The effect of disturbance regimes (fire and slashing) on populations of *Darwinia glaucophylla* on the Central Coast of NSW, Australia

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I hereby certify that the work embodied in this thesis is the result of original research, except where reference has been made to the work of others, and has not been submitted for a higher degree to any other University or Institution.

Signed: .................................................................

(Carmen Booyens)
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ABSTRACT

The effect of disturbance regime (slashing and time since last fire) was assessed for a vulnerable plant species (*Darwinia glaucophylla*) on the central coast of NSW, Australia. The species has a limited geographic range and high habitat specificity. Percentage cover, flowering, germination and seedling development of the species were measured across disturbance regimes. The habitat of *D. glaucophylla* was examined by measuring photosynthetically active radiation (PAR), cover and height of the surrounding vegetation, mycorrhizal colonisation and a suite of soil attributes (pH, electrical conductivity (EC), nitrogen, phosphorous, moisture and organic matter) within each of the different disturbance regimes. Results indicate significant differences among variables between disturbance regimes. This was less so at the macroplot level, although differences at this level suggest that site-specific characteristics may contribute to some of the variation reported. Slashing had a more significant effect than time since last fire on many of the variables investigated. These findings have implication for management of the species in terms of slashing practices and because the species requires fire-related cues to stimulate germination.
Chapter 1: General introduction

1.1 Literature review

1.1.1 Biodiversity and rarity

Throughout the past three decades, preservation of biodiversity has received considerable attention in popular media and scientific literature alike. A growing understanding of the value of biodiversity for humanity’s benefit forms much of the rationale behind conservation activities (Lindenmayer & Burgman, 2005). Biodiversity encompasses more than merely different types of species. However, at a basic level this is often as it is defined (Kirkpatrick, 1999; Gill et al., 1999). The often overlooked and unaccounted for services provided by biodiversity (Lindenmayer & Burgman, 2005) have, in their increasingly degraded state, become limiting factors to economic progress (Hawken et al., 1999). Consequently, conservation efforts have gained momentum at all levels of society in an effort to curb further degradation of the biosphere.

In an Australian context, floral biodiversity has historically been rich for a number of reasons: low soil fertility (Siddiqi & Carolin, 1975; Burrough et al., 1977), isolation (Lindenmayer & Burgman, 2005), aridity (Myerscough, 1998), and a unique fire regime (Gill, 1975; Fox & Fox, 1986; Keith, 1996) thought to have been partially influenced by the use of fire by Aboriginal peoples (Kershaw et al., 2002). This combination of factors has resulted in a high level of endemism on the Australian continent with about 10% of global higher order plants found only in Australia (Leigh & Briggs, 1992; Cropper, 1993; Kirkpatrick, 1999).

Australian heath represents a major vegetation biome possessing high species richness, with between 33 and 131 species recorded per hectare (Groves, 1994). Australian heath is defined by the presence of predominantly small-leaved, evergreen, sclerophyllous shrubs of varying height. Representative genera include Banksia, Leptospermum, Hakea, Acacia and
Xanthorrhoea. These plants are adapted to soils with low fertility and moisture, and possess an extensive root system capable of regenerating after fire (Siddiqi et al., 1975; Groves, 1994). The heath of the south-west of the continent (kwongan) contains the highest floral biodiversity and endemism, followed closely by the heath of the Sydney region (on Hawkesbury sandstone) (Siddiqi et al., 1972; Groves, 1994; Myerscough, 1998).

Rare plant species contribute to biodiversity and are usually more susceptible to extinction than common species, for reasons which will be explored shortly. Rare species are often the target of conservation activities, sometimes at the expense of ecosystem-wide approaches to conservation (Erhlich, 2005). Bevill & Louda (1999) state that the “conservation of rare plant populations is an important dimension to the preservation of biodiversity” (p. 493). Attempts to define and explain rarity of species are a challenging task, as the literature testifies. Rabinowitz’s (1981) various forms of rarity, based on traits relating to geographic distribution, habitat specificity and population size; provide one such attempt at definition. Small geographic range, high habitat specificity (Harper, 1981; Prober & Austin, 1990; McIntyre, 1995) and low abundance are characteristics which contribute to species rarity (Rabinowitz, 1981; Menges & Gordon, 1996). It is this set of features that make rare species more susceptible to extinction, especially when stochastic events arise (Harper, 1981; Lindenmayer & Fischer, 2006). Various studies have attempted to identify plant attributes which could provide an explanation for rarity or commonness (Noble & Slayter, 1980; Kunin & Gaston, 1993; Quinn et al., 1994; Bevill & Louda, 1999; Gross et al., 2003; Sjostrom & Gross, 2006). Some of these attributes include life history strategies such as dispersal ability, time to reproductive maturity, seeds per fruit, seed bank characteristics, seed viability, required photoperiod, flowering frequency, shoot height and drought tolerance. The rationale for such studies can be stated as, “if we can predict what type of species (by their attribute set) will
become rare, we can set priorities for species conservation efforts” (Schwartz, 1993, p.121). Investigating plant attributes is a very labour intensive exercise. However, an understanding of plant attributes (such as response to fire), can provide invaluable information for general approaches to conservation that aim to have plants represented in a landscape but not necessarily in every habitable site (Keith, 1996).

1.1.2 Threats and Disturbances

A species may be rare and remain so in space and time (Harper, 1981), but if threats (natural, anthropogenic or a combination of these) are imposed on these rare species (or on a common species rendering it rare), their continued presence could be jeopardised. It is worth noting here that the term disturbance is sometimes used synonymously with threat, but not all disturbances are threats. In fact, some species are considered disturbance-dependent, while others are disturbance-sensitive (in which case a disturbance may have become a threat) (McIntyre, 1995; Forman, 2003; Ross et al., 2004; Kirkpatrick, 2007). Threats are those processes which deleteriously interfere with the life cycle of a species (Silvertown & Doust, 1993; Keith, 1996) and whose sustained presence could lead to species extinction.

Loss of biodiversity has largely been attributed to overexploitation of natural capital by the direct and indirect effects of human population growth and resource use (Thompson & Jones, 1999; Ehrlich, 2005). Recent human-induced extinction rates are 1000 times that of natural background rates of extinction and are predicted to increase (Ehrlich, 2005). Preventing species loss requires effective action and long term efforts at local, regional and international levels which are directed toward protecting entire ecosystems. Many threatening processes have been identified that contribute to the extinction of plant species. These include habitat loss (which encompasses a plethora of associated effects), competitive weed invasion, introduced pathogens, poorly managed fire regimes and grazing pressures (Saunders et al., 1991; Leigh &
Land clearance is the main reason for loss of biodiversity where the number of habitable sites for species is reduced (Kirkpatrick, 1999; Lindenmayer & Fischer, 2006). In Australia, agricultural and urban development since European colonization have provided the main impetus for land clearance (Gilfedder & Kirkpatrick, 1998; Costin et al., 2001). Intensive land use (such as urban development in coastal areas) accounts for less than 1% of total land use, while extensive grazing activities account for about 50% of modified landscape (Australian Bureau of Statistics, 2010). Habitat loss may include degradation of habitat (such as reduction in heterogeneity), fragmentation of habitat (loss of connectivity) and increased edge-effects (Saunders et al., 1991; Gilfedder & Kirkpatrick, 1998; Clarke et al., 2006). The management of utilities easements often results in habitat loss through fragmentation and edge effects (Foreman, 2003).

Fire is an “ecological process that mediates between maintenance and loss of biological diversity” (Keith, 1996 p.37). Fire can represent either a disturbance and/or a threat to species. Much of the Australian flora has evolved physiological strategies to ensure long term survival following fire. Some of these include vegetative resprouting by epicormic shoots or lignotubers (Gill, 1975; Fox & Fox, 1986; Auld, 1987; Bond & van Wilgen, 1996), breaking of seed dormancy and germination cued by heat and/or smoke (Morris, 2000; Tierney, 2006), bradyspory (Groves, 1994) and post-fire flowering (Myerscough, 1998; Whelan et al., 2003). Gill (1975) and Bond & van Wilgen (1996) define a fire regime in terms of frequency, intensity and season of burn. Alteration of any one of these parameters could potentially affect long-term persistence of a species. If, for example, a species is killed by fire before it has matured sufficiently to set seed, that species could become extinct in that location, particularly
if its dispersal ability from neighbouring stands is poor (Keith, 1996). Whelan et al. (2002) suggest that it is difficult to predict the effects of particular fire regimes on any one species due to the variations that exist in characteristics of organisms, the fire itself, the landscape and the climate. This leaves resource managers in a difficult situation when faced with the challenges of predicting the response of endangered or threatened species to prescribed fires.

Understanding the life processes of a species helps predict possible responses to fire.

Historically, the issue of ‘appropriate’ fire regimes has been controversial. One school of thought is that fire regimes governing the growth of flora and procurement of food were initiated by indigenous human inhabitants (Kershaw et al. 2002). These historical fire regimes changed with European settlement and altered fire regimes now represent a threatening process to some rare species (Keith, 1996). Other discussions concerning the historical influence of fire on the Australian flora centre on climatic changes, particularly increasing aridity (Kershaw et al., 2002). The relative contributions of fire usage by aboriginal peoples, Europeans and the influence of climatic changes remain a contentious issue (Bowman, 1998; Mooney et al., 2001).

Cropper (1993) suggests that slashing is sometimes used in place of fire as a means to control vegetation where the use of fire is unsafe, such as within power line easements. The goal of slashing is to reduce biomass in order to prevent the dangers associated with wild fires (Clarke et al., 2006). However, slashing is a poor substitute for burning when recruitment and promotion of floristic diversity is the goal. This is because additional effects, such as deposition of ash, stimulation of flowering and breaking of seed dormancy can only be achieved with the use of fire (Cropper, 1993; Kenny, 2000).
1.1.3 The Target Species

During the past decade in Australia, legislation specifically concerned with the conservation of species has been enacted. At the national level, the *Environment Protection and Biodiversity Conservation Act* (1999) provides a list of extinct, critically endangered, endangered and vulnerable species; the definitions of these categories being derived from the *International Union for the Conservation of Nature* (IUCN) (1994). Species are assigned to these groups and subsequent action is taken to abate known threats. At the state level, the *NSW Threatened Species Act* (1995) classifies species under Schedules, with Schedule 1 relating to endangered species, Schedule 2 to vulnerable species (those species facing high risk of extinction in the wild in the medium-term i.e within 10 years) and Schedule 3 to threatening processes (Lindenmayer & Burgman, 2005; Dept of Environment, Climate Change and Water, 2009). Further, plant species of interest in NSW are placed on a Rare or Threatened Australian Plant (ROTAP) list.

Mokany & Adam (2000) have identified the North Coast and Central Coast botanical divisions of NSW as having the greatest number of threatened plant species when compared with western divisions. This is possibly due to a greater concentration of research efforts on the east coast and/or greater variety in habitat niches (rainforest & sclerophyll communities are represented on the east coast and are biologically diverse ecosystems, also containing a large number of threatened species) (Mokany & Adam, 2000).

The plant species of interest in the present study is *Darwinia glaucophylla* (Myrtaceae), an endemic of the Central Coast of NSW with a small geographic range estimated at less than 100 km (National Parks and Wildlife Service, 2009). It has been afforded vulnerable status under
Schedule 2 of the *NSW Threatened Species Act* (1995) and has also been placed on the ROTAP list. There are about 40 *Darwinia* species in Australia, with the vast majority of these endemic to the south-west of Western Australia (Cochrane *et al.*, 2002). Research by Briggs (1962) focused on describing the genus as it is represented on the east-coast and included characteristics such as morphological features, hybridization, breeding systems (mostly out-crossing), extent of range and chromosomal number. More recent research by Auld and colleagues (1995, 2009) focused on post-fire recruitment, germination and myrmecochory. Auld and Scott (1995) investigated the impact of fire interval on conservation of endangered plants in the Sydney region. *D. glaucophylla* was one of several such species included in this research. It was from this work that a 5-10 year fire interval was suggested as beneficial for conservation of this species and this was largely based on the appearance of viable fruits 2 years post-fire. This suggested fire interval has not been tested experimentally. Auld and Ooi (2009) found that heat in the 80-100 ° C range increased seed germination of *D. glaucophylla* and that seed viability was high. Their research did not investigate the impact of smoke on germination of *D. glaucophylla* seed. Auld (2009) also showed that ants remove seeds from *Darwinia* species in a matter of days. Other than these papers, much remains unknown about the species and its ecological interactions. The present study contributes by exploring interactions such as mycorrhizal colonization, components of disturbance regimes to which this species is exposed and flowering patterns.

