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The Effect of Disturbance Regime on *Darwinia glaucophylla* (Myrtaceae) and its Habitat

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The effect of disturbance regime (time since last fire or slashing) on the vulnerable plant species, *Darwinia glaucophylla*, was assessed on the Central Coast of New South Wales, Australia. The abundance, growth and flowering of *D. glaucophylla* adults and abundance and growth of seedlings was measured within sites that had either been recently burnt (≤ 5 years), long unburnt (≥ 14 years) or regularly slashed (30 cm above ground) along a utility easement. Our results showed that *D. glaucophylla* was most abundant at slashed sites, followed by recently burnt sites; it was present but not abundant at unslashed sites that were burnt ≥ 14 years ago. Seedlings were only found at one, recently burnt site. Disturbance regime had no significant effect on the timing or density of flowering. Fruit collected from sites with different disturbance regimes did not germinate after exposure to various combinations of heat, smoke-water and/or scarification. Recently burnt sites contained plants producing a significantly greater number of viable fruits compared to those from other disturbance regimes. Fire and slashing altered the habitat of *D. glaucophylla* in different ways. Our findings suggest that slashing promotes favourable conditions for adults by creating a habitat with higher light and less competition. However, it is not apparent whether these same conditions are favourable for seedling recruitment.

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KEYWORDS: conservation management, fire, flowering, germination, slashing, threatened, utilities easement

INTRODUCTION

Many Australian plant species are considered disturbance-dependent, while others are sensitive to significant disturbance (in which case the disturbance may become a threatening process) (Ross et al. 2004; Kirkpatrick 2007). To be a threat, the disturbance must deleteriously interfere with transfers in the life cycle of a species and/or significantly affect the number of individuals at a particular life stage (Keith 1996). Fire is a natural disturbance that can pose a threat to some species if the long-term regime is disrupted in some way (Keith 1996). Fire frequency, fire interval variability, fire intensity, season of burn and pattern of burn are all elements of a fire regime (Gill 1975; Bond and van Wilgen 1996) which, when considered on a landscape scale, affect biodiversity (Keith 1996). Keith (1996) identified twenty possible fire-driven mechanisms of plant extinction. He concluded that high and low fire frequency, as well as repeated fires with little heat penetration of the soil or the production of smoke derivatives, are fire regimes likely to result in plant population decline and extinction (Keith 1996). Therefore, management of rare plants in fire-prone habitats typically requires knowledge of life-cycle attributes critically involved in population processes and the population response to different fire regimes.

Other disturbances common to urban habitats include sewer, water, gas and electricity services, all of which require installation of hard infrastructure, often at the expense of biotic components of a landscape (Foreman 2003). Slashing, whether by hand or machinery, is the main means by which utilities easements are maintained. Slashing allows easy access for maintenance and surveillance, and reduces fuel loads in order to decrease the threat of fire on such services. Slashing of easements may advantage some plant species, such as those well represented in earlier successional stages and which would subsequently be less well represented in mature ecosystems. The current area of occupancy of *D. glaucophylla* includes regularly slashed gas pipeline and powerline easements located within National Parks, raising the question as to whether slashing is beneficial or detrimental to the species. Although there has been a great deal of research on the effects of slashing (hay-cropping) in grassland ecosystems in Europe, where it is used as a management tool to restore plant diversity to former agricultural land (see review by Walker et al. 2004), only one study (Ellis and Allen 2013) could be found on the impacts of slashing in coastal heathland vegetation in Australia.

Darwinia glaucophylla B.G.Briggs is listed as vulnerable under schedule 2 of the *NSW Threatened Species Conservation Act* 1995. It is a prostrate shrub found in fire-prone coastal heath where it occurs on skeletal soils surrounding Hawkesbury sandstone outcrops in the Gosford Local Government Area (Department of Environment Climate Change and Water 2009a). Its small extent of occurrence, high endemism and habitat specificity has afforded this vulnerable status (Department of Environment Climate Change and Water 2009b). Previous studies of *D. glaucophylla* include descriptive observations of its morphology and phenology (Briggs 1962), seed germination response to heat (Auld and Ooi 2009) and the role of myrmecochory (Auld 2009). Auld and Ooi (2009) found that heat (80°C) enhanced germination and reported that seedlings emerge in the field 2-3 years after fire. However, the effect of smoke on the seed germination of *D. glaucophylla* has not yet been determined.

The current study aims to increase our understanding of the ecology of *D. glaucophylla* in a way that informs the management of the species. As the species grows in fire-prone habitat and is conspicuous in slashed areas along sections of the Sydney to Newcastle gas/oil pipeline, but rarely detected in adjacent unslashed areas, we ask the following research questions: (1) Is the above ground abundance of *D. glaucophylla* in slashed easements and unslashed sites (adjacent to easements) similar ? (2) Does the above ground abundance of *D. glaucophylla* differ between sites that have been burnt in recent times compared with sites that were burnt more than a decade ago? (3) Do the physical characteristics of the habitat of *D. glaucophylla* differ among disturbance regimes (fire, slashing) and, if so, how? (4) Does the flowering phenology of *D. glaucophylla* differ between disturbance regimes (fire, slashing)? and (5) Does smoke water, heat and/or scarification enhance seed germination?

