

Seed dynamics of resprouting shrubs in grassy woodlands: Seed rain, predators and seed loss constrain recruitment potential

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Abstract Measuring the fate of seeds between seed production and seedling establishment is critical in understanding mechanisms of recruitment limitation of plants. We examined seed fates to better understand the recruitment dynamics of four resprouting shrubs from two families (Fabaceae and Epacridaceae) in temperate grassy woodlands. We tested whether: (i) pre-dispersal seed predation affected seed rain; (ii) post-dispersal seed predation limited seed bank accumulation; (iii) the size of the seed bank was related to seed size; and (iv) viable seeds accumulated in the soil after seed rain. There was a distinct difference in seed production per plant between plant families with the legumes producing significantly more seeds per individual than the epacrids. Seed viability ranged from 43% to 81% and all viable had seed or fruit coat dormancy broken by heat or scarification. Pre-dispersal predation by Lepidopteran larvae removed a large proportion of seed from the legume seed rain but not the epacrids. Four species of ants (*Notoncus ectatomoides*, *Pheidole* sp., *Rhytidoponera tasmaniensis* and *Iridomyrmex purpureus*) were major post-dispersal seed removers. Overall, a greater percentage of *Hardenbergia* (38%) and *Pultenaea* (59%) seeds were removed than the fleshy fruits of *Lissanthe* (14%) or *Melichrus* (0%). Seed bank sizes were small (<15 seeds m⁻²) relative to the seed rain and no significant accumulation of seed in the soil was detected. Lack of accumulation was attributed to seed predation as seed decay was considered unlikely and no seed germination was observed in our study sites. Our study suggests that seed predation is a key factor contributing to seed-limited recruitment in grassy woodland shrubs by reducing the number of seeds stored in the soil.

Key words: dispersal, seed bank, seed germination, seed predation.

INTRODUCTION

Measuring the fate of seeds between seed production and seedling establishment is critical in understanding mechanisms of recruitment limitation in many terrestrial ecosystems, including grassy woodlands (Yates & Hobbs 1997; Clarke 2000). Seed dynamics encompass seed production, dispersal in space and time, and losses through predation, decay and germination (Harper 1977). In fire-prone communities the accumulation of a persistent seed bank is important for post-fire recruitment and compensation of adult mortality in both obligate seeding and resprouting species (Tyler 1995; Keith 1996). Resprouting is the most common response to fire in temperate grassy woodlands (Knox & Clarke 2004) and resprouters tend to produce fewer seeds per season than species killed by fire (Keeley 1977; Carpenter & Recher 1979; Bell 2001; Pausas *et al.* 2004). Nevertheless, resprouting species with innate seed dormancy should accumulate

a persistent soil-stored seed bank, although empirical data are lacking.

Species with event driven recruitment have a pulse of germination following fire, but species lacking fire related germination cues may be expected to recruit seedlings between fire events. Recruitment of shrub seedlings in grassy woodlands has rarely been observed (Campbell 1999; Clarke 2000; Clarke & Davison 2001; Knox & Clarke 2006) and seed sowing experiments have demonstrated that recruitment is seed-limited rather than safe-site limited (Clarke & Davison 2001; Knox & Clarke 2006). Grassy woodland species in the families Epacridaceae and Fabaceae also have innate seed dormancy, implying that recruitment of seedlings may be event-driven and that diaspores should accumulate in the soil if plants are fecund in most years. Accumulation of these seed banks is however, also dependent upon seed losses through dispersal to unsafe sites by seed removal pathogen loss, and seed predation (Clarke & Davison 2001). In particular, predators can impose top-down effects on plant populations by limiting the number of seeds available for germination during recruitment events (Maron & Simms 1997; Maron & Gardner

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2000). Determining seed dynamics through the soil in grassy woodlands may therefore be pivotal in understanding the recruitment dynamics of understorey species and may explain the mechanisms for seed-limited recruitment.

This study examined seed dynamics of resprouting shrubs in temperate grassy woodlands. We investigated seed production, seed rain, viability, dormancy, seed removal and soil seed banks of four shrubs from two families (Fabaceae and Epacridaceae) that are widespread and abundant in grassy woodlands on the New England Tablelands. We tested whether: (i) pre-dispersal seed predation affected seed rain; (ii) post-dispersal seed predation limited seed bank accumulation; (iii) the size of the seed bank was related to seed size; and (iv) viable seeds accumulated in the soil after seed rain.

METHODS

Species

Seed dynamics were measured for four widespread and relatively abundant shrubs in grassy woodlands on the New England Tablelands (nomenclature follows Harden 1991, 1992): *Lissanthe strigosa* and *Melichrus urceolatus* (Epacridaceae), and *Hardenbergia violacea* and *Pultenaea microphylla* (Fabaceae) (Table 1), hereafter *Lissanthe*, *Melichrus*, *Hardenbergia* and *Pultenaea*. *Melichrus* and *Lissanthe* are erect, stiffly branched shrubs to 1.5 m in height. Both produce a succulent drupe with a woody endocarp that separates into a number of pyrenes each containing a small, soft seed. Both species resprout from basal root-stocks and root-

sucker after fire. *Hardenbergia* is a spreading twiner and *Pultenaea* is a mat forming to prostrate shrub. Both species produce arillate seeds in dehiscent pods and resprout from subterranean root-stocks following the passage of fire.