The habitat of *Darwinia glaucophylla* is sandy heath, where it is often found fringing rocky outcrops. It has a spreading prostrate form with maximum height in the order of 15 cm. Leaves are grey/green with reddish tips, although in dry conditions and very shallow soils tips may have a purple tinge. Leaves measure 10 – 15 mm in length and only 3 mm in width (Briggs, 1962). It has a woody main stem and central root system. However, anchorage roots grow from
branchlets that spread along the ground (Plate 1.1). These roots seem to appear only when appropriate and sufficient substrate is present (pers. obs, 2008). When growing amongst dense scrub or fallen branches, *D. glaucophylla* individuals use neighbouring structures as scaffolding and grow upwards, thus providing the plant some height and a possible means by which to avoid competition for light (pers. obs, 2008) such as occurs in the flush of post-fire secondary succession. *Darwinia glaucophylla* may flower a little in winter but peak flowering occurs in spring (Dept. Environment, Climate Change and Water, 2009). Flowers are tubular, occur in clusters of 2-4 and vary in colour from green, yellow or red. First flowering on juvenile plants tends to occur within 2-3 years after emerging (usually after a fire event) (Briggs, 1962; Auld & Scott, 1995). Fruits are small (4-5 mm), one seeded and abscise near the parent plant on maturation with possible secondary dispersal by ants (Auld, 2009).

![Plate 1.1: Prostrate branch of *D. glaucophylla*. Note anchorage roots along branchlet](photo by C.Booyens, 2009).

### 1.2 Purpose of the study

The main objective of the current study is to contribute to an understanding of the ecology of *D. glaucophylla* (a vulnerable endemic species) populations subject to differing disturbance regimes. The outcome of the research will hopefully see improved management of habitats in which the species occurs, thus ensuring its continued presence in the Australian landscape.
In recognition of *D. glaucophylla*’s listing as a ROTAP species, the Department of Environment, Climate Change and Water has identified 15 priority actions for the species, some of which include: education of landholders about the species, monitoring impact of fire, fencing and signage for site protection, weed control, habitat rehabilitation, monitoring the population, engaging in relevant research concerning the species, assessing genetic makeup of populations, mapping potential habitat particularly at limits of distribution and reviewing its conservation status (Dept. of Environment, Climate Change and Water, 2009). At the local level, National Parks and Wildlife Service (NPWS) have the responsibility for implementing some of these actions. Consequently, in consultation with the NPWS Biodiversity officer on the Central Coast of NSW (Gosford), questions were raised concerning *D. glaucophylla*. These included:

1) Are the Central Coast records of *D. glaucophylla* in the NSW National Parks Wildlife Atlas correct? Does this plant actually exist in these locations?

2) The species appears to be very abundant and conspicuous in slashed areas along the gas/oil pipeline. Does it have similar abundance in unslashed (or closed) scrub adjacent to these slashed areas or is it just more difficult to see in the unslashed adjacent bush?

3) Does the abundance of *D. glaucophylla* differ between areas that have been burnt in recent times compared with areas that were burnt more than a decade ago?

These questions provided a framework for the research that follows. The study has dealt with the species in a broad sense, exploring attributes of the species and its habitat. It has also provided baseline data on the relative abundance of the species within permanently marked macroplots, to which NPWS personnel will return in subsequent years to monitor the
populations over time and in so doing, fulfil their responsibilities under legislation concerning this species.

1.3 Study Aims and Hypotheses

The main aims of the study were to:

(i) Determine the response of *D. glaucophylla* to slashing and time since last fire. This was achieved by comparing the effects of different disturbance regimes (slashing and fire) on populations of *Darwinia glaucophylla* within the Central Coast region of NSW by measuring species characteristics such as percentage cover, flowers per unit area, mycorrhizal associations and seed germination.

(ii) Describe how the habitat of *D. glaucophylla* differs as a result of slashing and time since last fire. This was determined by measuring abiotic characteristics such soil attributes light penetration, aspect and slope within sites subject to different disturbance regimes.

**Hypotheses:**

1) There is a significant difference in mean percentage cover *D. glaucophylla* between disturbance regimes.

2) There is a significant difference in mean percentage cover of ‘associated’ vegetation between disturbance regimes.

3) There is a significant difference in mean photosynthetically active radiation (PAR) and mean maximum height of vegetation between disturbance regimes.

4) There is a significant difference in flowering of *D. glaucophylla* between different disturbance regimes.

5) There is a significant difference in seed quality and germination responses of *D. glaucophylla* between disturbance regimes.

6) There is a significant difference in soil attributes between disturbance regimes.

7) There is a significant difference in mycorrhizal associations between disturbances.
Chapter 2: Study sites and overall experimental design

2.1 Study sites

Records of *D. glaucophylla* observations within the Central Coast region of NSW were obtained from the NSW National Parks Wildlife Atlas and were plotted onto 1:25 000 topographic maps (Gunderman 9131-3S and Gosford 9131-2S). Co-ordinates for these records were in AGD66 format, therefore all readings were adjusted to GDA94 format before being plotted. Accessible sites (near tracks or roads) were chosen from those listed and ground-truthed over several days (about 4-5 days). Some of the records were found to be inaccurate with other eastern species of *Darwinia* (such as *D. fasicularis*) present where *D. glaucophylla* is listed.

Five main populations of *D. glaucophylla* were located and four of these were included in this study (Plate 2.1 & Table 2.2). These occur along the Sydney to Newcastle gas/oil pipeline in Popran National Park (NP), along the gas pipeline near Girrakool Trigonometric station (199 mASL) on the Pacific highway (Monstead & McMillan, 2007), opposite the Karring Rifle Range and at Lyre Trigonometric station (241 mASL) in Brisbane Water National Park (NP). Within the four sites, six macroplots (Figure 2.1) of 1000 m² each were placed over the known populations. The Popran and Girrakool sites contain two adjacent macroplots (‘unslashed’ and ‘slashed’) within each of these sites (Figure 2.2). Sites in this study were chosen on the basis that they provide examples of different disturbance regimes (slashing or burning) and therefore allow for comparison of the relative success of *D. glaucophylla* under these disturbance regimes.
Plate 2.1: Map showing study location (inset) and aerial photograph showing the four study sites (Created by Kindi Smith, NPWS, Gosford office).
Within the macroplots (10 m wide x 100 m long) at each site, thirty 1 m x 1m randomly placed quadrats were set up (Figure 2.1). The appropriate number and size of quadrats was determined by a pilot study (see section 3.2). The choice of a 10 m wide plot was dictated by the dimensions of the pipeline easement (10 m wide) and as such, consistency of macroplot size was maintained across all sites. Random placement of quadrats was carried out by generating a series of random numbers on a calculator and using measuring tapes to mark location of the 30 quadrats. A 0.5 m long peg was placed in either the top left hand or top right hand corner of the quadrat (depending on whether quadrats were placed to the left or right of the main 100 m transect line – see Figure 2.2) and a 1 m² conduit square was placed over these pegs. Pegs were marked with fluorescent pink paint (and numbered 1-30) and left in situ for the duration of the project. Where vegetation was dense and/or tall, marking tape was used to assist in relocating these pegs for subsequent data collection.

Figure 2.1: Placement of 30 random (1 m x 1 m) quadrats within 1000 m² macroplot at Lyre Trig and Rifle Range sites. Both macroplots are unslashed and had fire ≤ 5 years ago.
2.1.1 Climate

The climate of the region can be described as having a mean annual minimum temperature of 11 °C with occasional frosts in winter months and a mean annual maximum temperature of 23 °C with some temperatures over 40 °C during summer. Mean annual rainfall is 1280 mm with a summer rainfall distribution pattern (Bureau of Meteorology, 2009). Meteorological data for Gosford (Narara Research Station is approximately 100 m ASL) was obtained from the Bureau of Meteorology (BOM) website for long term averages and for the period of the present study and plotted below (Figures 2.3 & 2.4). The study sites are between 6 -12 kilometres from this station and range between 50 - 250 m ASL.
Figure 2.3: Minimum and maximum temperatures for Gosford (Narara Research Station). The red series indicate the temperatures for the study period of January 2008 to March 2009 whilst the blue series show the mean monthly temperatures for the last 12 years. (Source Bureau of Meteorology, 2009)

Figure 2.4: Total monthly rainfall for Gosford (Narara Research Station). The red series indicates the rainfall for the study period of January 2008 to March 2009 whilst the blue series shows the mean monthly rainfall for past 29 years. (Source: Bureau of Meteorology, 2009)
Figure 2.3 shows that temperatures during the period of this study were similar to the 12 year average except, that the mean annual minimum temperature for the study period was slightly above the longer term average (1.5 °C higher). Figure 2.4 shows that rainfall during 2008 was highly variable compared with long term averages. During the study period, rainfall was above average for the months of February (both in 2008 & 2009), April, June and September 2008 and below average for the months of March (both in 2008 & 2009) and May 2008.

2.1.2 Fire regimes

Fire history data for the study sites were obtained from the National Parks and Wildlife Service (NPWS) database in Gosford. With the use of GIS ArcView (version 8) software, maps were created to overlay fires from different years/seasons (See Appendix A for an example of such a map) to ensure the NSW NPWS Atlas records of *D. glaucophylla* populations were actually subjected to these fires. A summary of the fire history, including frequency and time since last fire was developed for each site (Table 2.1).

Most of the fires (12/15) in Table 2.1 were wild fires as opposed to ‘prescribed’ fires which form part of the management activities in these areas. Of the prescribed fires recorded, all occurred at the Lyre Trig site and within the last 9 years. This site is the most closely located to expanding residential areas, hence the reason for prescribed fires in more recent times (i.e. asset protection by reducing fuel load).
Table 2.1: Fire history for sites in this study. Fire interval is similar for all sites while fire frequency and time since last fire differs considerably (time since last fire referenced to the year 2008). Note: Girrakool and Popran data is relevant for both ‘slashed’ and ‘unslashed’ macroplots.

<table>
<thead>
<tr>
<th>Site</th>
<th>Year of fire</th>
<th>Fire Interval</th>
<th>Time since last fire</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lyre Trig</td>
<td>1969</td>
<td>-</td>
<td>2 years</td>
</tr>
<tr>
<td></td>
<td>1977</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1987</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2000</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2006</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>mean</td>
<td>9.25</td>
<td></td>
</tr>
<tr>
<td>Rifle range</td>
<td>1965</td>
<td>-</td>
<td>5 years</td>
</tr>
<tr>
<td></td>
<td>1969</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1989</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1994</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2003</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td></td>
<td>mean</td>
<td>9.5</td>
<td></td>
</tr>
<tr>
<td>Girrakool</td>
<td>1977</td>
<td>-</td>
<td>14 years</td>
</tr>
<tr>
<td></td>
<td>1980</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1994</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td></td>
<td>mean</td>
<td>8.5</td>
<td></td>
</tr>
<tr>
<td>Popran</td>
<td>1980</td>
<td>-</td>
<td>19 years</td>
</tr>
<tr>
<td></td>
<td>1989</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td></td>
<td>mean</td>
<td>9</td>
<td></td>
</tr>
</tbody>
</table>

2.1.3 Slashing

Slashing of vegetation along the Sydney to Newcastle gas pipeline (installed in 1978) is undertaken approximately biannually (Plate 2.2). Correspondence with the company (Alinta) that survey and maintain the pipeline, including control of vegetation along its length, confirmed that slashing is performed as required with the use of handheld brush-cutters. Operators are instructed to not cut below 30 cm from the ground (this should effectively preserve *D. glaucophylla* as it does not generally grow to such heights) (Monsted & McMillan, 2007). The main aim of the slashing is to remove tree saplings (Eucalypts); the root systems of
which could interfere with the pipeline, being only 1m below the ground surface. During the period of this study (July 07 – July 09) no slashing was done at either of the pipeline sites despite the protocol mentioned above. It is important to clarify that the use of the term ‘slashing’ in this study relates to removal of vegetation greater than 30 cm tall and for the most part does not include slashing of the species of interest.