METHODS

Study area

NSW National Parks and Wildlife Service (NPWS) atlas records were used to choose four main locations (Figure 1) within the extent of occurrence of the species: Popran National Park (151°13’05”E, 33°26’09”S), Girrakool Track (151°15’44”E, 33°25’46”S), Lyre Trig (151°17’51”E, 33°27’06”S) and Rifle Range road (151°16’34”E, 33°27’24”S). The latter three locations are all within Brisbane Water National Park. The four locations were no more than 12 kilometres apart and their elevation ranged between 50 to 250 m ASL (Table 1). The Central Coast region of NSW has a warm, temperate climate and a summer maximum rainfall distribution (Murphy 1993). Mean annual rainfall at Narara Meteorological Station (29 years of record) is 1280 mm (Bureau of Meteorology 2009). This station was the closest to most of the sites in this study. The mean maximum temperature of 23° C occurs in January and the mean minimum temperature of 11° C occurs in July (Narara Meteorological Station 12 years of record) (Bureau of Meteorology 2009). According to Murphy (1993), the Rifle Range, Lyre Trig and Girrakool locations belong to the Lambert soil landscape, having undulating to rolling hills on Hawkesbury sandstone. Slopes are typically < 20% and rock benches are common (Murphy 1993). Soils are shallow and sandy and within a pH 3.5 - pH 5.5 range (Murphy 1993). Benson (1986) has categorised the vegetation at these locations as consisting of open forest, woodland, open scrub, open heath and sedgeland. Characteristic flora present includes *Banksia* spp., *Hakea* spp., *Grevillea* spp., *Kunzea* spp., *Dillwynia* spp., *Acacia* spp. and *Leptospermum* spp. The Popran location differs in that it belongs to the Gymean soil landscape, but it also has a substrate comprising Hawkesbury sandstone with similar vegetation communities to the other locations (Benson 1986; Murphy 1993).

At each location, a plot measuring 10 m x 100 m (1000 m^2) was established in an area where *D. glaucophylla* was present. At two of the locations

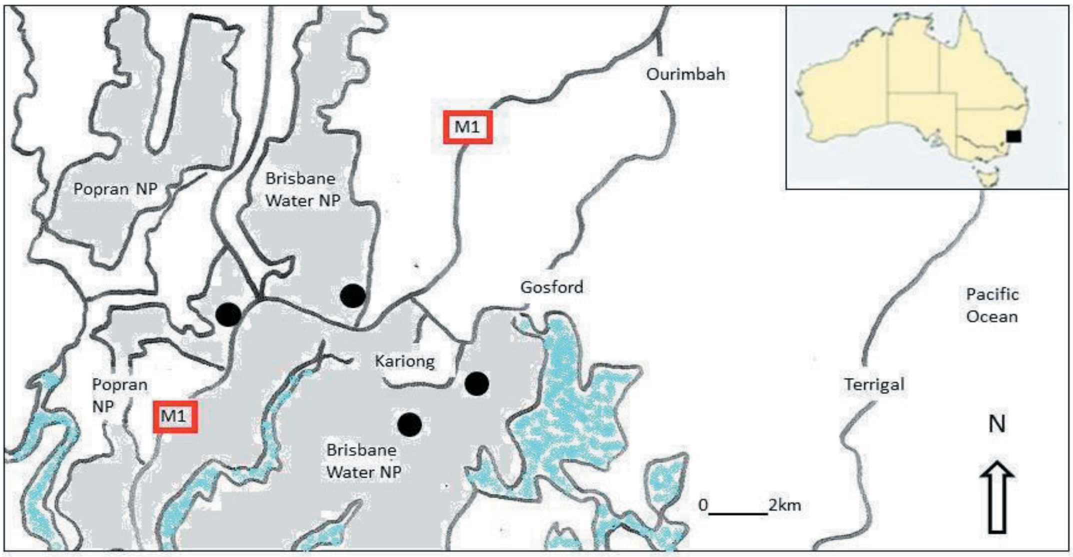

Figure 1. The four *Darwinia glaucophylla* sampling locations on the Central Coast of New South Wales.

(Girrakool and Popran), adjacent unslashed and slashed plots were set up, giving a total of six 1000 m^2 plots (hereafter referred to as sites). An orthogonal design, to test for interactive effects between slashing and fire, was not possible because there were no sites available that were both recently (≤ 5 years ago) burnt and slashed (Table 1). Thirty 1m^2 quadrats were placed randomly within each site. The size and number of quadrats was based on a pilot study by Booyens (2010) which found that 1m^2 quadrats showed less variance in the percentage cover of *D. glaucophylla* than 25m^2 quadrats. Post-hoc power analysis demonstrated that for a one-way ANOVA conducted on the percentage cover of *D. glaucophylla*, a high level of power (> 0.9) could be obtained with twenty-seven 1 m^2 quadrats (Booyens 2010). The pilot study also demonstrated that density could not be used as a measure of abundance because *D. glaucophylla* has a prostrate growth form and can root at the nodes. Percentage cover of the species was estimated using the projected foliage photos of MacDonald et al. (1990). Frequency of occurrence (mix of ramets and genets) within the 30 quadrats at each site was also determined. During the field component of the study (i.e. spring 2008) new apical growth of 30 randomly selected branchlets at each site was measured with a ruler each fortnight. New growth was easily recognised by its non-woody texture and pink/red colour at the tips of branches. Flowering density was estimated by counting the total number of flowers within each quadrat at fortnightly increments over a 3 month period (10/8/08 – 6/11/08). The number of *D.*

Table 1. Characteristics of the locations where *Darwinia glaucophylla* was sampled in this study.

Location	Slope (°)	Elevation (m)	Mean Fire interval (years)	Time since last fire (years)	Slashing
Girrakool NP	3.5	50	8.5[a]	14	biannually with hand-held brush cutters at 30 cm above ground
Popran NP	4.5	120	9[b]	19	biannually with hand-held brush cutters at 30 cm above ground
Lyre Trig	4	230	9.3[c]	2	No slashed sites available
Rifle Range	4	180	9.5[d]	5	No slashed sites available

a Burnt in 1977, 1980 & 1994; b Burnt in 1980 & 1989; c Burnt in 1969, 1977, 1987, 2000 & 2006; d Burnt in 1965, 1969, 1989, 1994 & 2003.

glaucophylla seedlings located within each quadrat was recorded and each seedling was marked and their survival monitored for the duration of the project. The sites and quadrats were permanently marked for the duration of the project.