Study sites

The four shrub species were examined on four rural properties on the New England Tablelands within 30 km of Armidale, north-east New South Wales, Australia (Table 1). The area has a summer dominated rainfall, with the mean annual rainfall ranging from 850 to 950 mm. The region is characterized by mild to warm summers and moderately cold winters with severe frosts occurring from April to September (Lodge *et al.* 1984). Sites were characterized by an overstorey of stringy-bark eucalypt species (*Eucalyptus caliginosa*, *Eucalyptus laevopinea*) to 25 m in height, and understorey vegetation was dominated by herbaceous plants with a sparse shrubby layer. At each study site three to five patches (20 × 25 m) of each species were studied using random stratified sampling, and population density was estimated by counting the number of individuals within each patch (Table 1).

Seed production and pre-dispersal seed losses

Seed production was measured from three reproductive individuals in three to five patches at each site (Table 1). Different seed sampling methods were used for each species due to differences in growth form, reproductive biology and seed dispersal. Individuals of

Table 1. Species and site characteristics of four resprouting woodland shrubs from the New England Tablelands that were sampled for this study

Family/species	Growth form	Diaspore type	Diaspore mass (mg) [†]	Sites (no. patches)	Population density (stems m ⁻²) [‡]
Epacridaceae					
<i>Lissanthe strigosa</i>	Erect shrub (to 1 m)	Succulent drupe	8.96	Site 1, Hillgrove Creek (5) Site 2, Tilbuster Creek (5) Site 3, Rocky River (3)	0.44 (0.09) 0.14 (0.02) 0.14 (0.05)
<i>Melichrus urceolatus</i>	Decumbent shrub (to 1.5 m)	Succulent drupe	4.52	Site 1, Hillgrove Creek (5) Site 2, Tilbuster Creek (5) Site 3, Rocky River (5)	0.06 (0.01) 0.15 (0.02) 0.07 (0.01)
Fabaceae					
<i>Hardenbergia violacea</i>	Twiner	Arillate seed	39.64	Site 1, Hillgrove Creek (5) Site 2, Tilbuster Creek (5) Site 3, Rocky River (3)	0.03 (0.002) 0.02 (0.002) 0.02 (0.002)
<i>Pultenaea microphylla</i>	Mat forming to prostrate shrub	Arillate seed	1.96	Site 2, Tilbuster Creek (5) Site 4, University Hill (5) Site 1, Hillgrove Creek (5)	0.08 (0.03) 0.13 (0.01) 0.04 (0.01)

[†]From Clarke *et al.* (2000). [‡]Data are mean (±SE).

Lissanthe and *Melichrus* were bagged with nylon mesh at the immature fruit stage, as indicated by the green mesocarp of developing fruits. Mesh was retained on the plants until fruits had matured and released from the parent plants. Seed production in *Hardenbergia* and *Pultenaea* was measured by harvesting mature fruits directly from reproductive plants prior to ballistic seed release. The relationship between seed production and plant size was determined by correlating seed production per plant against plant size. Owing to differences in growth form the following measures of plant size were considered appropriate for each species: plant height for the erect shrubs *Melichrus* and *Lissanthe*, circumference of the main stem for *Hardenbergia* and plant area for *Pultenaea*. The proportion of pre-dispersal seed loss due to insect predators for *Hardenbergia* and *Pultenaea* fruits was measured by counting the number of seeds in each fruit that showed evidence of insect attack, that is, either presence of larvae or the remains of consumed seeds.

Dormancy, germinability and viability of the seed rain

Dormancy and germinability of the seed rain were examined with a factorial experiment testing the effects of heat, smoke and light on germination, and also fruit coat imposed dormancy in the epacrids. Four replicates of 10 seeds or fruits (epacrids) were randomly assigned to eight treatments representing all combinations of two heat treatments (heat vs. no heat), two smoke treatments (smoke vs. no smoke) and two light treatments (light/dark vs. no light). Treatments were applied and seeds and fruits placed on germination pads on top of moisture retaining sponge in square germination trays. To test for fruit coat imposed dormancy in the epacrids, the 10 fruits in each replicate were gently cracked open without releasing the seeds before the application of treatments and an additional five uncracked fruits were added to each replicate. Replicate dishes were irrigated with distilled water, with two replicates of each treatment placed in separate incubators with a controlled environment of 12/12 h light/dark cycle at 25/15°C. Germination was considered to have occurred when the radicle or plumule had emerged and exceeded half the length of the seed.

In the heat treatment each replicate batch of seeds or fruits were separately exposed to dry heat (80°C) in a modified oven for 6 min and allowed to cool to room temperature before placement in germination trays. This temperature and duration were considered appropriate as they fall within reported ranges of maximum germination response in many plant species (e.g. Martin *et al.* 1975; Warcup 1980; Auld & O'Connell 1991), and the range of conditions experienced

by seeds in the soil during fire (Floyd 1966; Bradstock & Auld 1995). In the smoke treatment, freshly manufactured smoke water was applied to replicate trays after seeds were heated. Smoked water was prepared by slow combustion of dry plant litter collected from a local grassy woodland, in a metal drum, with the smoke bubbled through 10 L of distilled water for 30 min. Dark treatments consisted of wrapping germination trays in aluminium foil to exclude light in the germination cabinet.