Plate 2.2: Slashed vegetation along pipeline facing south (easement approximately 10 m wide) in Popran National Park. Vast spreads of *D. glaucophylla* are evident in the centre of the photo. Pink-topped stakes can be seen. Adjacent bush to the left represents the unslashed macroplot at this site. The last fire through this site was 19 years ago (as of 2008).

2.1.4 Other site features

At each site (Table 2.2), the following general features were observed and recorded: aspect (compass), slope (clinometer), elevation (GPS and topographic contours), soil landscape characteristics (Murphy, 1993) and landform descriptions. Where possible, the Australian Soil and Land Survey Field Handbook (McDonald *et al*., 1998) was used as a guide to describe site features.
Table 2.2: Site features

<table>
<thead>
<tr>
<th>Site</th>
<th>Latitude (top marker)</th>
<th>Northing (GDA94)</th>
<th>Longitude (top marker)</th>
<th>Easting (GDA94)</th>
<th>Elevation (m) of top marker of macroplot (GPS/contour)</th>
<th>Slope (°) from top marker of macroplot to lower marker</th>
<th>Aspect</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Rifle Range</td>
<td>33°27′24″ S</td>
<td>6296747</td>
<td>151°16′34″ E</td>
<td>339799</td>
<td>176/180</td>
<td>4</td>
<td>NE 45°/SW 225°</td>
</tr>
<tr>
<td>2. Lyre Trig</td>
<td>33°27′06″ S</td>
<td>6297297</td>
<td>151°17′51″ E</td>
<td>341783</td>
<td>234/230</td>
<td>4</td>
<td>E 90°/W 240°</td>
</tr>
<tr>
<td>3. Popran (slashed/unslashed)</td>
<td>33°26′09″ S</td>
<td>6298953</td>
<td>151°13′05″ E</td>
<td>334360</td>
<td>143/120</td>
<td>4.5</td>
<td>NE 45°/SW 225°</td>
</tr>
<tr>
<td>4. Girrakool (slashed/unslashed)</td>
<td>33°25′46″ S</td>
<td>6299709</td>
<td>151°15′44″ E</td>
<td>338399</td>
<td>169/50</td>
<td>3.5</td>
<td>NE 45°/SW 225°</td>
</tr>
</tbody>
</table>

According to Murphy (1993), the Rifle Range, Lyre Trig and Girrakool sites belong to the Lambert soil landscape, having undulating to rolling hills on Hawkesbury sandstone. Slopes are typically < 20% and rocky benches are common. Soils are shallow and sandy and within a pH 3.5 - pH 5.5 range. According to Benson (1986), vegetation at these sites is classed as the 10a series consisting of open forest woodland, open scrub, open heath and sedgeland consisting of Banksia spp., Hakea spp., Grevillea spp., Kunzea spp., Dillwynia spp., Acacia spp. and Leptospermum spp. The Popran site is a little different and belongs to the Gymean soil landscape, but also has a substrate comprising of Hawkesbury sandstone and similar vegetation communities (Benson, 1986; Murphy, 1993).
Plate 2.3: Top – Rifle range site (encircled by Eucalypts, rocky ledge in foreground). Middle – Lyre Trig site (facing upslope, *D. glaucophylla* in foreground). Bottom – Girrakool slashed site along pipeline (pink-topped stakes marking quadrats visible, service track conspicuous, adjacent bush to the left represents unslashed macroplot at this site).
**Rifle Range (Plate 2.3)**

The macroplot (1000 m²), containing quadrats, was situated perpendicular to a dirt road that provides access to a popular rifle range and Scout camp. The macroplot was approximately 20 m from the road edge and therefore remained inconspicuous. The area of occupancy (Keith, 2000) was an approximately 120 m x 120 m area encircled (excluding the road) by 10 m tall clumped Eucalypt species. These, along with blackened *Banksia* spp. cones and *Xanthorrea* spp. stumps provided evidence of recent past fire (NPWS fire database records fire at this site 5 years ago). The vegetation at the site was typical of heathland scrub and the majority of this vegetation did not exceed 2 m in height. Four terraced rocky outcrops, approximately 1.5 m wide (downslope) and 10-20 m long (across the macroplot) intercepted the macroplot at this site. The downslope edge of these outcrops ranged from a 0.15 m to 0.8 m drop off, while the upslope edge provided preferential habitat for colonisation by *D. glaucophylla*.

**Lyre Trig (Plate 2.3)**

The macroplot (1000 m²) at this site was on the right-hand side of a 4 m wide vehicular access track ascending to a trigonometric station at 241 m ASL. The macroplot was orientated perpendicular to the access track and only 2 m from its edge. Despite a locked gate to prevent access to this site, a vehicle drove through almost the entire length of the macroplot in late November 2008, uprooting two *Xanthorrea* individuals, one *Banksia ericifolia*, flattening grasses and destroying some *D. glaucophylla* seedlings. This action is particularly concerning because this was the only site in the study where *D. glaucophylla* seedlings were found. Evidence of recent fire was most conspicuous at the lower end of the macroplot where 3 m tall *B. ericifolia* shrubs were blackened and devoid of foliage. Amongst these burnt *Banksia* spp, was a dense understorey of graminoids up to 1.5 m tall. It was surprising to find *D. glaucophylla* amongst this dense grassy understorey. However, rather than its usual procumbent growth form, it presented vertically (and with fewer anchorage roots on
branchlets) by using the surrounding vegetation as scaffolding. Down slope of these burnt Banksia spp. was a dense stand of Leptospermum spp. growing in damp conditions and which appeared to have been unaffected by fire. The middle section of the macroplot consisted of a less dense array of graminoids (<1m tall) and very few shrubs. The upper section of the macroplot showed evidence of lignotuber growth of eucalypts. The soil was sandy and two rocky outcrops ran, at ground level, across the width of the macroplot (approx 60 m apart) with the lower platform containing of a large patch of D. glaucophylla.

Girrakool (slashed and unslashed) (Plate 2.3)
This site contained two macroplots adjacent to one another. One was a slashed plot while the other, was unslashed. Girrakool ‘slashed’ is an approximately 10 m wide slashed area, 4-5m of which contains a service track for the Sydney to Newcastle gas pipeline and for overhead powerlines along the side of this track. The macroplot is located roughly between two earthen culverts (running across the macroplot) directing runoff from the track and into the adjacent ‘unslashed’ macroplot. These culverts remain largely unvegetated and have provided an ideal terrain/substrate for the establishment of large ant colonies. The ‘unslashed’ site consists largely of scattered Eucalyptus spp. (10 m tall), with a middle stratum of Leptospermum spp. and B. ericifolia and an understory of graminoid species and leaf litter.

Popran (slashed and unslashed) (Plate 2.1)
Similar to the Girrakool site described above, the Popran site also consists of adjacent macroplots (‘slashed’ and ‘unslashed’) where slashing provides easy access to the gas pipeline. The slashed plot is flanked by unslashed vegetation on either side, however only one of these was sampled in this study. Unlike the Girrakool site, the Popran site does not have a service track running through it, nor does it have any power lines present. Rather, a service track runs perpendicular to the macroplot at its northern boundary. This service track also serves the F3
which runs parallel to this site and is approximately 200 m east of the macroplots described here. During the course of this study, motorbikes and mountain bikes were observed using this track, despite such activities being prohibited in the National Park. Fortunately, no evidence of damage to vegetation in the slashed or unslashed macroplots at this site was observed during the course of this study. The slashed macroplot is dissected by a small creek at 50 - 60m from its northern boundary. The creek flows in a westerly direction and the channel bed consists of sand and bedrock. It varies in width from several metres (3m) to <1m. One large ant nest (2 m wide, 0.3m high) was observed in the slashed macroplot. Apart from an abundance of *D. glaucophylla*, the slashed macroplot also contains several graminoid species and young *Acacia, Grevillea, Banksia and Eucalyptus* spp. The unslashed macroplot consists of Eucalypt emergents, a canopy of large *Banksia* spp. and *Leptospermum* spp. and an understorey of dense *Gahnia* spp., other grass-like plants and leaf litter. Unslashed plots at both the Girrakool and Popran sites are dense and, in some sections, difficult to penetrate.

2.2 Overall experimental design

One of the main aims of this study is to determine whether different disturbance regimes affect populations of *D. glaucophylla* and the abiotic variables to which the species is exposed. An ideal design would have been to examine the effects of fire and slashing, and their interaction in an orthogonal design (Underwood, 1997a). This however was not possible because of the absence of particular combinations of disturbances. That is, a slashing/fire ≤ 5 years ago regime does not occur in the field. This limited the design of the study, rendering the following schema:
Figure 2.5: Experimental design showing combinations of disturbance regimes. Numbers 1 - 6 are the macroplots.

An orthogonal design was used to examine the effect of slashing at Girrakool and Popran where both sites had experienced fire $\geq 14$ years ago (that is, comparisons between macroplots 1, 2, 5 and 6 above). At these sites, macroplots were adjacent to one another, providing further justification for an orthogonal design to investigate the effect of slashing or not slashing under similar fire regimes. To test differences with respect to time since last fire, a nested design was used to incorporate all unslashed macroplots (that is, a comparison between plots 3, 4, 5 and 6 above). The nested design being appropriate here because all 4 sites were discrete from one another (Underwood, 1997a). A series of univariate, two-factor ANOVAs were conducted to test for significant differences at the disturbance level, the macroplot level and to test for any interactions between these factors.
Chapter Three: The effect of disturbance regime on population structure and reproductive characteristics of *D. glaucophylla*

3.1 Introduction

In studies concerning rare plant species, a common approach has been to investigate sets of plant characteristics which may explain rarity and patterns of distribution (Kunin & Gaston, 1993; Schwartz, 1993; Quinn *et al.*, 1994). Noble and Slatyer (1980) identified vital attributes which contribute to the continued presence of a species at a given location. These include the method of arrival or persistence, the ability of a species to establish to maturity in the face of resource competition (Craine, 2005) and the time taken to reach critical life stages before adverse disturbances may jeopardise persistence at a given location (Keith, 1996). Other studies (Coates *et al.*, 1999; Gross, 2001; Rymer *et al.*, 2005) consider factors such as phylogeny, pollinator effectiveness, site histories and disturbance regimes as critical in explaining the distribution of common or rare species. A combination approach that incorporates both individual plant attributes and species assemblage attributes may provide the more comprehensive explanation for rarity and best facilitate practical management of rare species.

An important characteristic of rare plants to consider is population structure or stand demographics. The demographics of a given plant population may vary in space and time (succession) and reflect both the attributes of the species (including its reproductive potential) and/or those of its environment, including substrate properties as well as the indirect influences of intra- and interspecific competition (Luken, 1990; Clarke *et al.*, 1996; Craine, 2005). Disturbances (e.g. fire, grazing, slashing), and the resource enrichment or decline that follows, may also affect plant populations both in terms of species composition and in the relative proportions of age cohorts. Disturbances often result in a change in the vertical stature of a population (Keith & Bradstock, 1994) and/or percentage foliage cover. The perpetual cycle of
disturbance through secondary succession to disturbance typically sees a transition from predominantly taller and larger mature individuals to shorter and smaller juveniles then back to mature individuals. Perturbations may occur as a single event or patchy continuous events that provide gaps for recruitment of new individuals, so long as the necessary recruitment cues have occurred (e.g fire breaking dormancy of seeds) (Pickett & White, 1985; Morgan, 1995; Morris, 2000).