For each quadrat, the mean height and percentage cover of surrounding vegetation was recorded. Photosynthetically active radiation (PAR) was also measured 1 m above the ground using a LI-190SA quantum light photometer and expressed as µmol/sec/m^2. Soil samples (0.1 m deep) were collected from a stratified random subset of the 1 m x 1 m quadrats (n = 30). Soil pH was determined using a 1:5 dilution and a Hanna pH meter and electrode (Rayment 1992). Electrical conductivity (EC) was measured in a similar manner with a Hanna meter and electrode (Rayment 1992). Soil moisture was determined using the gravimetric method of Rayment (1992). Total nitrogen (mg/kg), phosphorous (mg/kg) and percentage organic matter tests were performed by Sydney Analytical Laboratories using Australian Standard (AS) methods. Samples were dried, split and crushed to 150 microns prior to testing. Phosphorus levels were determined using H_2SO_4 digestion (APHA 4500BF), nitrogen by the APHA 4500B method and organic matter by the AS method 1289.4.1.1.

The indehiscent fruits (containing one ‘large’ seed) of *D. glaucophylla* were collected shortly after the majority of flowering had occurred in late November and early December 2008. Fruits were collected with forceps from the ground at the base of plants in the 1 m^2 quadrats and those fruits from each site were pooled. A total of about 1800 fruits were collected across the four sites and represented < 10% of what was available. Fruits were not collected from unslashed plots as low numbers of fruits meant that collection would have been ecologically irresponsible. Fruits were stored in paper envelopes in a cool, dry place until a germination experiment could be conducted (about six months).

The treatments chosen for the germination experiment (Figure 2) were based on previous studies (Auld and Scott 1995; Kenny 2000; Cochrane et al. 2002; Tierney and Wardle 2005) which showed that smoke water, heat and piercing the fruit coat enhanced germination in other species, including other *Darwinia* species. The fruit/seed coat of half of the collected fruit was pierced with a fine needle to reduce any impedance to germination imposed by the seed coat (Cochrane et al. 2002). The fruit were placed on agar (15g/L) plates to minimise desiccation and the need for repeated watering during the experimental period. Twenty-five fruit per plate were set up in duplicate for each treatment and placed in a germination cabinet (set at 12 hrs light/dark and 25°C / 15°C).

Fruits were soaked for four hours in a 0.1% (w/v) solution of Thiram (a fungicide) or in a second solution containing both Thiram and 2% commercial smoke water (Regen 2000) according to the methods of Tierney (2006). Where a heat treatment was performed, fruits were heated in an equilibrated glass Petri dish at 80° C for 10 mins and then the appropriate solution (Thiram or Thiram plus smoke water) was added (Baskin & Baskin, 1998; Tierney & Wardle 2005). Fruits were placed equidistant on the agar Petri dishes, sealed with Petri film and placed one layer deep in the germination cabinet. Germination was then monitored for a period of two weeks (Baskin and Baskin 1998; ISTA 2003; Mt Annan staff pers. comm. 2009). At the conclusion of the experiment, the viability of the ungerminated fruits was assessed using the ‘cut’ test (Baskin and Baskin 1998; Cochrane et al. 2001; Ooi et al. 2005; Mt Annan staff pers. comm., 2009).

Statistical analyses

Univariate two-factor analysis of variance (ANOVA) was used to test for significant differences (at the 0.05 level) among means for each of the variables measured and to test for any significant interactions (Tabachnick and Fidell 1996). The available combinations of disturbance in the field meant that the ANOVAs involved both orthogonal and nested designs. The orthogonal design

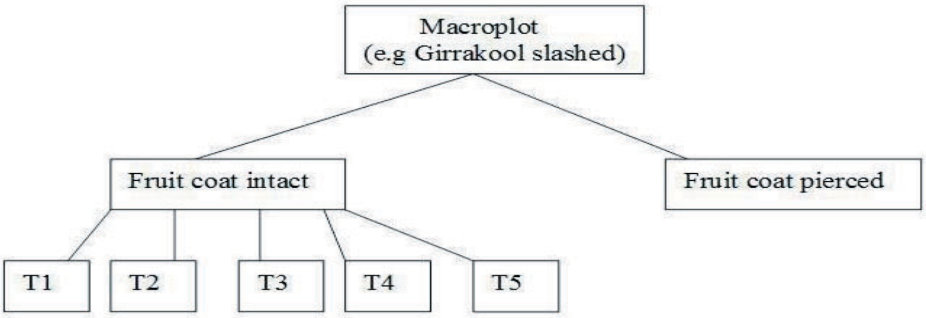

Figure 2. Germination experiment design. T1 = buffered Thiram, no smoke water, no heat; T2 = buffered Thiram, smoke water, no heat; T3 = buffered Thiram, no smoke water, heat; T4 = buffered Thiram, smoke water, heat; T5 = distilled water. Each treatment was performed in duplicate (Note: Macroplot = Site).

tested the effect of slashing or not slashing on sites within the same fire regime (i.e. time since last fire ≥ 14 years), while the nested design tested the effect of time since last fire at unslashed sites. For both types of ANOVAs, homogeneity of variance was tested using Cochran's test and normality was tested using the Shipiro-Wilk test. Where necessary, the data were transformed (arcsine or ln) to improve homogeneity. If transformation did not improve homogeneity, ANOVA was conducted on untransformed data as ANOVA is reportedly fairly robust to departures from this assumption (Underwood 1997a). *Post hoc* comparisons were made using Tukey –Krammer tests. As the flowering data was not independent from one sample time to the next, a repeated measures ANOVA was conducted to test for differences in flowering over time (Tabachnick and Fidell 1996) and *post hoc* comparisons were made using Scheffe (Ho 2006). Correlations between variables were tested using non-parametric Spearman's *rho* as a number of variables were not normally distributed. Chi-squared (χ^2) tests were used to test for significant differences among categorical data such as seed viability. All statistical analyses were conducted with JMP (version 8), SPSS (version 17) or GMAV (Underwood 1997b).