Treatment effects on germination were monitored for 20 days. At this time additional treatments were used to determine seed dormancy mechanisms and viability of ungerminated seeds. *Hardenbergia* and *Pultenaea* seeds in light treatment trays that had failed to germinate were scarified with a razor blade and germination monitored for a further 35 days. Foil coverings on all dark treatment trays (all species) were removed and seeds exposed to the light/dark regime and germination monitored for a further 35 days. *Hardenbergia* and *Pultenaea* seeds in dark treatment trays were exposed to 20 days of the light/dark regime, before ungerminated seeds were scarified with a razor blade and germination monitored for the remaining 15 days. Viability of ungerminated seeds was determined by visual inspection under a dissecting microscope for the presence of a firm, white embryo (Roberts 1981). Because of a slow germination response in *Lissanthe* and *Melichrus*, germination trays of these species were incubated for a total of 70 days. To determine the viability of ungerminated epacrid seeds each fruit was carefully dissected and the presence or absence of a seed within each pyrene scored. Viability of intact seeds was determined in the same fashion as for the legumes.

Post-dispersal seed losses

Post-dispersal removal of seeds and fruits (diaspores) was assessed by presenting a known number of diaspores to ants and other seed removers and recording their fates. The experiment was conducted at Hillgrove Creek (Site 1, Table 1), in two plots over 2 days. Plots consisted of a 6 × 6 m grid, with a total of 36 seed depots, one depot located at each 2 × 2 m intercept. Seed depots were a small clearing on the soil surface (no larger than a thumb print) marked with wooden skewer. Nine diaspores of each species were randomly placed at individual depots, such that there was only one diaspore per depot at any given time. The removal of diaspores at each plot was monitored over three sessions on each day: morning (7.30–9.30 hours), afternoon (13.30–15.30 hours) and evening (16.30–18.30 hours). A diaspore was only considered removed if it was dispersed more than 5 cm from a depot.

Table 2. Seed attributes and seed fates of four resprouting shrub species in grassy woodlands on the New England Tablelands

	No. seeds per fruit	Seed production per plant per year	Seed production per m ²	Viability of seed (%) [†]	Estimated seed rain per plant after pre-dispersal predation	Pre-dispersal seed loss per plant (%) [‡]	Estimated viable seed rain (per plant)	Post-dispersal seed removal (%)	Estimated seed entering the seed bank after predation
<i>Lissanthe</i>	5.1 (0.17)	158.3 (36.7)	42.6 (13.6)	43.0 (2.37)	68.1	n.a.	68.1	14.8 (4.68)	51.1
	4–6	16.0–243.5	0.7–177.0	18.8–75.0	3.0–182.6		3.0–182.6	0.00–33.3	2.0–182.6
<i>Melichrus</i>	4.1 (0.38)	103.2 (19.1)	12.1 (2.9)	66.7 (2.08)	69.1	n.a.	69.1	0	69.1
	1–6	16.0–269.3	0.8–35.0	45.8–95.4	7.4–255.8		7.4–255.8		7.4–255.8
<i>Hardenbergia</i>	7.1 (0.32)	416.3 (65.1)	9.2 (1.3)	81.6 (2.91)	341.3	64.3 (3.86)	122.9	38.8 (8.49)	75.0
	4–8	109.9–1038.1	2.0–18.7	40.0–100	43.9–1038.1	0.00–94.4	2.6–1038.1	11.1–66.7	0.8–923.9
<i>Pultenaea</i>	1.9 (0.07)	586.7 (131.5)	63.5 (20.0)	71.6 (4.09)	422.4	43.8 (3.57)	236.6	59.2 (8.45)	97.0
	1–2	36.0–1572.9	1.2–254.8	10.0–100	3.6–1572.9	0.00–89.5	0.4–1572.9	44.4–88.9	0.1–1038.1

Data are means (\pm SE).

[†]Data from germination experiment. [‡]Data are percentages of total seed crop. n.a., not available.

Seed bank sizes and change

Detecting the size and change in soil seed banks is prone to Type II error because of the innate spatial variation associated with seed distribution in the soil (Major & Pyott 1966; Bigwood & Inouye 1988). A pilot study was conducted and a combination of cost-benefit, precision and *a priori* power analysis used to determine an appropriate sample size (i.e. core size) and number to measure seed bank density of the four study species effectively. A sampling regime of five replicate samples using a soil core 5 cm in diameter was found to achieve a precision of greater than 0.5 at an acceptable cost of less than 5 h processing time (Campbell 1999). This sampling regime was also found to detect a difference of four seeds between sample means with more than 80% power (Campbell 1999). The pilot study also showed that the seed bank of each species was concentrated beneath the canopy of adult plants (Campbell 1999) and therefore seed bank density before and after seed rain was only sampled beneath adult plants. Three reproductive plants from each patch within each site were randomly selected at each sampling time. The five soil samples were taken from beneath each parent plant to a depth of 3 cm, as most seeds can be expected to be found in the first 0–5 cm of soil (Carroll & Ashton 1965; Roberts 1981; Auld 1986). All soil samples were washed under high water pressure through three sieves decreasing in mesh size (4.0 mm, 0.7 mm, 0.5 mm, respectively). The remaining portion of silt retained in each sieve was hand sorted under a magnified lamp for recovery of seeds and fruits. Viability of recovered seeds was determined by visual inspection under a dissecting microscope for the presence of a firm, white embryo (Roberts 1981).