Siddiqi et al. (1975) investigated the effects of fire disturbance on heath vegetation on the Central Coast of NSW. Changes were noted in the floristic composition and in the pattern of regrowth with both soil substrate and time since last fire contributing significantly to regrowth patterns. Less time between fire events saw a greater prevalence of lignotuberous species than obligate seeders in the plant population. In a study of Victorian coastal heath, Russell & Parsons (1978) reported rapid post-fire growth within 2 years after fire and a decline in species richness in stands older than 10 years. Commensurate with the findings of Siddiqi et al. (1975), Russell & Parsons (1978) also showed that shorter fire intervals selected vegetative regrowth over obligate seeders. The mechanism for such a trend no doubt reflects the time required for a seed bank to form, from which obligate seeders regenerate. Disruption to the replenishment of the seedbank can potentially eliminate a species from a site (Keith, 1996).

At Myall Lakes in NSW, Fox & Fox (1986; Fox, 1998) found that in post-fire vegetation, height of regrowth increased linearly with time since last fire and that understory cover re-established quickly. Species richness was high in just a few years after fire and peaked at 10 years, after which it declined. Shrub density was higher in areas burnt twice in a 12 year period compared with those areas only burnt once in 12 years. Yet again, a trend toward more vegetative resprouters than obligate-seeders was found in more frequently burnt areas.
Watson & Wardell-Johnson (2004) also demonstrated that floristic composition was affected by different fire regimes. However, species richness was not as affected and other site factors such as soil moisture may significantly affect floristics. Clarke (1988) showed that fire frequency, season of burn and post-fire weather (rainfall), significantly altered understorey structure and composition of vegetation on Hawkesbury sandstone. Clarke (1988) suggests that spring fires could effectively remove an entire season’s potential seed set and as such should be avoided. Similar logic could be applied to the timing of slashing regimes. Morrison et al. (1995) investigated the effects of fire on species composition in the Sydney region and found that fire frequency accounted for 60% of the floristic variation in sclerophyllous vegetation. Increasing time since last fire was associated with a decline in fire-tolerant species or conversely, short inter-fire periods saw a reduction in fire-sensitive species (Morrison et al., 1995).

While Australian heath flora has evolved mechanisms to cope with fire disturbances, obligate-seeders are perhaps the most vulnerable group especially if mature individuals are destroyed. This is because re-colonisation is entirely dependent on the stored seed bank whether transient or persistent; aerial or soil bound (Baskin & Baskin, 1998). Sufficient seed, seed viability, availability of an appropriate mechanism to break seed dormancy and conditions conducive to germination are all required before successful establishment and/or continuance at a given location can occur (Baskin & Baskin, 1998; Lloyd et al., 2000; Auld & Ooi, 2009). Clarke et al. (1996) investigated disturbance and competition in the post-fire environment within coastal heath. They found that ‘dry’ heath had more ‘safe sites’ for seedling establishment due to disturbance in the post-fire environment (bandicoots scratching) compared with ‘wet’ heath. They also found that inter-specific competition was low in the post-fire community and suggested this may be due to an unsaturated seed bank.
Tyler (1995) showed that in Californian chaparral, factors contributing to post-fire seedling establishment included direct heating of soil and a temporary reduction in competition or herbivory. The effect of fire on the seedling recruitment of the critically endangered Western Australian species, *Calytrix breviseta* showed positive responses to both heat and smoke treatments in field experiments (Nield *et al*., 2009). The juvenile period for this species was found to be 3 - 4 years and yet the recommended fire interval for habitats in which this species occurs has been suggested at between 15-20 years (Nield *et al*., 2009). Tierney’s (2006) study on the effects of smoke and heat on the germination of the rare plant species, *Prostanthera askania*, suggests that low intensity fires are best for the germination of this species and that smoke plays an important role in breaking germination. Auld and Ooi (2009) found that for *D. glaucophylla*, germination was cued by heat treatments of around 80 °C but that higher temperatures were lethal to seeds. The effects of smoke on this species are yet to be investigated. *Darwinia* spp. possess a persistent seed bank with some seeds released in a dormant state (Auld *et al*., 2000). Auld *et al*., (2000) showed that seeds of various *Darwinia* species have high viability, except for *D. glaucophylla* which showed variability in seed viability between sites. *Darwinia biflora* showed a short life span for seeds in soil with rapid decay over 6 months (Auld *et al*., 2000). Auld and Scott (1995) found that *Darwinia* species in the Sydney region were killed by fire and that only a few flowers and fruits appeared within 2 years after a fire event. They suggested that a fire-free period of 5 -10 years is required for many Sydney heath species in order to allow seedlings to mature and for replenishment of the soil seedbank before a second disturbance occurs (Auld & Scott, 1995; Kubiak, 2009).

Fire and its effects on flowering of heath species on the Central Coast of NSW were investigated by Pyke (1983) who found that average number of flowers per inflorescence peaked just 2 years after fire. For some resprouting species, flowering was virtually absent in
populations that had not been recently burnt (Auld, 1986; Denham & Auld, 2002). Auld (1987) and Tierney (2004) showed that for some rare Angophora spp., low levels of flowering occurred at sites of low fire frequency.

Mymercochoric interactions are beneficial to ants for the eliasome rewards (Anderson & Ashton, 1985) and for the plant as secondary dispersal provides an avenue whereby seedlings can exploit new areas. Even though the secondary dispersal distance in heath is relatively low, typically no more than 10 m from the parent plant (Myerscough, 1998; Keith et al., 2002), this movement is sufficient to reduce resource competition with the parent plant. Auld (2009) found that for some Darwinia species in Sydney heath, consumption of petals rather than a lipid body reward may be the incentive for secondary dispersal by ants which can occur within days.

Co-evolutionary relationships between terrestrial angiosperms and invertebrates have been known and partially understood for centuries. The role that invertebrates play in the pollination of flowers that leads to fruit and seed production, and the subsequent dispersal of these (Kay, 1987), is imperative to the long term survival of many plant species including those that provide humanity with much of its food. Gross et al. (2003) investigated the reproductive biology of Tetratheca juncea (a vulnerable Australian shrub with a contracted range) and found that the flower morphology was suggestive of bee pollinators but that no such pollinators were observed in proximity of the vulnerable species. With low self-fertilisation rates, the breeding system of Tetratheca juncea is reliant on insect vectors to ensure continued presence in the landscape. Perhaps a lack of suitable pollinators is a response to introduced insect generalists or to inappropriate fire regimes which may reduce the density of stands, making these less attractive to pollinators and/or reducing pollinator effectiveness (Gross et al., 2003).
Aim of this chapter – to examine population structure and reproductive characteristics of *D. glaucophylla* across disturbance regimes. To ascertain if any differences in these characteristics are significant (at the 0.05 level) between disturbance regimes, macroplots or the interactions of these.

3.2 Pilot Study

The appropriate size and number of quadrats to adequately sample percentage cover of *D. glaucophylla* was determined through a pilot study. The Rifle Range site (unslashed/fire ≤ 5 years ago) was chosen for the pilot study because of its accessibility and because it was believed to represent a site containing a ‘medium’ abundance of *D. glaucophylla*. Two quadrat sizes were used (25 m$^2$ and 1 m$^2$) and a t-test conducted to ascertain significant differences (at the 0.05 level) in percentage cover of *D. glaucophylla* between the two sizes. A significant difference was found (t = 2.33, df = 1, p = 0.03), with the smaller area reporting lower mean percentage cover of *D. glaucophylla* but less variance than the larger quadrat size (Table 3.1). The 1 m$^2$ quadrats proved most useful as it was easier to visualise and estimate percentage cover over a smaller area, easier for one operator to lay out and would also be more time efficient for future monitoring efforts (Cropper, 1993). Such a size is in keeping with Barker’s (2001) suggestion for understorey strata; the predominant vegetation type in this study. Power analysis (Foster, 2001) was conducted to determine the number of 1 m$^2$ quadrats required to detect a significant difference (at the 0.05 level) when conducting an ANOVA on percentage cover of *D. glaucophylla* (Figure 3.1). This analysis revealed a minimum number of 27 quadrats (line plateaus in Figure 3.1 at power of 0.95) to show significant difference. This is consistent with the suggestion by Barker (2001) that 30 x 1m$^2$ quadrats are sufficient for understorey strata to detect significant differences (at the 0.05 level) in abundance. Although
this number of quadrats only represents about 3% of the area of each macroplot (1000 m²), a number of studies have sampled comparable areas (Russell & Parson, 1978; Fox, 1988).

Table 3.1: Results of pilot study testing different quadrat sizes

<table>
<thead>
<tr>
<th>Quadrat Size</th>
<th>Mean % cover of D. glaucophylla</th>
<th>SD</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>1x1m</td>
<td>4.66</td>
<td>6.16</td>
<td>30</td>
</tr>
<tr>
<td>5x5m</td>
<td>10.17</td>
<td>12.07</td>
<td>30</td>
</tr>
</tbody>
</table>

Figure 3.1: Power to detect significant difference ($\alpha = 0.05$) in % cover of *D. glaucophylla* for different number of quadrats (size 1 m²).

3.3 Methods

3.3.1 Estimating abundance

The growth form of the species (prostrate, spreading and in clusters, see Plate 3.1) made counting individuals per unit area a difficult task and so percentage cover was used as a measure of abundance. Attempts to determine individuals could have potentially damaged plants as anchorage roots on branches would have to be uprooted. Percentage cover of *D.*
D. glaucophylla was determined visually. A photograph (Plate 3.1) was taken of each quadrat which may prove useful for future comparisons (see Appendix B).

Plate 3.1: (Left) Quadrat # 8 at Popran slashed (90% cover of D. glaucophylla); (Right) Quadrat # 10 at Popran slashed (25% cover of D. glaucophylla).

3.3.2 Apical growth

New apical growth on mature D. glaucophylla plants (Plate 3.2) was observed in early 2009 at four of the six macroplots. This growth was observed as a pink colour on the tips of branches and was softer and more flexible (less woody) than the rest of the branch. This feature had previously not been observed and was not part of the original set of plant features to be measured. Apical growth of 30 haphazardly selected branches at each macroplot was measured using a ruler (Plate 3.2).

Plate 3.2: Pink tips – new apical growth on D. glaucophylla
3.3.3 Seedlings

Seedlings were identified at each macroplot within the 30 randomly placed quadrats. Seedlings were defined as discrete, individually rooted plants not connected with another individual, less than 10 cm tall (or in length) and on which flowers did not develop through the duration of the study. That is, seedlings included juvenile plants that were not thought to be mature. Growth and mortality of seedlings was monitored over a twelve month period.

3.3.4 Flowering

Initially, flowering rate was to be measured by counting only newly emerged flowers with each successive visit. Flowering rate was to be monitored by marking each flower with a daub of non-toxic paint so that old flowers could be distinguished from new ones. This however proved too difficult given the progressively large numbers of flowers present (> 800 per m²). A lack of previous studies on the flowering of *D. glaucophylla*, combined with prior observations of relatively low numbers of flowers, had suggested that following flowering rates within 1 m² quadrats would be feasible. This was found not to be the case. Accordingly, rather than counting flowering rate, the total number of flowers in each quadrat during each visit was recorded. The 1m² quadrat was divided into four long strips and flowers were counted using a hand operated counter. To minimise the chance of counting flowers twice, flowers on the right-hand boundary of each strip were not counted (Elzinga *et al.*, 2001). Flowers were counted as flowers when the style had emerged from the petals and could be clearly seen. Such a definition could have contributed to an underestimation of flower numbers. Flowers lacking a style (structure is quite large and pronounced relative to the whole flower) were not counted as it was assumed these were not reproductively viable. Dead/dried flowers were not counted
either. Consistent with other studies (Auld, 1987), macroplots were visited fortnightly during peak flowering.