RESULTS

Effects of disturbance on abundance and growth

Darwinia glaucophylla was present in 59 of the 180 (33%) quadrats sampled in this study, with the highest frequency occurring in the slashed/fire ≥ 14 years ago disturbance regime (Table 2). Percentage cover ranged from 1% to 90%, with a mean percentage cover of 5.2% (± 1.2) across all quadrats. Mean percentage cover was significantly ($p < 0.0001$) higher in the slashed/fire ≥ 14 years ago disturbance regime compared to the two other disturbance regimes (Figure 3). There was no significant difference in cover between unslashed/fire ≤ 5 years ago locations and the unslashed/fire ≥ 14 years ago locations (Figure 3 and Table 2). The effect of slashing on percentage cover was consistent across the sites (i.e. no interaction between slashing and site).

Only 5 (3%) of the 180 quadrats in this study contained seedlings, with a total of 12 individuals being recorded. All of these seedlings occurred at the Lyre Trig site, which had an unslashed/fire ≤ 5 years ago regime. Seven of the 12 seedlings survived over the 12-month monitoring period, with five being killed by off-road vehicular damage. Those remaining showed an average increase in height/length of 1.3 cm (range 0.7 cm to 2 cm) over the 12-

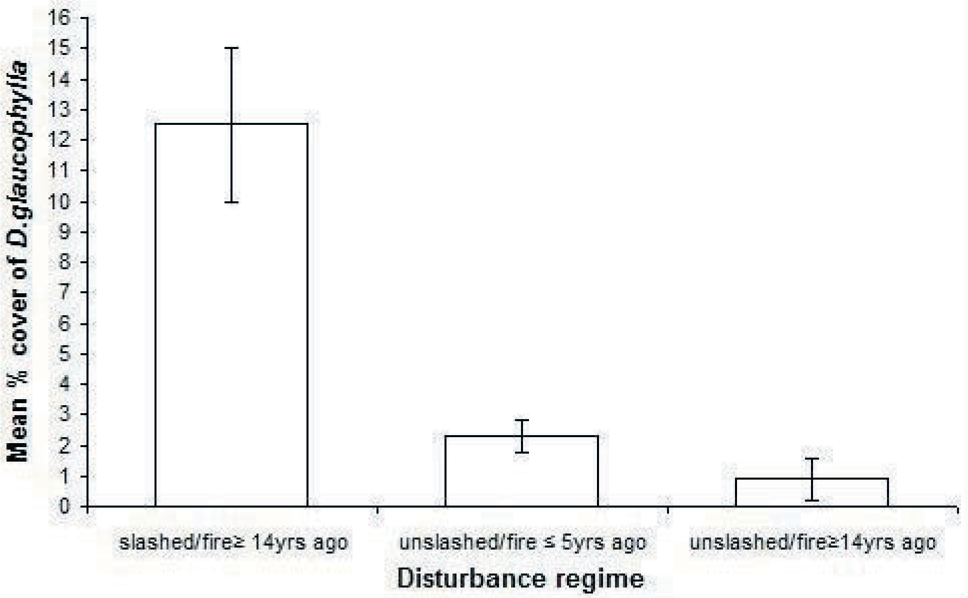

Figure 3. Mean percentage (%) cover of *Darwinia glaucophylla* for each disturbance regime (n = 60). Columns with the same letter are not significantly ($p \leq 0.05$) different. Bars represent ± 1 standard error of the mean.

Table 2. Frequency of occurrence (%), mean percentage (%) cover and mean apical growth of *Darwinia glaucophylla* at the six study sites (n = 30 at each site). Within rows, means with the same letter are not significantly different from one another (at the 0.05 level). The effect of slashing (orthogonal design) compares the Girrakool and Popran sites, while the effect of fire (nested design) compares the unslashed sites at Girrakool and Popran with the last two columns in the table.

	Girrakool NP fire ≥ 14 years		Popran NP fire ≥ 14 years		Lyre Trig fire ≤5 years	Rifle Range fire ≤5 years
	unslashed	slashed	unslashed	slashed	unslashed	unslashed
Frequency (% of quadrats at each site)	3.3	60	3.3	53.3	30	46.7
Mean (± SE) percentage cover (%)	0^{a}	$13\ (\pm 4)^{b}$	$1(\pm 1)^{a}$	$12\ (\pm 3)^{b}$	$2\ (\pm 1)^{a}$	$3\ (\pm 1)^{a}$
Mean (± SE) apical growth (cm/month)	0^{a}	$5.5\ (\pm 0.3)^{b}$	0^{a}	$4.0\ (\pm 0.2)^{c}$	$4.5\ (\pm 0.4)^{b/c}$	$3.7\ (\pm 0.2)^{c}$

month period and none produced flowers during this period. By comparison, the mean apical growth rate (in one month) of mature, established plants was 4.4 cm. Disturbance had a significant effect (p= 0.0007) on mean apical growth and was largely attributable to the absence of growth under the unslashed/fire≥ 14years ago regime (Table 2).

Flowering

Of the quadrats containing *D. glaucophylla* (59), all but one contained individuals producing flowers. At the height of flowering intensity in spring, around 9000 flowers were counted within a combined area of 58 m^2. Repeated measures ANOVA showed no significant difference in the mean number of flowers among disturbance regimes, nor a significant interaction between sampling time and disturbance regime. However, there was a significant difference in mean number of flowers among sampling times (Figure 4). The mean number of flowers significantly increased with subsequent visits until flowering peaked in September, after which it began to decline. *Post hoc* pair-wise analysis showed no significant difference in mean number of flowers between time 1 & 7 but flowering at these times were significantly different from time 2 & 6 (which were similar to one another) and from time 3, 4 & 5 (which were similar to each other). There was a significant difference in the mean number of flowers between sites under the same fire regime, which was due to the absence of flowers in the Girrakool unslashed site (data not shown).

Seeds

No seeds germinated despite the various treatments used. Microscope examination of fruits collected from the ground beneath and adjacent to established adults revealed that only 94/1600 (5.9%) were filled. Cut tests showed that of those fruits containing material, 21/94 (22%) had potentially viable seed. Across the four sites from which fruits were collected, greater than 90% of fruits were empty. The percentage of fruits containing *potentially viable seed* was significantly greater (χ^2 =14.8; d.f. = 1; p = 0.01) in the unslashed/fire ≤ 5years ago sites compared to the slashed/fire ≥ 14years ago sites. Of the four sites able to be sampled, Popran had no viable material within the collected fruits.