Data analysis

Seed production in all species was estimated by dissecting 15 fruits and calculating the mean number of intact seeds per fruit (Table 2). Population densities were then used to convert the number of seeds produced per plant to number of seeds produced per squared metre. Differences in seed production per plant and per squared metre between species were analysed with a one-factor ANOVA in Statview (SAS 1998), homogeneity of variances and normality were improved by log transformation of the data. Scheffe's test was used to compare significantly different means. The relationship between seed production and plant size was tested using Pearson's correlation coefficient.

Orthogonal logit-linear models with binomial error structures were used to analyse: (i) the proportion of seed loss to insect predators between species and sites; (ii) the effects of heat, smoke and light on germination (number of germinates recorded at 20 days were used for analysis); and (iii) the proportion of diaspores removed between species (diaspore removal experiment, data pooled over days and plots). Linear models were constructed in GLMStat (Beath 2001). The assumption of homogeneity of variances was validated using Cochran's test on the binomial variances (Zar 1984). The significance of the models was determined using Pearson's Chi-square function in GLMStat (Beath 2001) and pairwise contrasts for multiple comparisons between means.

Soil seed bank density per plant was determined by allocating each species a standard plant area and multiplying the mean number of seeds exhumed per sample by the number of samples per plant area. Seed bank density at the landscape scale was calculated using population density as for seed production. The difference in viable seed bank density at both scales

between species was analysed using three-factor (site \times time \times species) orthogonal general linear models (GLM) with a Poisson error structure. In both cases site was not found to be a significant factor and data were pooled accordingly. Further analysis was used to compare seed bank density between the families using a two-factor GLM with a Poisson error structure where species and sites were pooled such that family and time were the factors used in the analysis. Linear models were constructed in GLMStat (Beath 2001).

RESULTS

Seed production and pre-dispersal losses

All species produced seed in the sampling period, but the number of seeds produced per plant was highly variable within species, ranging over an order of magnitude (Table 2). There was a distinct difference in seed production per plant between plant families with *Hardenbergia* and *Pultenaea* plants producing significantly more seeds per individual than *Melichrus* and *Lissanthe* ($F_{3,52} = 9.93$, $P < 0.0001$) (Table 2). However, when plant density was taken into account, *Pultenaea* and *Lissanthe* populations produced significantly more seeds than *Melichrus* populations, and *Hardenbergia* populations had a significantly lower seed rain than *Pultenaea* populations ($F_{3,52} = 3.13$, $P < 0.05$) (Table 2). All species had a significant positive relationship between seed rain and plant size with *Lissanthe* having the weakest relationship ($r = 0.51$, $P < 0.05$), followed by *Melichrus* ($r = 0.62$, $P < 0.05$), *Hardenbergia* ($r = 0.66$, $P < 0.05$) and *Pultenaea* being the strongest ($r = 0.81$, $P < 0.05$).

Lepidopteran larvae (family Pyralidae) were responsible for pre-dispersal seed losses in both leguminous species. *Hardenbergia* plants lost 64% of their seed crop to these larvae, which was a significantly greater loss than the 43% experienced by *Pultenaea* plants ($F_{1,82} = 19.25$, $P < 0.0001$) (Table 2). Insect larvae were not detected in the epacrid fruits, and while this could have been an artefact of bagging, freshly fallen fruits appeared to have no insect larvae present.

Dormancy, germinability and viability of the seed rain

Overall, the legumes had more seeds germinate over a shorter time period than the epacrids (Fig. 1), and *Hardenbergia* (82%) and *Pultenaea* (72%) had greater overall seed viability than *Lissanthe* (43%) and *Melichrus* (67%) (Table 2). There was no germination of *Lissanthe* or *Melichrus* seeds from fruits that had not

been cracked at the start of the experiment, indicating that the fruit coat was preventing imbibition and germination of seeds. At 20 days there was no significant incubator effect detected and data were pooled accordingly. Exposure to an alternating light regime significantly increased germination of *Lissanthe* seeds in cracked fruits relative to trays incubated in total darkness ($F_{1,24} = 14.47$, $P < 0.0001$, Fig. 1). There was no significant effects of other treatments and none of the interactions between treatments were significant ($P > 0.05$). There was no germination of *Melichrus* seeds at 20 days (Fig. 1) and hence analysis was not possible. Cumulative germination was greater in *Lissanthe* (<40%) than *Melichrus* (<10%) after 70 days in the germination cabinet (Fig. 1).

The heat treatment significantly increased germination of *Pultenaea* seeds ($F_{1,24} = 18.22$, $P < 0.001$, Fig. 1), but had no significant effect on germination of *Hardenbergia* seeds ($F_{1,24} = 0.08$, $P > 0.05$, Fig. 1). None of the interactions between treatments were significant for either of the legumes ($P > 0.05$). Within the initial 20-day period germination of both species was low, with only 21% of *Pultenaea* and 7% of *Hardenbergia* seeds having germinated (Fig. 1). However, after scarification of the seed coat 100% of viable seeds of both species had germinated by 55 days of incubation (Fig. 1). The primary dormancy mechanism of these two species was therefore probably imposed by the seed coat.