Insect interactions were observed in spring of 2008 at each macroplot. This was performed upon arrival at macroplots (10 am) and involved watching a quadrat with a high percentage cover of *D. glaucophylla* for 30 mins and recording the types and approximate number of different insect visits (Gross *et al.*, 2003). This variable was not intended to be a quantitative measure but rather to serve as observational/anecdotal data that may confer with the observations made by others and provide a basis for possible avenues of future investigation.

### 3.3.5 Germination experiment

The indehiscent fruits (abscised flowers containing one ‘large’ seed) of *D. glaucophylla* were collected shortly after the majority of flowering had occurred in late November and early December of 2008 (Plate 3.3). Fruits were collected with forceps from the ground at the base of plants in the 1 m² quadrats and those fruits from each macroplot were pooled. A total of about 1800 fruits were collected across four macroplots. Collection of fruit was in keeping with the NPWS scientific license specifications obtained for this project. Fruits were not collected from unslashed macroplots as insufficient fruit was available and collection of such was deemed ecologically irresponsible. Fruits were stored in paper envelopes in a cool, dry place for approximately six months until a germination experiment could be conducted. While this differs from the recommendations of Baskin & Baskin (1998) to germinate seeds as soon as possible after collection, Tierney & Wardle (2005) showed that thin-walled fruits of *Kunzea rupestris* (another Sydney region heath species) germinated after being stored for seven months.
The purpose of the germination experiment was to determine if there were any differences in seed germination across disturbance regimes. Further, the experiment aimed to ascertain what cues (smokewater and/or heat) might enhance germination. The schema presented in Figure 3.2 outlines the germination experimental design. The fruit/seed coat of half of the fruit set was pierced with a fine needle to reduce any impedance to germination imposed by this structure (Cochrane et al., 2002). The treatments chosen for the germination trials were based on previous studies (Auld, 1995; Kenny, 2000; Cochrane et al., 2002; Tierney, 2002) which showed that smoke water, heat and piercing the fruit coat enhanced germination in other species, including other Darwinia species. It was decided to use agar (15g/L) rather than filter paper in order to minimise desiccation and the need for repeated watering during the germination experiment. Twenty-five seeds per plate were set up in duplicate for each treatment (Plate 3.4). This differs from the recommended 50 seeds per plate by Baskin & Baskin (1998); however fewer numbers of seeds is acceptable for rare species (Cochrane et al., 2002). Fruit quality was not assessed at the set up stage as the only way to determine this conclusively would have been to cut open the fruit, rendering it useless for further experimentation. Swelling at the base of the fruit did not always indicate presence of seed. A germination cabinet was programmed for a 12 hour light/dark period with respective temperatures of 25 ° C and 15 ° C. It was believed that this temperature/light regime best emulated field conditions (Baskin & Baskin, 1998). The experiment ran for two weeks (Baskin & Baskin, 1998) on the advice of staff from Mt Annan Botanical Gardens and the standards set out in the International Seed Testing Association (2003). At the conclusion of the experiment, fruits were cut longitudinally with a scalpel (Plates 3.5 & 3.6) and contents assessed visually i.e the ‘cut’ test. This post-germination technique is a simple and recognised method (Baskin & Baskin, 1998; Cochrane et al., 2001; Ooi et al, 2005; Mt Annan pers. comm., 2009).
The treatments performed were:

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

A 0.1% (w/v) solution of Thiram (a fungicide) was made and buffered using 0.3 % (w/v) KH$_2$PO$_4$ and 0.06 % (w/v) KOH (Tierney, 2006). A second solution of the same was made but with the addition of a 2% commercial smoke water solution (Tierney, 2006). Fruits treated with these solutions were soaked for 4 hours (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 Thiram solution above was added (Auld & Connell, 1991; Baskin & Baskin, 1998; Tierney & Wardle, 2005). Seeds were placed equidistant on agar Petri dishes, sealed with Petri film (Plate 3.4) and placed one layer deep in the germination cabinet.
Plate 3.3: (Left) *D. glaucophylla* fruits with persistent styles, (Centre) fruits with petals and style removed, (Right) single fruit (4.5mm) from base to narrowed section at top.

Plate 3.4: (Left) *D. glaucophylla* fruits in glass petri (90mm) soaking in one of the solutions (Right) *D. glaucophylla* fruits (25) on agar (0.15% w/v) in plastic petri (90mm) sealed with Petri film.

### 3.3.6 Statistical Analysis

Statistical analysis was conducted using JMP (version 8), GMAV (Underwood, 1997b) or SPSS (version 17.0). The following analyses were performed:
1) Descriptive statistics (mean, SD, SE) were determined using JMP software. Hypothesis testing ($p \leq 0.05$) was used to determine the distribution (normal or skewed) of data using the Shapiro-Wilk ($W$) test to ensure the assumptions of ANOVA were met.

2) Univariate two-factor ANOVAs (in GMAV) were 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. The combination of disturbance regimes and macroplots in the field meant that ANOVAs were run using orthogonal and nested configurations (see Chapter 2: Overall experimental design). In both types of ANOVA, data were tested for homogeneity of variance using Cochran’s test and were transformed ($\text{arcsin } \%$ or log $n$) to improve homogeneity where necessary. ANOVA was conducted on transformed data if transformation resulted in homogenous variance. If transformation did not improve homogeneity, ANOVA was conducted on untransformed data because ANOVA are robust to departures from this assumption (Underwood, 1997a). Post hoc comparisons were made using Tukey-Krammer.

3) Repeated measures ANOVA was conducted using SPSS (v17.0) to test for differences (at the 0.05 level) in flowering over time. Post hoc comparisons were made using Scheffe (Ho, 2006). The use of repeated measures ANOVA was necessary as flowering data was not independent from one sample time to the next (SPSS, v17.0). Due to multiple comparisons, a Bonferroni type adjustment was also made for flowering data (Ho, 2006).

4) Chi-squared ($\chi^2$) tests in SPSS were used to test for significant differences (at the 0.05 level) of categorical data such as seed viability (Tabachnick & Fidell, 1996).

5) Correlations between variables were tested using non-parametric Spearman’s rho ($\rho$) as some variables were not normally distributed (Tabachnick & Fidell, 1996).
3.4 Results

3.4.1 Mean percentage cover of *D. glaucophylla*

One-hundred and eighty (180) quadrats across three disturbance regimes were sampled in this study. *Darwinia glaucophylla* was present in 59 (32.7%) of these quadrats, with the highest frequency in slashed/fire ≥ 14 years ago regimes (Table 3.2). Percentage cover of *D. glaucophylla* across quadrats ranged from 1% to 90% with a mean percentage cover of 5.2% across all quadrats. Figure 3.3 shows that mean percentage cover was significantly (F=17.7, df =2, p<0.0001) higher in the slashed/fire ≥ 14 years ago disturbance regime compared to the other two disturbance regimes (Figure 3.3 & 3.4). *Post hoc* analysis showed no significant differences between the unslashed/fire ≤ 5 years ago and the unslashed/fire ≥ 14 years ago regimes (Figure 3.3).

<table>
<thead>
<tr>
<th>Disturbance</th>
<th>Macroplot</th>
<th>% of quadrats containing <em>D. glaucophylla</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>slashed/fire ≥14 years ago</td>
<td>Girrakool slashed</td>
<td>60</td>
</tr>
<tr>
<td>slashed/fire ≥14 years ago</td>
<td>Popran slashed</td>
<td>53</td>
</tr>
<tr>
<td>slashed/fire ≤5 years ago</td>
<td>Lyre Trig</td>
<td>30</td>
</tr>
<tr>
<td>slashed/fire ≤5 years ago</td>
<td>Rifle range</td>
<td>47</td>
</tr>
<tr>
<td>slashed/fire ≥ 14 years ago</td>
<td>Girrakool unslashed</td>
<td>3</td>
</tr>
<tr>
<td>slashed/fire ≥ 14 years ago</td>
<td>Popran unslashed</td>
<td>3</td>
</tr>
</tbody>
</table>
Figure 3.3: Mean percentage (%) cover of *D. glaucophylla* within disturbance regimes. Columns with the same letter are not significantly (p ≤ 0.05) different from one another. Vertical lines show ± 1 standard error of the mean (n =60 for each disturbance regime). The effects of slashing can be seen by comparing columns 1 and 3 (orthogonal design) while the effects of fire can be seen by comparing columns 2 and 3 (nested design).

The effect of slashing or not slashing within the same fire regime (an orthogonal design) on mean percentage cover of *D. glaucophylla* was significant, with slashed areas reporting greater cover (Table 3.3 & Figure 3.3). No significant differences were detected at the macroplot level and there was no interaction between slashing and macroplot (Figure 3.4 & Table 3.3). That is, the effect of slashing was consistent between macroplots.
Table 3.3: Summary of ANOVA comparing effect of slashing on *D. glaucophylla* (orthogonal design) *p*<0.05, **p*<0.01, ***p*<0.001. γ Data was arcsin % transformed but this did not improve homogeneity of variance (Cochran’s test was significant) so ANOVA was conducted on untransformed data. φ Data transformation (log n) did not improve homogeneity of variance, so ANOVA was conducted on untransformed data.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>df</th>
<th>% cover <em>D. glaucophylla</em> γ</th>
<th>Apical growth <em>D. glaucophylla</em> φ</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>MS</td>
<td>F</td>
</tr>
<tr>
<td>Slashing</td>
<td>1</td>
<td>4036</td>
<td>20.23***</td>
</tr>
<tr>
<td>Macroplot</td>
<td>1</td>
<td>0.300</td>
<td>0.000</td>
</tr>
<tr>
<td>Slashing x Macroplot</td>
<td>1</td>
<td>20.83</td>
<td>0.010</td>
</tr>
<tr>
<td>Residual</td>
<td>116</td>
<td>199.59</td>
<td>1.04</td>
</tr>
<tr>
<td>Total</td>
<td>119</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Figure 3.4: Mean percentage (%) cover of *D. glaucophylla* within macroplots. Columns with the same letter are not significantly different from one another (at the 0.05 level). Vertical lines on columns represent ± 1 standard error of the mean (n = 30 for each macroplot). L-R: Columns 1 and 2 are macroplots with slashed/fire ≥ 14 years ago disturbance regimes, columns 3 and 4 are macroplots with unslashed/fire ≤ 5 years ago and columns 5 & 6 are macroplots with an unslashed/fire ≥ 14 years ago disturbance regime.

The effect of different fire regimes (within unslashed sites) on mean percentage cover of *D. glaucophylla* (in the nested design) showed no significant differences between those sites that had been burnt ≤ 5 years ago and those that had been burnt ≥ 14 years ago (Table 3.4 &
Figure 3.3). Mean percentage cover was not significantly different between the macroplots (nested within different fire regimes) (Table 3.4 & Figure 3.4). That is, the effect of fire was consistent between different macroplots.