Effects of disturbance on the habitat of *D. glaucophylla*

Mean percentage cover of associated vegetation (1m above ground level) across the 180 quadrats was 44%. Significant differences among the three disturbance regimes were found for this variable, with slashed/fire ≥ 14 years ago areas having significantly lower percentage cover of associated vegetation compared to that of the other two disturbance regimes (Table 3). For sites burnt ≥ 14 years ago, mean percentage cover of associated vegetation (1 m above the ground) was significantly ($p<0.01$) lower in the slashed compared to the unslashed areas. However at Girrakool, slashing had no significant effect on percentage cover of associated vegetation. Further, the unslashed site at Girrakool had significantly ($p < 0.05$) less cover of associated vegetation compared

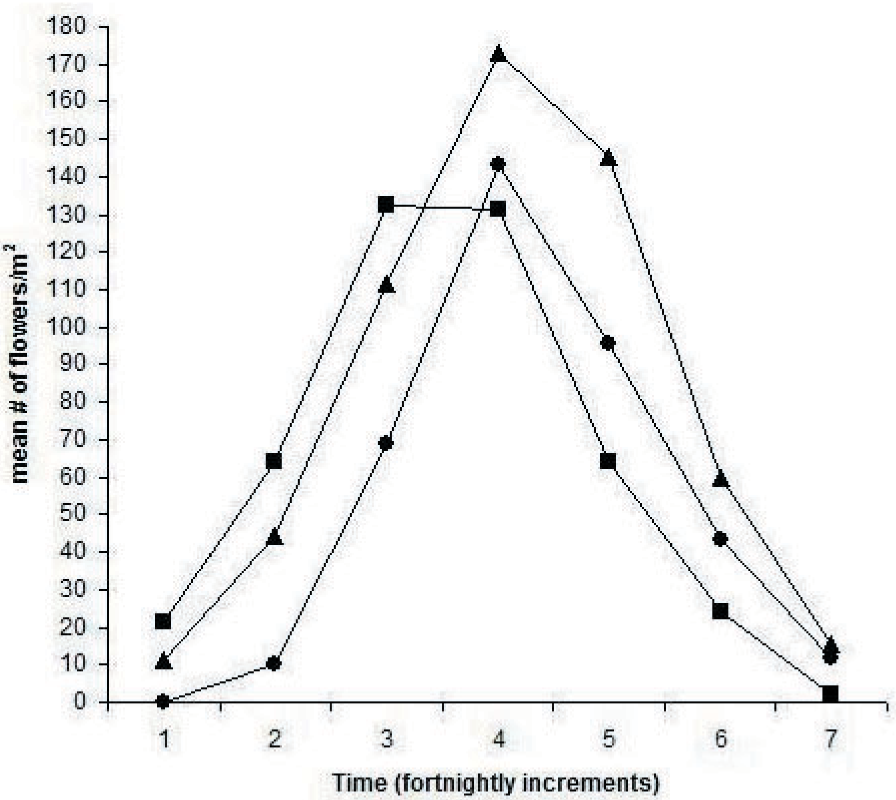

Figure 4. Flowering density of *Darwinia glaucophylla* over time for each disturbance regime (▲= slashed/fire≥14years ago, n= 33; ■ = unslashed/fire≤5years, n=22; ● = unslashed/fire≥14 years ago, n= 2). Monitoring commenced (Time 1) on the 10/8/08 and ceased (Time 7) on the 6/11/08.

to the unslashed site at Popran even though the time since last fire was similar (≥ 14 years ago). There was no significant effect of time since last fire (unslashed) on the percentage cover of associated vegetation (Table 3).

Mean maximum height of vegetation across all quadrats (n=180) was 1.7 m (range 0.15 m to 10 m). Mean maximum vegetation height differed significantly amongst the three disturbance regimes ($p<0.0001$) with vegetation under an unslashed/fire ≥ 14 years ago regime being significantly taller than the other two regimes (Table 3). Within the slashed/fire ≥ 14 years ago regime, the maximum vegetation height at Girrakool was significantly greater than at Popran. Within the unslashed sites, time since last fire had a significant effect with maximum vegetation height being lower when fire was ≤ 5 years ago compared to fire ≥ 14 years ago (Table 3). Photosynthetic active radiation (PAR) was significantly affected by time since last fire (Table 3). Among the unslashed sites, mean PAR was lower in areas burnt ≥14years ago compared to those burnt ≤ 5years ago (Table 3). The effect of site was due to PAR being similar at the two unslashed/fire ≤ 5 years ago sites (Lyre Trig and Rifle range), while within the unslashed/fire ≥ 14 years ago regime, Girrakool had significantly higher PAR compared to Popran. PAR of the slashed sites was similar to sites with an unslashed/ fire ≤ 5years ago regime (Table 3).

Of the suite of soil variables investigated, most showed significant differences between disturbance regimes and/or sites, but there were no significant interactions between these two factors (Table 3). Time since last fire had a significant effect on percentage soil moisture (Table 3), with areas burnt ≤ 5 years ago having lower mean percentage soil moisture compared to areas burnt ≥ 14 years ago (within unslashed sites). Soil pH, electrical conductivity, percentage organic matter, total soil nitrogen and phosphorus were not significantly affected by time since last fire (Table 3). However, the effect of fire on soil nitrogen was close to significance ($p = 0.052$). Slashing had a significant effect on soil pH, electrical conductivity, total soil nitrogen and percentage of organic matter in the soil (Table 3). Mean soil pH was significantly higher while EC, organic matter and total soil nitrogen was

Table 3. ANOVA results (F-values) for percentage cover of associated vegetation, mean height of vegetation, photosynthetically active radiation (PAR) and soil chemistry within quadrats. Sl (slashing), St (site), F (fire) . L – R: the effects of slashing (orthogonal design) are conveyed in columns 2, 3 & 4 of the table while the effects of fire (nested design) are shown in columns 5 & 6. *p<0.05, **p<0.01, ***p<0.001.