Post-dispersal removal

Ants were the only organisms observed removing seeds during the dispersal trial with four species being the major removers: *Notoncus ectatomoides*, *Pheidole* sp., *Rhytidoponera tasmaniensis* and *Iridomyrmex purpureus*. Overall, a greater percentage of *Hardenbergia* (39%) and *Pultenaea* (59%) seeds were removed than the fleshy fruits of *Lissanthe* (15%) and no *Melichrus* diaspores were removed during the experiment (Table 2). Significantly more *Pultenaea* seeds were removed than both *Melichrus* and *Lissanthe* fruits, but the removal rate of *Hardenbergia* seeds was only significantly greater than removal of *Melichrus* fruits ($F_{3,20} = 21.31$, $P < 0.0001$) (Table 2).

Soil seed banks

Seed bank density beneath parent plants of all species was low and variable at both sampling times (Table 3). There was a significant difference between species in the soil seed bank density beneath parental plants (two-factor GLM, species: $F_{3,328} = 10.61$, $P < 0.001$) (Table 3). Seed bank density per plant did not increase over time (two-factor GLM, time: $F_{1,328} = 0.10$,

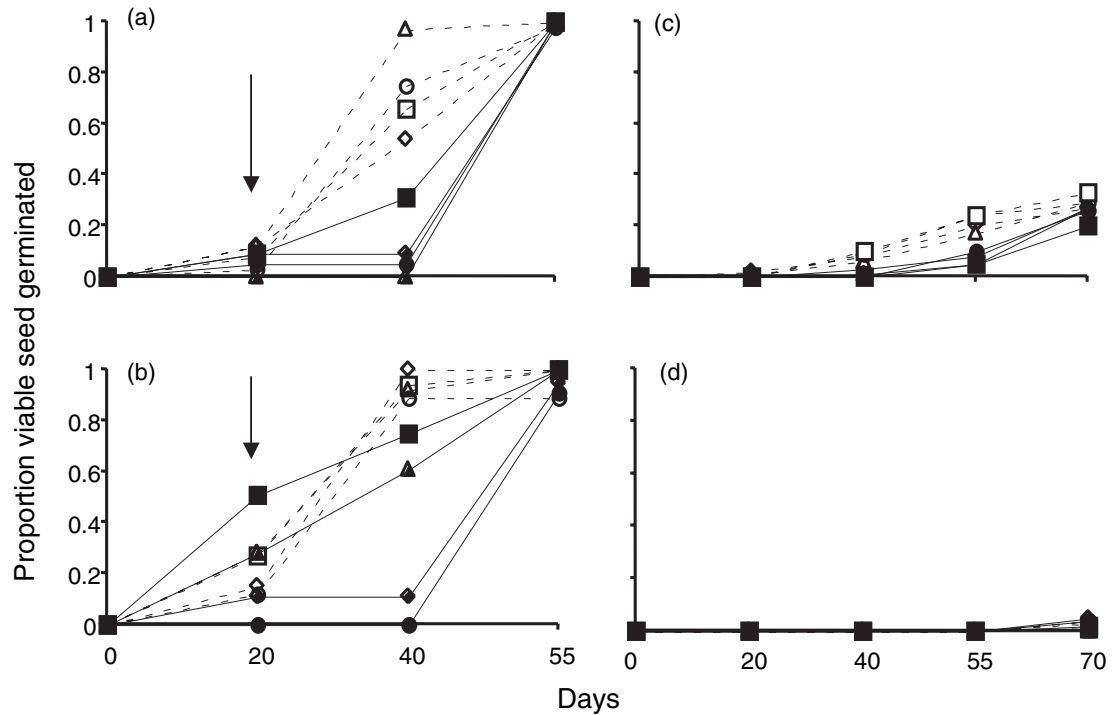


Fig. 1. Mean cumulative germination of viable seeds for (a) *Hardenbergia*, (b) *Pultenaea*, (c) *Lissanthe*, and (d) *Melichrus*. Treatment combinations: Light (open), Dark (solid) (◆) No heat, no smoke; (■) Heat, smoke; (▲) Heat, no smoke; (●) No heat, smoke. Arrows indicate the imposition of seed scarification after 20 days in (a) and (b).

Table 3. Estimated soil seed bank density at the individual (seeds plant⁻¹) and landscape (seeds m⁻²) scale for four resprouting shrub species in grassy woodlands on the New England Tablelands

Family/species	Seed bank density per plant			Seed bank density per m ²		
	Pre-seed fall	Post seed fall	Grand mean [†]	Pre-seed fall	Post seed fall	Grand mean [†]
Epacridaceae	4.47 (1.26)	6.87 (1.34)	5.67 (0.92)	9.60 (3.01)	10.31 (2.22)	9.96 (1.86)
<i>Lissanthe</i>	3.92 (2.15)	3.92 (1.66)	3.92 (1.34)	11.71 (5.94)	7.88 (3.71)	9.80 (3.49)
<i>Melichrus</i>	4.95 (1.45)	9.43 (2.00)	7.19 (1.25)	7.77 (0.30)	12.41 (2.60)	10.09 (1.74)
Fabaceae	8.63 (3.69)	7.20 (3.79)	7.94 (2.43)	5.58 (2.09)	2.26 (0.66)	3.92 (1.10)
<i>Hardenbergia</i>	17.41 (7.75)	15.23 (8.03)	16.33 (5.54)	1.44 (0.63)	1.31 (0.67)	1.37 (0.45)
<i>Pultenaea</i>	1.02 (0.38)	0.32 (0.11)	0.67 (0.21)	9.17 (3.81)	3.09 (1.08)	6.13 (1.99)

Data are means (\pm SE).

