	46	

and Main's (1979) study, like our own, showed that the effects of fire on seedling herbivory could last for well over a year following the original burn. However, the same authors also make the point that this pattern of invertebrate response to fire is not universal and note that small fires actually attract herbivores from surrounding unburned vegetation when seedling regeneration occurs.

Similar variability in the activity of small mammals following fire has also been reported. Leigh and Holdgate (1979) found that mammal activity increased in recently burned plots in Australian eucalypt woodland. Other studies, by contrast, have reported decreased mammal activity in burned plots (Bond 1984; Tyler 1995), and there may be significant variability even within a single study (Newsome et al. 1975). Our results are somewhat inconclusive given the small number of (non-herbivorous) mammals caught. However, the fact that they were restricted to unburned plots, coupled with the reduced rates of herbivore attack on experimental seedlings at both field sites, is circumstantial evidence for a reduction in mammal activity in burned plots.

Serotiny, seedling defence and seedling attack

The ability of weakly serotinous species to establish between fires is well documented (Lamont et al. 1991; Witkowski et al. 1991; Whelan et al. 1996) and our results add further support to these studies. However, despite clear differences in inter-fire establishment success, we found no evidence to suggest that the degree of serotiny has any influence over seedling defence or susceptibility to herbivory.

From an adaptive viewpoint, weak serotiny may be considered a means of securing against losses, in which most stored seeds are released after fire, but where some seeds are continually released in order to take advantage of favourable, yet unpredictable, germination microsites (Lamont et al. 1991; Whelan et al. 1996). Enright et al. (1998) concluded that highly stochastic fire frequencies, and mean frequencies close to the average lifespan of the species, favoured incomplete serotiny. Climatic factors may also be important, with improving inter-fire establishment conditions (e.g. higher annual rainfall) favouring weakly serotinous species (Enright et al. 1998). It has also been suggested that many weakly serotinous species are small seeded, a characteristic that facilitates their penetration of litter (Witkowski et al. 1991). This possibility is in fact supported by four out of six of our pair-wise comparisons. Whatever the reasons favouring inter-fire seed release, it seems clear from the present study that seedling palatability is not a factor that differentially affects the establishment success of weakly and strongly serotinous Proteaceae species.

Seedling attributes and herbivory

Although selective removal of preferred seedling species by herbivores is relatively well documented (see Hanley 1998), the precise mechanism by which this selection occurs is poorly understood. In this study we have shown that the level of chemical defence possessed by cotyledon-stage seedlings does have significant effects on seedling selection by herbivores. While the phenomenon of selective seedling removal is not in doubt, there is some question as to whether it has significant long-term implications for plant community development. Crawley (1988) and Edwards and Crawley (1999) suggest that even a substantial reduction in seedling populations as a consequence of herbivory, simply reduces subsequent losses due to density-dependent mortality. In a short-term study such as the one described here, we cannot say whether the selective removal of (Proteaceae) seedlings

manifests itself in the composition and structure of mature vegetation. However, in many fire-prone ecosystems, selective seedling removal is important in influencing the plant species composition of mature vegetation (Mills 1986; Tyler 1996).

Although percentage phenolic concentration was the only attribute significantly correlated with seedling herbivory in our study, this does not imply that other attributes are not important in different circumstances. For example, seedling size, nutritional quality (percentage nitrogen), leaf hardness and thickness, and possession of cyanogenic glycosides also influence herbivore-feeding behaviour (Grime et al. 1997; Hanley 1998). In addition to highlighting the importance of seedling characteristics and their influence on herbivore-feeding behaviour, Hanley (1998) also points out the need to recognise herbivory as a factor shaping the evolution of seedling life-history traits.

A number of studies have identified ecophysiological tradeoffs that constrain the evolutionary responses of plants as they interact with their biotic and abiotic environments (Grime et al. 1997; Wardle et al. 1998; Westoby 1998). Significant among these tradeoffs are those between traits conferring herbivore resistance and those that influence plant growth (Herms and Mattson 1992). It is clear from the present study that well-defended seedlings are less likely to be attacked than those with relatively lower cotyledon phenolic concentrations. Although not quantified here, increased allocation of resources to defence is also likely to reduce growth and competitive ability for our 12 Proteaceae species. This well-established trade-off between defence and growth has the potential to significantly affect patterns of plant community development in temperate grasslands (Hanley et al. 1995b). Nevertheless, a rather sparse, and often conflicting literature on seedling herbivory prevents ecologists making generalisations about the effect of selective seedling removal on plant community development. More research is required if we are to determine whether the long-term effects of selective seedling removal are negligible in comparison with competition (Crawley 1988), or whether the phenomenon represents an important plant community process in *certain* circumstances (Hanley 1998). However, by demonstrating an interaction between chemical defence and seedling removal, this paper highlights one mechanism by which selective seedling removal may influence tradeoffs between plant life-history traits displayed within the seedling stage.

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