Variable	Sl x St	Sl	St	St (F)	F
d.f	1	1	1	2	1
Percentage (%) cover of associated vegetation	3.00	7.03**	5.31*	4.00*	1.85
Mean height (m) of vegetation	0.03	106.38***	7.19**	1.50	41.88*
PAR	25.39**	134.23***	18.05***	20.78**	183.3***
pH of soil	0.97	10.98**	1.18	0.72	0.65
Electrical conductivity (µS) of soil	0.26	11.79**	5.49*	1.17	0.02
Percentage (%) soil moisture	0.00	2.30	15.52**	0.0018	7.20*
Total soil nitrogen (mg/kg)	1.28	7.98*	2.00	3.16	1.01
Total soil phosphorous (mg/kg)	0.17	1.27	3.91	9.32*	0.07

significantly lower in the slashed areas compared to the unslashed areas. Total soil phosphorus and percentage soil moisture were not significantly affected by slashing (Table 3).

Several environmental variables were significantly correlated with quadrats containing *D. glaucophylla* and with percentage cover of the species (Table 4). Presence of, and/or percentage cover of, *D. glaucophylla* was negatively correlated with mean maximum height of vegetation, percentage cover of associated vegetation, soil moisture content and soil EC , and positively correlated with PAR and soil pH. Average apical growth was positively and negatively correlated with PAR and maximum height of vegetation, respectively. Seedling presence was positively corrected with soil N, P, percentage organic matter and EC, while percentage of viable seeds was positively correlated with the maximum height of vegetation, percentage cover of associated vegetation and soil N (Table 4). Although these correlations were significant, some of the correlation co-efficients were small indicating that the relationship between some of the variables was weak and therefore should be treated with caution.

DISCUSSION

Abundance and habitat

While 33% of quadrats surveyed in this study contained *D. glaucophylla*, mean percentage cover was only 5%, indicating that the above ground abundance of the species is lower than initial field observations suggested. The patchy nature of growth in specific habitats such as rocky shelves often gives the impression of local abundance (Booyens pers. ob. 2007). Large spreading mats of *D. glaucophylla* at slashed sites (along the pipeline easement) also give the impression of abundance, but our results show that the mats in this location are due to the intentional management of the over-storey. *Darwinia glaucophylla* was also found in unslashed areas where fire had passed through more than 14 years ago, but it was less frequent compared to sites burnt less than 5 years ago. This finding is consistent with the reported decline of heathland sub-shrubs during long fire intervals, as a result of density-dependent interactions (Keith 1996). Species that are subordinate in stature are particularly prone to competitive elimination (in the absence of disturbance) but these competitive

Table 4. Significant correlations between measured environmental variables and attributes of *D. glaucophylla*. PAR – photosynthetically active radiation; EC = electrical conductivity; N = total nitrogen; P = total phosphorus; OM = organic matter. *p<0.05, **p<0.01, ***p<0.001.

Environmental Variable	*D.glaucophylla* attribute	Spearman ρ	p-value
PAR (µmol/sec/m)	% cover	0.3565	***
PAR (µmol/sec/m)	Average apical growth (cm)	0.6510	***
Max. height of vegetation (m)	% cover	-0.4482	***
Max. height of vegetation (m)	Average apical growth (cm)	-0.5597	***
Max. height of vegetation (m)	% viable seeds	0.6365	***
% cover associated vegetation	% cover	-0.4121	***
% cover associated vegetation	% viable seeds	0.2893	**
pH	Presence in quadrat	0.4110	*
pH	% cover	0.5578	**
EC (µS/cm)	% cover	-0.4112	*
EC (µS/cm)	quadrats containing seedlings	0.4218	*
% moisture (field)	Presence in quadrat	-0.3746	*
N (mg/kg)	% viable seeds	0.4802	*
N (mg/kg)	quadrats containing seedlings	0.4661	*
P (mg/kg)	quadrats containing seedlings	0.3941	*
% OM	quadrats containing seedlings	0.4081	*

interactions only affect the standing plant life stages of populations (Keith 1996). Most plant species from fire-prone communities are expected to have soil seed banks (Auld et al. 2000). Depending on the longevity of their dormant seeds, these species may persist in the community long after standing plants have been eliminated (Keith 1996). Although not examined in this study, it is expected that *D. glaucophylla* is also present in the soil seed bank at the long unburnt sites. *Darwinia* species are known to have persistent soil seed banks (Auld and Ooi 2009), which would allow hidden (below ground) populations of *D. glaucophylla* to persist during fire intervals typical of the current study.

Both slashing and time since last fire had a significant effect on the attributes and habitat of *D. glaucophylla* (Table 5). Overall our results show that there were more similarities between the sites with differing times since last fire (unslashed) than between sites with different slashing regimes (Table 5). That is, the presence of slashing had a greater number and magnitude of effects than time since last fire. However, slashed habitats did resemble areas burnt less than five years ago in the following ways: vegetation of lower stature, greater light penetration and less soil moisture compared with those areas burnt more than 14 years ago and not slashed. The effects of slashing were not all negative though; slashing resulted in the greatest frequency and percentage cover of *D. glaucophylla*.

As soil variables are inter-related, it is difficult to isolate the importance of individual soil factors to the abundance of *D. glaucophylla* (especially because correlation does not confer causality). However, the results confirm that the soil characteristics of the species habitat were typical of that found in heathland vegetation on Hawkesbury sandstone (Murphy 1993) and that mature *D. glaucophylla* individuals can tolerate a range of nitrogen levels (230 – 880mg/kg). The species ability to tolerate low nitrogen may be possible because of existing ectomycorrhizal associations (Booyens 2010). *Darwinia glaucophylla* was more likely to be present, and more abundant, in quadrats with higher soil pH and lower soil moisture (Table 5). The fact that phosphorus levels were similar across the different disturbance regimes

Table 5. Summary of effects of disturbance regime (time since last fire or slashing) on *D.glaucophylla* and its habitat. * Due to presence of permanent creek through this site.