[†]Data pooled across pre- and post seed fall.

$P > 0.05$) and there was no significant interaction between species and time (two-factor GLM, species \times time $F_{3,328} = 0.52$, $P > 0.05$), indicating no accumulation of seeds in the soil beneath parent plants after seed rain. Overall there was no difference in the seed bank density beneath plants between the epacrids and the legumes (two-factor GLM, family: $F_{1,332} = 0.62$, $P > 0.05$; time $F_{1,332} = 0.8$, $P > 0.05$; time \times family: $F_{1,332} = 0.41$, $P > 0.05$) (Table 3). At the landscape scale with plant density factored into seed bank calcu-

lations, there was a significant difference in seed bank density between species regardless of sampling time (two-factor GLM, species: $F_{3,328} = 5.44$, $P < 0.01$; time: $F_{1,328} = 0.63$, $P > 0.05$; time \times species: $F_{3,328} = 1.91$, $P > 0.05$) (Table 3). Similarly, epacrid populations consistently had significantly greater seed bank densities than populations of leguminous species (family: $F_{1,332} = 8.72$, $P < 0.01$; time: $F_{1,332} = 0.53$, $P > 0.05$; time \times family: $F_{1,332} = 1.94$, $P > 0.05$) (Table 3).

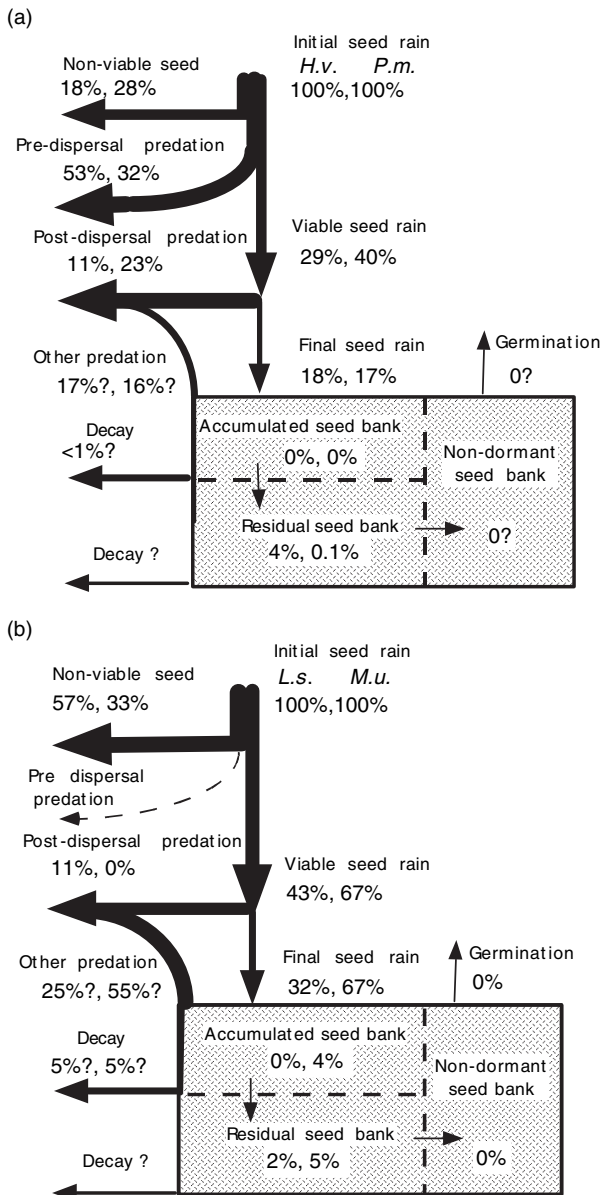


Fig. 2. Estimated seed fates as a proportion of annual seed rain for (a) the legumes *Hardenbergia* (*H.v.*) and *Pultenaea* (*P.m.*), (b) the epacrids *Lissanthe* (*L.s.*) and *Melichrus* (*M.u.*), Decay of pre-existing seed banks, and germination are assumed to be zero.

Seed dynamics

Seed bank densities were low in all species with less than 10% of the seed rain being maintained in the soil (Fig. 2). For the leguminous species there were significant pre-dispersal losses of seed due to insect predation (53%, *Hardenbergia* and 32%, *Pultenaea*) and subsequent losses to ant removal reduced accumulation of seed on the soil to less than 20% of seeds produced (Fig. 2a). At this stage there appears to be

further attrition as few seeds appear to be incorporated into the soil-stored seed bank (Fig. 2a). Most of this seed appears to be removed by predators rather than by decay as no decayed seed remains were detected in the soil or on the soil surface. Similarly, no germinated seeds or seedlings were seen at the study sites when the population structure was surveyed. Even if all the seeds decayed prior to new seed rain and were replaced then a further 14% (*Hardenbergia*) and 15% (*Pultenaea*) of seeds appear to be lost through predation. In contrast to the legumes, pre-dispersal losses to insects were not detected in the epacrids and post-dispersal removal was also less (Fig. 2b). Thus, there were larger proportions of viable seeds potentially available to enter the seed bank. However, most of this seed also appears to be removed by predators as little accumulation of seed in the soil was detected and no germination or decay was observed (Fig. 2b).