Table 3.4: Summary of ANOVA comparing the effect of fire on *D. glaucophylla* (nested design) *p*<0.05, **p*<0.01, ***p*<0.001. γ Data was arcsin % transformed to improve homogeneity of variance (Cochran’s test was not significant) so ANOVA was conducted on transformed data. φ Data transformation (log n) did not improve homogeneity of variance so ANOVA was conducted on untransformed data.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>df</th>
<th>% cover <em>D. glaucophylla</em> γ</th>
<th>Apical growth <em>D. glaucophylla</em> φ</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MS</td>
<td>(F)</td>
<td>MS</td>
</tr>
<tr>
<td>Fire</td>
<td>1</td>
<td>525.7841</td>
<td>15.44</td>
</tr>
<tr>
<td>Macroplot (Fire)</td>
<td>2</td>
<td>34.0443</td>
<td>0.81</td>
</tr>
<tr>
<td>Residual</td>
<td>116</td>
<td>42.0029</td>
<td>1.6450</td>
</tr>
<tr>
<td>Total</td>
<td>119</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

3.4.2 Apical growth on mature *D. glaucophylla*

What was assumed to be new apical growth from one visit to the next, a month later, was recorded at four of the six macroplots. The two macroplots within the unslashed/fire ≥ 14 years ago disturbance regime (Girrakool unslashed and Popran unslashed) did not show obvious new apical growth during this same period (Figure 3.5). Of the quadrats containing *D. glaucophylla*, not all plants exhibited apical growth but of those that did, mean apical growth was 4.4 cm (in one month) across the four macroplots with a range between 1.9 and 10.9 cm.
Disturbance regime had a significant effect (F= 7.6, df = 2, p= 0.0007) on mean apical growth, largely attributable to the absence of such growth at the unslashed/fire ≥ 14 years ago sites (Figure 3.5). The effect of slashing or not slashing under the same fire regime (orthogonal design) on apical growth was significant (Table 3.3 & Figure 3.5). Unslashed areas did not show the level of apical growth observed in adjacent slashed macroplots. There was a significant difference in apical growth at the macroplot level (Table 3.3), where longer mean apical growth was reported at Girrkool slashed compared to Popran slashed (Figure 3.6). A significant interaction between slashing and macroplot was also found (Table 3.3), suggesting the effect of slashing is not consistent between macroplots.
Fire regime (within unslashed sites) had a significant effect (Table 3.4 & Figure 3.5) on apical growth, with those areas burnt less than 5 years ago reporting apical growth compared with those sites burnt 14 or more years ago at which no apical growth was reported. No significant differences at the macroplot level were detected for this variable (Table 3.4 & Figure 3.6).

Figure 3.6: Mean length of apical growth (cm) of *D. glaucophylla* in one month for macroplots. L-R: Columns 1 and 2 are macroplots with slashed/fire ≥14 years ago disturbance regimes, columns 3 and 4 are macroplots with unslashed/fire ≤5 years ago and columns 5 & 6 are macroplots with unslashed/fire≥14 years ago disturbance regime. Girrakool and Popran unslashed macroplots did not show apical growth of *D. glaucophylla* on mature plants.
3.4.3 Seedlings

Only 5 (0.03%) of the 180 quadrats in this study contained *D. glaucophylla* seedlings (12 total). These were identified during preliminary investigations of sites (August, 2007). All of these occurred within the same macroplot (Lyre Trig) with an unslashed/fire ≤ 5 years ago disturbance regime. Of the twelve seedlings identified at this macroplot, seven survived over a 12-month monitoring period with five being destroyed as the result of off-road vehicular damage. Those remaining recorded an average increase in height/length of 1.3 cm (range 0.7 cm to 2 cm) over a 12-month period. None of these plants produced flowers during this period. The growth rate reported here is considerably slower when compared with apical growth rates (in one month) on some mature, established plants.

3.4.4 Flowering

Of the quadrats containing *D. glaucophylla* (59), all except one produced flowers. At the height of flowering intensity, just over 9000 flowers were counted within a combined area of 58 m² in the spring of 2008. Mean number of flowers for each disturbance regime, each macroplot and each visit were calculated (Figures 3.7 & 3.8).
Repeated measures ANOVA showed no significant differences in the mean number of flowers between disturbance regimes. However, significant differences were detected over time (Table 3.5 & Figure 3.7). 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 flowering numbers between time 1 & 7. These 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). No significance was found for the interaction of time and disturbance regime (Table 3.5). A significant interaction was found between macroplot and time (Table 3.5).
Figure 3.8: Flowering over time for macroplots (Time 1 = 10/8/08, Time 7 = 6/11/08). Slashed/fire ≥14 years ago (Popran slashed Δ and Girrakool slashed ○). Unslashed/fire ≤5 years ago (Lyre Trig ▲ and Rifle Range ●). Unslashed/fire ≥ 14 years ago (Popran unslashed +). Sample sizes vary (n= 9, 14, 16, 18) with n=1 for Popran unslashed + and as such caution should be exercised. No flowers were recorded at Girrakool unslashed (no line on graph for this macroplot).

Table 3.5: Summary of ANOVAs for mean number of flowers of *D. glaucophylla* between disturbance regimes over time and between macroplots over time.

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>df</th>
<th>Flowering</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>MS</td>
</tr>
<tr>
<td>Time</td>
<td>6</td>
<td>45715.652</td>
</tr>
<tr>
<td>Disturbance regime</td>
<td>2</td>
<td>1706.958</td>
</tr>
<tr>
<td>Time x disturbance regime</td>
<td>12</td>
<td>9685.215</td>
</tr>
<tr>
<td>Residual</td>
<td>336</td>
<td>5865.229</td>
</tr>
<tr>
<td>Total</td>
<td>355</td>
<td></td>
</tr>
<tr>
<td>Time</td>
<td>6</td>
<td>49086.092</td>
</tr>
<tr>
<td>Macroplot</td>
<td>5</td>
<td>9860.473</td>
</tr>
<tr>
<td>Time x macroplot</td>
<td>30</td>
<td>10333.090</td>
</tr>
<tr>
<td>Residual</td>
<td>318</td>
<td>5587.883</td>
</tr>
<tr>
<td>Total</td>
<td>358</td>
<td></td>
</tr>
</tbody>
</table>

A comparison of slashing or not slashing on flowering (under the same fire regime), showed no significant difference (Table 3.6). However, a significant effect of time on flowering was
demonstrated (Table 3.6, Figure 3.7). No interaction between time and slashing was found, suggesting little effect of this disturbance regime on flowering. No significant differences in flowering were detected between macroplots under the same fire regime, despite the absence of flowers in the Girrakool unslashed macroplot (Figure 3.8). No interaction between macroplot and time was detected (Table 3.6).

Table 3.6: Summary of ANOVAs comparing effect of slashing (orthogonal design) on mean number of flowers of *D. glaucophylla* over time and between macroplots over time. *p*<0.05, **p*<0.01, ***p*<0.001. Data was adjusted using a Bonferroni type adjustment.

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>df</th>
<th>Flowering</th>
<th>MS</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time</td>
<td>6</td>
<td></td>
<td>25939.919</td>
<td>3.848**</td>
</tr>
<tr>
<td>Slashing</td>
<td>1</td>
<td></td>
<td>1050.238</td>
<td>0.205</td>
</tr>
<tr>
<td>Time x Slashing</td>
<td>6</td>
<td></td>
<td>836.366</td>
<td>0.124</td>
</tr>
<tr>
<td>Residual</td>
<td>204</td>
<td></td>
<td>6740.496</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>216</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time</td>
<td>6</td>
<td></td>
<td>26004.041</td>
<td>3.880**</td>
</tr>
<tr>
<td>Macroplot</td>
<td>3</td>
<td></td>
<td>5477.645</td>
<td>1.104</td>
</tr>
<tr>
<td>Time x macroplot</td>
<td>18</td>
<td></td>
<td>5180.210</td>
<td>0.773</td>
</tr>
<tr>
<td>Residual</td>
<td>192</td>
<td></td>
<td>6702.269</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>218</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Comparing flowering under different fire regimes (in unslashed macroplots) revealed no significant differences between areas burnt 5 years ago and those burnt more than 14 years ago. However, a significant difference in flowering over time was detected (Table 3.7). No significant interaction between time and fire regime was found for flowering (Table 3.7 & Figure 3.7). At the macroplot level, a significant interaction was found between macroplot and time (Table 3.7 and Figure 3.8).
Table 3.7: Summary of ANOVAs comparing the effect of time since last fire (nested design) on mean number of flowers of *D. glaucophylla* over time and between macroplots over time. *p*<0.05, **p*<0.01, ***p*<0.001. Data was adjusted using a Bonferroni type adjustment.

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>df</th>
<th>Flowering</th>
<th>MS</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time</td>
<td>6</td>
<td></td>
<td>17613.969</td>
<td>3.865**</td>
</tr>
<tr>
<td>Fire</td>
<td>1</td>
<td></td>
<td>158.865</td>
<td>0.028</td>
</tr>
<tr>
<td>Time x Fire</td>
<td>6</td>
<td></td>
<td>2483.356</td>
<td>0.545</td>
</tr>
<tr>
<td>Residual</td>
<td>138</td>
<td></td>
<td>4557.539</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>150</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time</td>
<td>6</td>
<td></td>
<td>19772.112</td>
<td>5.083***</td>
</tr>
<tr>
<td>Macroplot</td>
<td>3</td>
<td></td>
<td>12104.314</td>
<td>2.740</td>
</tr>
<tr>
<td>Time x macroplot</td>
<td>18</td>
<td></td>
<td>8540.521</td>
<td>2.196**</td>
</tr>
<tr>
<td>Residual</td>
<td>126</td>
<td></td>
<td>3889.771</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>152</td>
<td></td>
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</tr>
</tbody>
</table>

The insect interactions observed were many, ranging from non-native bees to four different ant species, through to the occasional spider. Ants were by far, the most common invertebrate visitors to quadrats containing *D. glaucophylla*. However, these were not observed removing fruit or other material from established *D. glaucophylla* plants, despite the presence of large conspicuous sandy ant mounds (2 m diameter) within the slashed macroplots at Girrakool and at Popran. At both of these macroplots, *D. glaucophylla* was growing in close proximity to these ant mounds. During spring, at the height of flowering, non-native bees were observed inserting proboscis’ into flowers of *D. glaucophylla*. At the Popran site, a single non-native bee remained at quadrat number 8 (90% cover of *D. glaucophylla*) for 10 minutes visiting *D. glaucophylla* flowers.
3.4.5 Germination

Unfortunately, the effort invested in the germination experiment was not proportional to the outcome, as none of the seeds germinated. Across the entire germination experiment, 94/1600 (5.9%) fruits contained material (creamy, grey or white; mushy to firm) with 22% of these (21/94) being potentially viable (contents firm and white, Plate 3.5). Greater that 90% of fruits were empty (Plate 3.6, Figure 3.9, Tables 3.8 & 3.9) across the four macroplots from which fruits were collected. The slashed/fire $\geq 14$ years ago areas had a slightly greater percentage of fruits containing material compared with unslashed/fire $\leq 5$ years ago areas (Table 3.8) with the Girrakool slashed macroplot contributing most to this difference (Table 3.9). The percentage of fruits containing potentially viable material, was greater in the unslashed/fire $\leq 5$ years ago plots compared with the slashed/fire $\geq 14$ years ago regimes (Table 3.8). A $\chi^2$ test revealed a significant difference ($\chi^2 = 14.8$ df = 1, $p = 0.01$) in percentage viable fruits between these two disturbance regimes.
Plate 3.5: Potentially viable (firm and white) seed inside longitudinally cut *D. glaucophylla* fruit (Magnification 40x).

Plate 3.6: Empty ovule of *D. glaucophylla* fruit (Magnification 40x). > 90% of fruits (n= 1600) were like this.
Table 3.8: Characteristics of fruit collected from different disturbance regimes assessed after completion of the germination experiment (ns = not sampled).

<table>
<thead>
<tr>
<th>disturbance regime</th>
<th>% of empty fruits</th>
<th>% of fruits containing material</th>
<th>% of fruits containing potentially viable material</th>
<th>% of fruits containing potentially viable material as a % of fruits containing any material</th>
</tr>
</thead>
<tbody>
<tr>
<td>slashed/fire ≥ 14 years ago</td>
<td>93.3</td>
<td>6.7</td>
<td>1.0</td>
<td>14.9</td>
</tr>
<tr>
<td>unslashed/fire ≤ 5 years ago</td>
<td>95.0</td>
<td>5.0</td>
<td>1.7</td>
<td>34.0</td>
</tr>
<tr>
<td>unslashed/fire ≥ 14 years ago</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
</tbody>
</table>

A significant difference ($\chi^2 = 15.7$, df = 3, p = 0.001) with respect to percentage of viable fruits was found between macroplots sampled. This difference was due largely to the Popran slashed macroplot (Table 3.9 & Figure 3.9) where no potentially viable fruits were found.