Slashed/fire
≥ 14 years ago

- High frequency of quadrats containing *D .glaucophylla*
- High % cover of *D. glaucophylla*
- High apical growth
- No seedlings
- Similar flowering density
- Low seed viability
- No overstorey
- Low stature vegetation
- High PAR
- Mean pH 5.1
- Low EC
- Medium soil moisture (field)*
- Low N
- Similar P
- Low % OM

Unslashed/fire
≤ 5 years ago

- Low frequency of quadrats containing *D. glaucophylla*
- Low % cover of *D. glaucophylla*
- High apical growth
- Seedlings present
- Similar flowering density
- Higher seed viability
- No overstorey
- Low stature vegetation
- High PAR
- Mean pH 4.9
- High EC
- Low soil moisture (field)
- High N
- Similar P
- High % OM

Unslashed/fire
≥ 14 years ago

- Low frequency of quadrats containing *D. glaucophylla*
- Low % cover of *D. glaucophylla*
- No apical growth
- No seedlings
- Similar flowering density
- No seeds collected
- Overstorey present
- Tall stature vegetation
- Low PAR
- Mean pH 4.8
- High EC
- High soil moisture (field)
- High N
- Similar P
- High % OM

(Table 5) is not surprising, as most studies have found that soil chemical properties return to pre-fire conditions within a year (Raison 1979) and two years had elapsed since the most recent fire in the current study. Any nutrient pulse resulting from soil heating and ash residues would have since been taken up by the existing vegetation, been re-immobilised by microbes or lost by leaching (Raison 1979). Despite this, there was a weak positive correlation between the presence of *D. glaucophylla* seedlings in a quadrat and total soil nitrogen and phosphorus levels. As seeds of the species contain no endosperm (Auld and Ooi 2009) the ash-bed effect, which provides a temporary nutrient-rich substrate allowing enhanced seedling growth (Hobbs 2002), may be particularly important.

Flowering

Slashing had no significant effect on flowering, with mean density of flowers and progression of flowering over time similar across the different disturbance regimes. Flowering fecundity and timing may be affected by resource availability such as adequate soil moisture (Craine 2005). However in our study, differences in soil resources (i.e. moisture and nitrogen) between the treatments (Table 5) appeared to have little effect on flowering. Differences in flowering response between individual sites under the same disturbance regime (e.g. unslashed Popran and unslashed Girrakool) may be attributable to other site-specific features such as aspect and degree of shading from vegetation surrounding the pipeline easement. Despite being surrounded by vegetation, the Popran unslashed site showed much higher levels of flowering compared to the unslashed Girrakool site. This may be explained by the maximum height of vegetation at the latter site being greater and site elevation being considerably lower; both factors likely to increase the degree of shading. In agreement with previous studies (Briggs 1962; Myerscough 1998) we found that peak flowering occurs in September. Peak flowering came earliest to the Rifle Range (fire ≤ 5 years) site and latest to the Girrakool slashed site, further indicating some site specific differences. Fecundity could not be ascertained as an unexpectedly large number of flowers developed, preventing each marked flower being followed over time. It is recommended that future studies of flowering in this species use the density estimates presented here to determine a suitable quadrat size or number of branchlets to sub-sample.

Seeds and seedlings

Whilst one sampling period is insufficient to make conclusions about seedling recruitment in this species, the only site containing seedlings was Lyre Trig, which was burnt two years prior to the study. *Darwinia glaucophylla* is an obligate-seeder with a soil-stored seed bank and therefore fire is important for the recruitment of this species (Auld and Ooi 2009). Auld and Ooi (2009) found that the viability of fresh seed collected from *D. glaucophylla* was high (85-94%), irrespective of site and the year of collection. Our seed viability results aren't comparable because it was tested at the end of the germination experiment, by which time the seeds were at least 7 months old. Greater than 90% of fruits collected in the current study were empty at the end of the germination trial (as determined by a cut test). Possible reasons for empty fruits include: abscission of immature fruits due to weather conditions, lack of resources, competition with developing fruit for limited resources, genetic abnormalities or lack of appropriate insect pollinators, post-dispersal decomposition of fruits deposited on the soil surface, pre- or post-dispersal insect predation of fruit contents, or decomposition while on agar plates despite addition of fungicidal agents (Stephenson 1981; Baskin and Baskin 1998). Myrmecochory has also been reported for *Darwinia* spp. in south-eastern Australia, with removal of abscised fruits being rapid (within 4 - 5 days) (Auld 2009). As fruits in the current study were collected post-dispersal, our findings raise the question as to whether ants could be selectively removing filled, and potentially viable, fruits. We also found that twice as many fruits containing viable seed were collected from the unslashed recently burnt sites compared to the unslashed long unburnt sites. This finding is worthy of further study.

For most of the south eastern Australian *Darwinia* species, a large proportion (80-100%) of the seed is dispersed in a dormant state (Auld and Ooi 2009). However for *D. glaucophylla*, the proportion of fresh seed that were dormant was lower (40-75%) and varied considerably between sites and slightly between years (Auld and Ooi 2009). The findings of the current study confer with Auld & Ooi (2009) in that most of the annually produced seed were dormant (100% in our study). However, Auld and Ooi (2009) also found that heat elicited a germination response in *D. glaucophylla* although the response was quite variable. Three of the four seed crops (two sites over two collection years) germinated at low rates (20 - 40%) without heat treatment (i.e. controls), one seed crop showed a temperature response after being exposed to 60 – 110°C and two seed crops showed a response to 80 – 100°C (Auld and Ooi 2009). The fact that no viable seeds in the current study germinated (with or without treatment) cannot be explained by differences in methods between the two studies, with the exception of the seed collection method. The different methods of seed collection meant that post-dispersal environmental conditions would have differed between the two studies, raising the possibility that a short period (about 1 month) of exposure to ground surface conditions in the current study may have induced secondary dormancy. This hypothesis is worthy of further study because Auld *et al.* (2000) suggested that *D. biflora* may exhibit seasonal secondary dormancy.