DISCUSSION

Seed rain

We found that the number of seeds produced in the four shrub species ranged from fewer than 20 to more than 1500 seeds per plant per year but actual seed rain was reduced by the intrinsic viability of seeds and, in the legumes, by pre-dispersal losses to seed predators. The wide range in seed production appears to reflect size effects on fecundity rather than site effects as plant size and seed rain were correlated but a major challenge remains in determining factors affecting plant size and population age structures. Seed viability varied by an order of magnitude and when the additional effect of pre-dispersal predation was factored into the legume seed rain, the range in viable seed rain increased by several orders of magnitude.

We detected a large pre-dispersal seed loss to Lepidopteran larvae in the leguminous species, but not in the epacrids. Pre-dispersal seed loss to insect predators is not unusual in the Fabaceae and similarly large losses have been reported for other species (Auld 1983; Auld & Myerscough 1986; Auld & O'Connell 1989). However, our methods are likely to have underestimated losses in the Fabaceae because we did not account for whole fruit loss and developmental damage to embryos (Andersen 1988). A persistent seed bank is thought to buffer against losses to seed predators as future plant recruitment is determined more by safe-site availability than actual seed number (Harper 1977; Andersen 1989; Maron & Gardner 2000). However, there is some emerging evidence that increasing seed bank longevity may amplify the population level effects of seed loss to predators as annual reduction in seed inputs may accumulate in the seed

bank (Maron & Gardner 2000). The translation of reduced fecundity across generations and the effects on the abundance of *Hardenbergia* and *Pultenaea* populations is an area that may provide vital information on the recruitment dynamics of these two species. The lack of pre-dispersal seed predation in the epacrids could be an artefact of bagging green fruits, however, unbagged samples of epacrid fruits collected from the ground showed no signs of seed predation. It is possible that the hard mesocarp of the drupaceous fruits prevents oviposition in adults of potential pre-dispersal predator species.

Our expectation was that seed rain would be relatively small as all study species are known to resprout following fire (Knox & Clarke 2004) and low seed production may be expected because of the trade-off in allocation between the ability to resprout and sexual reproduction (Keeley & Zedler 1978; Bell 2001; Pausas *et al.* 2004). Contrary to expectations, the estimated number of seeds produced per plant was within ranges reported for obligate seeding shrubs such as *Bossiaea aquifolium* (9–680) (Hansen *et al.* 1991) and exceeded estimates for *Acacia suaveolens* (1.5–51.1) (Auld & Myerscough 1986) and *Acacia elongata* (0.5–34.1) (Auld & O'Connell 1989). Direct estimates of seed production per plant of drupaceous epacrids are lacking, but on an area basis, obligate seeding species in Western Australia were reported to have four to five times greater seed production than the resprouting species measured here (Meney *et al.* 1994), but differences in methods of estimation make comparisons tenuous. Our study was limited because we only measured seed production in 1 year and reproductive output can fluctuate greatly on an annual basis (Keeley 1977; Morrison & Myerscough 1989). Qualitative observations over several years suggest, however, that the year in which we sampled was not unusual in terms of flowering and fruiting.

Seed bank size

We found that the four species of shrubs had low numbers of seeds in the soil seed bank relative to the seed rain. The mean seed bank sizes ranged from 1.3 to 12.4 seeds m^{-2} and we did not detect accumulation of seed banks. Our expectation was that seed bank density would be relatively low because of low levels of seed rain rather than failure to enter the seed bank. For example, Keeley (1977) found that seed bank density of a resprouter shrub was 8.3 seeds m^{-2} whereas its congener obligate seeder had a density of 26 seeds m^{-2} . Currently, there are few comparative examples for Australian plants with soil-stored seed banks, although similar patterns have been reported for serotinous species (Enright & Lamont 1989; Bellairs & Bell 1990; Lamont & Groom 1998).

At a landscape scale the seed bank densities for resprouting shrubs that we found were generally fewer than 15 seeds m^{-2} and this is consistent with shrub seed densities reported in eucalypt forests in Western Australia (Vlahos & Bell 1986; Ward *et al.* 1997) and in grassy woodlands on the New England Tablelands (Grant & MacGregor 2001). Our findings of seed bank densities fewer than 15 seeds m^{-2} are consistent with the notion that low seed numbers in the soil may contribute to a lack of seedling recruitment after fire and other disturbances that stimulate germination and emergence (Clarke & Davison 2001; Knox & Clarke 2004). However, we may have underestimated the seed bank size because seed removers may have dispersed the seeds (horizontally and vertically) beyond the sampling domain. For example, seeds of myrmecochorous species are reported to be more widely distributed throughout 0–20 cm of the soil profile and can be concentrated in and around ant nests (Auld 1986; Wang 1997).

What limits seed bank accumulation?

We expected to detect an accumulation of seed in the soil because: (i) our pilot study suggested we had sufficient statistical power to detect small changes; and (ii) seeds had innate dormancy. The leguminous species had seed coat imposed dormancy, a characteristic that is reported to contribute to long-lived seed-stores in other members of the Fabaceae (Gilbert 1959; Auld 1987, 1996). Dormancy release and germination are likely to be episodic in these species as heat was found here to induce germination of *Pultenaea* seeds, and similar results are reported for *Hardenbergia* elsewhere (Auld & O'Connell 1991). Hence, accumulation of soil seed reserves has been demonstrated in legume species in fire-prone vegetation (Auld 1987), but we found no evidence of this.