Table 3.9: Characteristics of fruit collected from different macroplots after completion of the germination experiment (ns = not sampled).

<table>
<thead>
<tr>
<th>macroplot</th>
<th>% of empty fruits</th>
<th>% of fruits containing material</th>
<th>% of fruits containing potentially viable material</th>
<th>% of fruits containing potentially viable material as a % of fruits containing any material</th>
</tr>
</thead>
<tbody>
<tr>
<td>girrakool slashed</td>
<td>90.9</td>
<td>9.1</td>
<td>1.5</td>
<td>16.4</td>
</tr>
<tr>
<td>popran slashed</td>
<td>95.7</td>
<td>4.3</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>lyre trig</td>
<td>95.3</td>
<td>4.7</td>
<td>1.5</td>
<td>31.9</td>
</tr>
<tr>
<td>rifle range</td>
<td>94.6</td>
<td>5.4</td>
<td>2</td>
<td>37</td>
</tr>
<tr>
<td>girrakool unslashed</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>popran unslashed</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
</tbody>
</table>

The germination experiment was designed to reveal responses of *D. glaucophylla* seeds to fire-related cues (smokewater and/or heat). Unfortunately, because very few seeds contained material, let alone germinated, it is not possible to report on the effects of these treatments on seed collected between different disturbance regimes. Better seed quality would need to be
obtained and the experiment repeated to ascertain effects of treatments on seed collected from different disturbance regimes and different macroplots.

Figure 3.9: Percentage (%) of fruits empty, containing material or potentially viable for the different macroplots (L-R: left columns are from the slashed/fire \( \geq 14 \) years ago, centre columns are unslashed/fire \( \leq 5 \) years ago and the unslashed/fire \( \geq 14 \) years ago sites were not sampled (ns = not sampled).
3.5 Discussion

Within its relatively small geographic distribution, *Darwinia glaucophylla* has been described as locally abundant (Dept of Environment, Climate Change and Water, 2009). While 33% of quadrats surveyed in this study contained *D. glaucophylla*, mean percentage cover was only 5%, suggesting that it is perhaps not as abundant as previously described. The patchy nature of growth in specific habitats such as rocky shelves could give the impression of local abundance (pers. ob., 2007). Large spreading mats of *D. glaucophylla* at slashed macroplots burnt 14 or more years ago are only possible and maintained because of intentional management of the overstorey. Presumably, a reduction in resource competition with more light available to the species has enabled it to thrive (*D. glaucophylla* may be shade intolerant), suggesting that some form of disturbance to the overstorey is beneficial for the long-term success of this vulnerable species. Further, slashed macroplots may emulate the reduced competition that occurs on rocky shelves, producing conditions ideal for proliferation of *D. glaucophylla*.

*Darwinia glaucophylla* was also found in areas that are not slashed and in which fire had passed through either less than 5 years ago or more than 14 years ago. Despite this difference in time since last fire, percentage cover of *D. glaucophylla* was surprisingly similar. However, frequency of occurrence was dramatically different, with unslashed plots burnt less than 5 years ago reporting a much greater number of quadrats containing *D. glaucophylla* compared to unslashed macroplots burnt more than 14 years ago. This serves to illustrate the significance of fire to this species. Further, the importance of fire for recruitment of this species can not be ignored given that it is an obligate-seeder, with heat proving an important cue for germination (Auld and Ooi, 2009). There may be seed in the soil of unslashed macroplots adjacent to slashed ones, but over time the viability and size of these seed banks may have decreased...
(Baskin & Baskin, 1998) to the point that even if these unslashed macroplots were cleared and/or burnt, *D. glaucophylla* may not regenerate. Little is known about the soil seed bank of this species, including the depths to which seed is buried or the longevity of seeds in the soil. Auld & Scott (1995) did, however, find that the viability of *D. glaucophylla* seeds varied significantly between locations and that successful germination was possible with 6-month old seed. This contrasts with the seed germination trials of the current study which will be discussed further below. While current slashing practices do not offer recruitment conditions, they do contribute to the continued success of *D. glaucophylla* individuals already along the pipeline, at least in the short term.

The two recently burnt macroplots could be described as secondary successional systems with re-sprouters apparent and an array of graminoid and understorey species typical of post-fire recruitment in heath (Keith *et al*., 2002). The relative abundance of *D. glaucophylla* at these macroplots compared with the slashed pipeline macroplots, was low, although the difference in terms of frequency was not so great. *D. glaucophylla* appears to be a pioneer species that is able to capitalize on the nutrient flush/competition-poor surrounds following disturbances such as fire. Lyre trig was burnt 2 years ago and this may explain why this macroplot was also the only plot containing any *D. glaucophylla* seedlings. The appropriate fire-related cues allowed germination of a soil seed bank at this site, resulting in emergence of seedlings within 2-3 years since the fire (Auld & Scott, 1995). This raises another question. Did the large spreads of *D. glaucophylla* around rocky outcrops at both the Rifle range and Lyre trig macroplots (recent fire), grow from seed in just 3-5 years? This seems unlikely, given the slow growth of juveniles observed under the favourable climatic conditions in this study. Although, once established, the rapid apical growth also witnessed in this present study, could see the development of vast spreads in a relatively short time (3-5 years). Alternatively, perhaps the fires at these sites were
of low intensity and patchy in nature so that mature *D. glaucophylla* plants survived. This is quite feasible given that, of the macroplots investigated in this study, Lyre Trig was the only site where fire events were controlled, hazard reduction burns. Further, the area is burnt frequently given its close proximity to residential areas. Frequent burning leads to less fuel and lower intensity fires (Bond & van Wilgen, 1996). All other macroplots in this study experienced unmanaged wildfires.

Apical growth was observed and measured in March of 2009, well after peak flowering had occurred, suggesting that plant metabolic process are seasonally assigned to allocate available resources to different plant parts. Higher than average rainfall in February 2009 combined with slightly higher than average temperatures during the preceding month of January, may explain this growth surge. The absence of apical growth at the unslashed/fire ≥ 14 years ago macroplots may have been due to the species being in very low abundance compared with other macroplots in the study and therefore the likelihood of encountering a plant with apical growth was greatly reduced. Alternatively, it may have been due to the shading effect of overstorey plants. The significant interaction between slashing and macroplot for this variable may be due to a number of reasons, namely differences in macroplot attributes such as aspect and degree of shading by adjacent vegetation.

Mean flowering response across the different sites was surprisingly similar, both in terms of mean density of flowers and progression of flowering over time, despite different disturbance regimes and populations being kilometres apart. This suggests that flowering fecundity and timing may occur in response to resource availability such as adequate photoperiod and soil moisture (Craine, 2005). However, the presence of significant interactions between macroplots and time under the various disturbance regimes suggest that any differences in mean flowering
response between macroplots depended on time. Peak flowering came earliest to the Rifle range macroplot and latest to the Girrakool slashed plot. This may also be attributable to site features such as greater shading from the taller vegetation surrounding the pipeline easement which was not an issue at either the Rifle range or Lyre Trig macroplots.

The quality of seed was poor, with very few fruits containing viable material. Some fruits had slightly bulging ovules suggesting presence of seed, but this was only verifiable by cutting them open, thus reducing available seed for germination trials. While the work here was unable to confirm whether cues of smoke, heat and scarification stimulate germination of *D. glaucophylla* seeds, it did serve to show that the viability of *D. glaucophylla* seed collected was very low. Reasons for this are unknown, however some speculative ideas include problems related to inadequate pollinators (Gross *et al*., 2003; Rymer *et al*., 2005), the role of myrmecochory (Auld, 2009) or climatic conditions prior to or during the study. The September prior to seed collection saw an above average rainfall for that month and therefore high soil moisture could have contributed the greater rates of decay of fruits/seeds.

While not directly observed carrying *D. glaucophylla* fruits, the presence of ants and large ant mounds in the vicinity of *D. glaucophylla* plants, may provide a possible explanation for a lack of viable seed. It is well documented eliasome rewards are attractive to ants and may provide an explanation for secondary dispersal by ants to underground stores (Baskin & Baskin, 1998; Auld, 2009) after fruits have been abscised (usually close to the parent). Auld (2009) suggests that petals of *Darwinia* spp. may also be attractive to ants. Ants may preferentially harvest seed-containing fruits, the fate of which is unknown, leaving under-developed or rapidly deteriorating fruits behind. However, if it is an eliasome or some other superficial reward (petals) that ants are attracted to, surely they would harvest fruits irrespective of their seed content and as such, some filled fruits should remain close to parent plants. If myrmecochory is
indeed involved in the current study, then ant-harvesting occurs relatively quickly (possibly within days to a few weeks of fruits being abscised). Auld (2009) described a period of 4-5 days in which 100% of available seed was harvested by ants. A major difference between Auld’s (2009) study and the current one was the use of bags on branches of *D. glaucophylla* to capture fruit before myrmecochory could occur. Seed collected for this study was removed from the ground at the base of plants. It was perhaps incorrectly assumed that this method of collection would suffice simply because the species is reported as an obligate-seeder and post-fire recruitment is via a stored soil seedbank (Auld & Scott, 1995). A recent study by Auld & Ooi (2009), published after the germination trials in the present study, successfully achieved 85% germination with 6 month old *D. glaucophylla* fruit. In their experiment, fruits were exposed to similar heat (80 °C) and soaking treatments as performed in this present study. Clearly the discrepancy between the results of this study and that of Auld & Ooi (2009) requires further investigation.

In conclusion, the work described in this chapter addresses hypotheses 1, 4 and 5 as stated in chapter one. Namely that the percentage cover of *D. glaucophylla* differs significantly between disturbance regimes, with slashing having a greater impact than time since last fire. Apical growth of established individuals also differs considerably with respect to disturbance, with a lack of slashing contributing to less apical growth. Seed quality appears to be marginally affected by fire regime, with slightly more fruits containing potentially viable material harvested from areas with 5 years since last fire. The results of this study raises the important question as to how much longer isolated populations along the pipeline can sustain themselves when the species has a limited lifespan of between 20-30 years (Auld & Scott, 1995) and where fire is required to break dormancy and promote germination (Auld & Scott, 1995; Auld & Ooi 2009) of the soil seed bank. Without deliberate management initiatives to promote new
genetic inputs, the persistence of such populations is more vulnerable to the effects of irregular perturbations and to the longer term effects associated with climate change; the combinations of which could increase the risk of extinction of such populations.
Chapter 4: The effect of disturbance regime on the habitat of *D. glaucophylla*

4.1 Introduction

It is widely accepted that climate and edaphic factors are the most influential in the biogeographic distribution of plants (Burrough, 1977; Myerscough, 1998; Kirkpatrick, 1999). Superimposed at smaller spatial scales are factors such as competition from surrounding vegetation and the microclimatic variations that particular assemblages create, the interactions of other biotic components such as mycorrhizal fungi and insect associations, and disturbance regimes. In a Canadian study investigating the relationship between relative abundance and species traits on an infertile ridgetop, Reader (1998) showed that three main factors, namely mycorrhizal infection, shoot mass and herbivory explained 99% of variation in abundance of grasses and forbs at disturbed sites. In Australia, Keith & Myerscough (1993) found that the floristic composition in upland swamp vegetation in the Sydney region was related to height and cover of the herbaceous stratum, to the nutrient status and moisture content of soil, and to the influence of fire regimes.