The maximum age of the seedlings at Lyre Trig is two years and is therefore consistent with Auld and Scott (1995) who found that seedlings of this species emerge within 2-3 years after fire. Auld and Ooi (2009) suggest that most seedlings don't establish as a result of over storey competition. The post-fire soil nutrient status and above average rainfall during the current study may have been favourable for the growth of seedlings (Keith and Tozer 2012). Unfortunately few seedlings were available to monitor in this project and a more extensive search for seedlings across the species range, followed by a longer monitoring period, is needed to improve knowledge of seedling recruitment and survival rates in different habitats. Adults growing in sites that had either been slashed or burnt less than 5 years ago showed a growth spurt well after peak flowering, which may have been associated with the higher than average rainfall experienced in February 2009. It cannot be determined whether the absence of apical growth of individuals in the unslashed sites that were burnt ≥ 14 years ago was due to competition for resources or an artefact of species abundance being so low that it was less likely that a plant with apical growth was encountered. However, it has been previously noted (Hobbs 2002) that a post-fire environment in Australian heath is conducive to high rates of growth at ground level.

Management implications

One of the disturbances to which *D. glaucophylla* is currently indirectly exposed is slashing of overstorey vegetation along the Sydney to Newcastle gas/oil pipeline. Slashing or mowing under power lines and within other utility easements serves to reduce biomass in an area, limiting fuel for potential fires and improving visibility of, and access to, the easement. In cases where biomass is removed, soil nutrient status and levels of organic matter may be adversely affected (Walker 2004). Findings generally vary as to whether nitrogen (N) or phosphorous (P) decline and the extent of the decline (Walker et al.

2004). The current study found that slashed sites had significantly higher soil pH and lower EC, nitrogen and organic matter compared to unslashed sites (Table 5). It is proposed that removal of biomass reduced inputs of organic matter into soil, which would have also reduced available nitrate and ammonium ions, contributing to lower electrical conductivity of soil water. Disturbance of the soil profile during the initial laying of the pipeline in 1978 (> 30 years ago) may also explain the lower levels of nitrogen and organic matter along the easement if the subsoil material was brought to the surface and left exposed.

While current slashing practices (hand-held cutter 30 cm above ground) along the Sydney to Newcastle gas/oil pipeline easement appear to promote the survival and growth of existing mature individuals, it is not known how long these practices have been in place. Given that the species has a life expectancy of around 20 - 30 years (Auld and Scott 1995), there has been insufficient time to see the effects of slashing over several generations. Further, it is not known whether slashing can provide conditions necessary for future recruitment. The current study found that slashing did not affect flowering but we were unable to determine whether fruit production was affected. Although Auld and Scott (1995) demonstrated that 6-month old seed of *D. glaucophylla* is viable, the long-term persistence of seeds in the soil is not known. If fire related cues are the only mechanism by which seed dormancy is broken and the species doesn't produce any non-dormant seeds, then the population along the pipeline at Popran may only be temporary. Keith et al. (2002) reports that "most heath species with persistent seed banks also produce a fraction of non-dormant seeds" (p. 214) but Auld and Ooi (2009) show that the proportion of *D. glaucophylla* seeds that germinate without treatment is low. Increased nitrogen levels in recently burnt areas often contribute to more successful establishment of seedlings (Bell et al. 1999). Thus even if seeds were to germinate in the slashed sites in the absence of fire, the lower soil nutrient levels together with a lack of a nutrient pulse after fire may limit seedling establishment. The life-span of *D. glaucophylla* is around 20 - 30 years (Auld and Scott 1995) and the site has not experienced a fire event for around 20 years. Auld and Scott (1995) recommend a 5-10 year minimum interval between fires for this species, but this may not be practicable under power lines or above the pipeline. It is likely that existing populations in Popran outside the unburnt easement are too far away to allow natural dispersal and recolonisation if the slashed population (including the soil-stored seed bank) was to reach the end of its life. If smoke alone can promote germination, this may provide a management alternative for populations where ecological burns cannot be conducted. Field trials using aqueous smoke extracts, pelletised smoke products or pile burns could then be conducted and recruitment monitored.

CONCLUSION

The current study confirms that fire and slashing both have positive effects on the above ground abundance of *D. glaucophylla*. Our results demonstrate that the above ground abundance of *D. glaucophylla* was very low in long unburnt sites, unless they have been slashed. The above ground abundance of *D. glaucophylla* was greater at sites that were recently burnt compared to those burnt more than a decade ago, and seedlings were only found at one site that was burnt < 5 years ago. Fire and slashing affected the habitat of *D. glaucophylla* differently. While both types of disturbance reduced the biomass of the surrounding vegetation and increased light penetration, slashing also resulted in lower levels of soil nutrients and organic matter. Ideally, components of the fire regime other than time since last fire should be investigated to refine fire management strategies. However given the species' restricted distribution, finding sites with suitable fire regimes may not be possible. Flowering in *D. glaucophylla* peaked in September and flowering density followed a similar pattern over time, irrespective of the disturbance regime. The results of the germination experiment indicated that further study of the seed ecology of this species is required.

The finding that the above ground abundance of *D. glaucophylla* is higher at slashed sites and flowering rates are unaffected by slashing goes some way to support the conclusion of Monsted and McMillan (2007) that current slashing practices along the Sydney to Newcastle pipeline in habitats containing *D. glaucophylla* are not adversely affecting existing mature individuals. However, insufficient time has elapsed to examine the effects of slashing over several generations. Although the soil seed bank of *D. glaucophylla* has the potential to allow the population to persist after the above ground plants reach the end of their life span, it was not apparent from the current study whether slashing creates conditions that are favourable for seedling recruitment.

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