We also found that the woody endocarp in the epacrids inhibited seed germination, a dormancy mechanism that has been observed in other drupaceous species (Allan *et al.* 2004; O'Brien & Johnston 2004). As fruits remain intact, accumulation of seed in the soil may therefore be expected, although persistence of the woody endocarp would be critical. We found germination in the epacrids was slow and was not affected by fire-related cues, suggesting that there is the potential for germination to occur between fires. Ooi *et al.* (2004), observed that combinations of fire-related and seasonal factors maximized germination and emergence of drupaceous *Leucopogon* species in the southern Sydney region. Specifically, post-fire seedling emergence was delayed until late autumn and winter periods, a pattern that coincided with recruitment in unburnt vegetation (Ooi *et al.* 2004). Seasonal factors may have a stronger influence on species in the

New England Tablelands as this region has seasonal rainfall and a winter characterized by severe frosts. Therefore, the delay in germination observed in the epacrids in our study may reflect a seasonal pattern of germination influenced by rainfall and temperature fluctuations.

Paradoxically, there is little evidence of germination after fires of the legume species as minimal seedling recruitment in the post-fire environment and in unburnt vegetation has been observed (Knox & Clarke 2006). In that study systematic searches for seedlings of legumes and epacrids in replicated experimentally burnt areas and adjacent unburnt vegetation over 2 years and two seasons, found no natural seedlings of the target species. Post-fire monitoring after an intense wildfire in the region in 2002 revealed emergences of *Hardenbergia* in dry sclerophyll forest but not in adjacent grassy woodlands (P. J. Clarke, pers. obs. 2003). Seedling emergences of epacrids were also recorded in this study, but they were restricted to rocky outcrops and dry sclerophyll forests with three seedlings of *Leucopogon lanceolata* (Epacridaceae) recorded in a grassy woodland site. In seed sowing experiments hard-seeded species have been shown to recruit in grassy woodlands (Clarke & Davison 2001; Knox & Clarke 2006), although the sowing of *Lissanthe* and *Melichrus* failed to result in post-fire recruitment (Knox & Clarke 2006).

Since seeds do not appear to be lost from the soil seed bank through germination, accumulation of dormant seeds in the soil would be expected if no other losses are occurring. Hence, we think that seed predators rather than seed germination and post-dispersal removal are the likely cause of the lack of accumulation in seed banks. Some ant species such as *Pheidole*, a major remover of *Pultenaea* in this study, are known seed predators (Auld 1986; Hughes & Westoby 1990). Seed loss to granivorous mammals are also known to affect seed bank accumulation in other systems (de Steven 1982; Schupp 1988; Maron & Simms 1997), although there is limited evidence of the importance of this interaction in eastern Australia (Auld & Denham 1999). Potentially larger losses of seed to ant removers and granivores may not have been detected by our short-term removal experiment and could explain the low seed bank densities observed in this study.

Compared with the legumes, fewer epacrid diaspores appear to be incorporated into the residual seed bank, assuming past seed rain was similar to that measured in our study. This difference may reflect the size of the diaspores as the larger fruits may not easily move into the soil profile and may be more vulnerable to granivory from ground dwelling vertebrates. Keeley and Hays (1976) concluded that post-dispersal predation by mammals of fleshy fruits in Chaparral shrubs was a major factor affecting the size of seed

banks in soils. Predation and ingestion of fruits directly off parent plants by birds or other vertebrates present sources of unmeasured seed loss in our study. Further studies on the magnitude of pre- and post-dispersal seed loss in fleshy fruited species are required to identify the mechanisms contributing to the large losses of diaspores between the parent plant and the soil seed bank.

CONCLUSION

Post-disturbance persistence of shrub species in grassy woodlands relies on combinations of the ability to resprout and accumulate seed banks in space or time (Clarke 2000). Most shrub species in grassy woodlands resprout and the four focal species in this study typify this response in the grassy woodlands and forests of the New England Tablelands (Knox & Clarke 2004). Their ability to resprout after fire, and other disturbances, reduces the reliance on seed banks for persistence but long-term mortality must be balanced by seedling recruitment if populations are to be maintained. Such natural recruitment has rarely been observed either in the post-fire environment or in the intervals between fires (Campbell 1999; Knox & Clarke 2006), which contrasts with observations of mass seedling establishment of epacrids and legumes in dry sclerophyll forests. Nevertheless, in grassy woodlands, seedling recruitment has been documented after seed sowing experiments into artificial seed banks (Clarke & Davison 2001; Knox & Clarke 2006) giving rise to the notion of a seed limited rather than safe-site limited recruitment for shrubs in these communities. We have established that, despite considerable variation within and among plant species, the annual seed rain of the focal species should be enough to saturate safe sites. However, substantial seed loss to pre- and/or post-dispersal predators translated into a low seed bank density that may remain low after seed rain. Our study suggests that seed predation is contributing to seed limited recruitment in grassy woodland shrubs by reducing the number of seeds in the soil seed bank available to germinate during recruitment events.

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