The broad effects of overstorey vegetation on understorey vegetation have been described in the previous chapter. The purpose of revisiting this theme here is to further explore the relationship between light availability and vegetation. Whelan *et al.* (2002) comments on the inability of seedlings to compete with established plants for resources (including light) unless fire or some other disturbance enhances resource supply and reduces competition. With increasing time since fire, overstorey vegetation reduces access to light for those plants in the understorey, which may reduce their fecundity and survival. Specht & Morgan (1981) found that in Australian heath, disturbances reduce the overstorey allowing increased diversity in the understorey. Keith & Myerscough (1993) showed that species richness in understorey
vegetation of upland swamps was positively related to light penetration through canopy vegetation. Prolonged exposure to shade has been implicated in high seedling mortality in some ecosystems, with moisture and fungal attack being associated factors (Keith, 1996). Davis et al. (1998), in a study of competition intensity between tree seedlings and herbaceous ground cover along a moisture/light gradient, found that tree seedling success was strongly influenced by the intensity of competition from herbaceous vegetation for water and light resources. Keith et al. (2002) suggests that within heath ecosystems, it is likely that species with low stature, intolerance for shade, short-lived seed banks and slow growth rates are most susceptible to extinction. While the studies mentioned above focus primarily on the role of vegetative competition most, by default, include the influence of soil in determining population dynamics. It is to soil that attention will now be directed in the remainder of this introduction.

In a comparison of Californian chaparral, French garrigue and Australian heath, Specht (1968) noted similarities in climate, soil and overlying vegetation. A notable exception however was the low nutrient status and rate of growth in Australian systems compared to the others (Specht, 1968). Despite greater amounts of solar radiation recorded in Australian settings, the relative lack of soil moisture impacted annual rates of growth compared with northern hemisphere counterparts (Specht, 1968). The naturally depauparate nutrient status of Australian soils, particularly in sandy heath, is well known (Groves & Specht, 1965; Siddiqi et al., 1975; Groves, 1994; Myerscough, 1998). Low phosphorus, nitrogen and moisture levels in such soils are often the limiting factors to vegetative succession and biomass development and give rise to a predominately sclerophyllous flora (McLaughlin, 1996). Despite the naturally poor nutrient status of many Australian soils, the floristic diversity on such substrates is high. Myerscough (1998) suggests this could be because low fertility provides more gaps for exploitation by a wider range of plants which have a range of adaptations for low fertility, low
water and high fire resistance. Natural seasonal fluctuations in soil nutrient availability may also influence floristic composition and rates of succession, further emphasising the importance of climate and soil attributes in determining the distribution of vegetation types (Luken, 1990).

Fire not only affects the biotic components of an area by removing them, but it also serves to change abiotic components, namely the chemistry of the soil, which in turn influences vegetation regrowth in the post-fire environment. Hobbs (2002) states that the nutrient rich ashbeds that result after fire allow for enhanced seed germination, particularly with subsequent rainfall. Further, Pickett & White (1985) report on studies that show a generally higher soil nutrient load after fire, despite volatilisation and increased runoff common during and immediately after a fire event. The nutrient flush that occurs in post-fire environments does not occur with slashing. In fact, the opposite may be true especially when biomass is removed from the site. If slashed material is allowed to decay on-site, soil nutrient status is not as compromised.

Other than neighbouring vegetation, a given plant species in a community is exposed to other biotic features of the environment. These may include association with fungi and with invertebrates. The symbiotically beneficial association that forms between soil fungi (both ectomycorrhiza and vesicular-arbuscular or VAM) and plant roots appear in many higher order plants including the Myrtaceae (Warcup, 1980; Myerscough, 1998; Chen, 2000). These greatly increase the surface area between fungi and host plants and serve to amplify the rate of transfer of nutrients and waste between the two entities (Alex et al., 1996). Mycorrhzal fungi have been used to facilitate establishment of plants at disused mine sites, the likes of which represent very disturbed areas (Gardner & Malajcuk, 1988; Alex et al., 1996). The ability of
fungi to tolerant a wide pH range contributes to their colonisation success (Wang et al., 1993). However, a narrower tolerance range to light provides added challenges both when large scale disturbances occur (e.g. slashing) and when a lack of disturbance produces conditions of over-shading (Furlan & Fortin, 1977; McGee, 1990). Although studies on the effects of fire on mycorrhizal fungi are limited, Harnett et al. (2005) showed that reduced fire frequency on African savannah, significantly increased VAM associations. Further, frequent fire appeared to heighten phosphorous levels, the presence of which deterred colonisation of mycorrhizal fungi in plant roots (Miranda et al., 1989). Pattinson et al. (1999) showed that subjecting surface soils to temperatures over 200 °C, significantly reduced the density of arbuscular mycorrhizal fungi.

**Aim of this chapter** – to examine the environmental factors influencing *D. glaucophylla* within sites subject to different disturbance regimes. To ascertain if environmental variables differ significantly (at the 0.05 level) between disturbance regimes and between macroplots. To determine if correlations exist amongst the measured environmental variables and the attributes of *D. glaucophylla* examined in chapter 3.

**4.2 Methods**

4.2.1 Estimating percentage (%) cover of associated vegetation

The term ‘associated’ vegetation will be used here to refer to a measure of the percentage vegetation cover at \( \leq 1 \text{m} \) above ground level in each \( 1 \text{m}^2 \) quadrat (including leaf litter), minus the cover of *D. glaucophylla* previously estimated (see Appendix C). For example, if total vegetation cover had been estimated at 75 \% and cover of *D. glaucophylla* had been estimated at 12\%, then the ‘associated’ vegetation cover would be 63\%. This approach was used because some quadrats consisted predominately (up to 90\%) of *D. glaucophylla* and an estimate of total vegetation cover in this case would have missed the fact that competition from associated
vegetation was minimal. In other cases the associated vegetation dominated the total vegetation cover in the quadrat and therefore provided greater competition for *D. glaucophylla*. A point worth noting here is that because of the inclusion of leaf litter, the percentage cover of associated vegetation may have been overestimated. In quadrats with overstorey vegetation ≥ 3m tall, (such as in the unslashed macroplots of Girrakool and Popran adjacent to the pipeline) projective foliage cover (PFC) was estimated visually using the crown-type plates of McDonald *et al.* (1990). All vegetation cover estimates for quadrats (n=180) were made once between August 2007 and February 2008.

4.2.2 *Mean maximum height of vegetation*

The tallest vegetation (whether a tree, shrub or herb) in each quadrat was measured using meter rulers for shorter vegetation and a surveyors extendable pole and binoculars for taller and denser vegetation. Heights were recorded in metres to two decimal places. Where height of some vegetation (e.g. of trees) exceeded that of measuring instruments, estimates (to the nearest half metre) were made using a height reference. The rationale here was to estimate the relative differences in vegetation height across the three disturbance regimes, similar to that undertaken in other studies (Fox, 1988; Keith & Bradstock, 1994; Tyler, 1995).

4.2.3 *Photosynthetically active radiation (PAR)*

Photosynthetically active radiation (‘light’) was measured at each quadrat using the averaging function on a LI-190SA quantum sensor that was within calibration. Units were recorded as µmol per second per m². PAR was always measured at 1m above the ground in the centre of each quadrat, on clear days, in early summer and between the hours of 11am and 1pm.
4.2.4 Soil attributes

Soil was collected (~300 g) from the top 10 cm of 30 randomly chosen quadrats of previously marked quadrats (i.e. 5 samples from each of the 6 macroplots) using an auger of 8 cm diameter. Limiting soil sampling to only 30 out of a possible 180 quadrats was primarily a budget constraint. Soils were collected in August of 2008, 48 hours after the last rainfall event and stored in sealed plastic bags in a refrigerator for approximately one week before analyses were conducted. Prior to removal of any sub-samples, soil was thoroughly mixed within sealed plastic bags. Two soil sub-samples of approximately 100 g each were removed from these bags, spread out on metal trays and allowed to air-dry in the laboratory for approximately one week. One of these sub-samples was used to determine pH (4A1 soil: water, 1:5) (Rayment, 1992) and electrical conductivity (3A1 soil: water, 1:5) (Rayment, 1992) while the other air-dried sub-sample was bagged and sent to Sydney Analytical Laboratories (SAL) for analysis of organic matter (AS1289.4.1), total phosphorous (mg/kg) (determined by APHA 4500BF) and total nitrogen (NH$_4^+$ and NO$_3^-$) (mg/kg) (determined by APHA 4500B). Prior to all analyses, air-dried soils were crushed and then sieved (to 150µm at SAL and < 2mm for other tests) to achieve homogeneity in the sample. Percent moisture of soil directly from the field (not air dried) was also determined using method 2B1 of Rayment (1992) to provide an estimate of the relative moisture retention properties of soil after a rainfall event. This sample was derived from the original soil stock and is referred to in this study as percentage field moisture.

4.2.5 Mycorrhizal associations

Root material was collected from four of the macroplots. It was decided not to sample the unslashed/ fire ≥ 14 years ago macroplots as the total amount of *D. glaucophylla* at these sites
was small and removal of material may have been detrimental to these plants. Sampling of quadrats was not random but targeted at those quadrats containing *D. glaucophylla*. A total of five quadrats containing *D. glaucophylla* from each of the four macroplots were sampled. A spade was used to extract an approximately 15 cm x 15 cm x 15 cm triangular block of soil/root material of *D. glaucophylla* from a selected quadrat. Extracted material was stored in sealed plastic bags in the fridge until mycorrhiza testing could be performed (i.e. about one week). Root samples were washed under running water to remove soil. Fine root material was preferentially selected and cut into 1-2 cm lengths, resulting in about 40 such fragments from each quadrat. Root material from the five quadrats within each macroplot was pooled, resulting in about 200 cuttings per macroplot. The rationale here for combining root material was to arrive at a percentage mycorrhiza colonisation for the macroplot. Individual quadrat colonisation was not the objective, but rather the effect of disturbance regime (at macroplot level) on mycorrhiza colonisation. After combining, root cuttings were divided into four sets of 50 root cuttings per glass vial. Within these vials, root material was brought to the boil in a 0.05% (w/v) trypan blue solution containing equal parts (33% each of) glycerol/water/lactic acid (Giovannetti & Mosse, 1980; Reich & Barnard, 1984; McGee, 1990). After cooling, root cuttings were rinsed in water and then decolourised with 50% glycerol (Giovannetti & Mosse, 1980; Reich & Barnard, 1984). To ascertain percentage root colonisation by mycorrhiza at the macroplot level, 10 randomly selected root cuttings from each of the four vials were placed on a glass slide in 50% glycerol and a large-sized coverslip placed over these (Giovannetti & Mosse, 1980). Under a compound microscope (magnification 100x), the ends of each root segment were investigated for presence of mycorrhiza fungi (ectomycorrhiza and/or vesicular arbuscular mycorrhiza). A distinction in the relative proportions of mycorrhiza types was not an objective in this present project. A total of 80 points were investigated across four slides (20 points x 4 slides) and a percentage of
presence of mycorrhiza (e.g. $8/80 = 10\%$) was recorded. This gave a percentage colonisation per macroplot (not per quadrat).

4.2.6 Statistical analysis

Statistical analysis was conducted using JMP (version 8), GMAV (Underwood, 1997b) and SPSS (version 17.0). The following analyses were performed:

1) Simple descriptive statistics (mean, SD, SE) were determined using JMP software. Hypothesis testing was used to determine the distribution (normal or skewed) of data using the Shipiro-Wilk (W) test to ensure the assumptions of ANOVA were met.

2) Univariate two-factor ANOVAs (in GMAV) were used to test for significant differences among means for each of the variables measured and to test for any significant interactions (at the 0.05 level). Data were tested for homogeneity of variance using Cochran’s test and were transformed (arcsin % or log n) to improve homogeneity if necessary. ANOVA was run on transformed data if transformation resulted in an insignificant C value. If transformation did not improve homogeneity, ANOVA was run on untransformed data because analyses of variance are robust to departures from this assumption (Underwood, 1997a). Post hoc comparisons were run using Tukey-Krammer in JMP or SPSS.

3) Chi-squared ($\chi^2$) tests in SPSS were used to test for significant differences (at the 0.05 level) among mycorrhizal colonisation between disturbance regimes (Howell, 2007).

4) Correlations between variables were tested using the non-parametric Spearman’s rho ($\rho$) (Howell, 2007) in JMP.
4.3 Results

4.3.1 Mean percentage (%) cover of associated vegetation

Mean percentage cover of associated vegetation at ≤ 1m above ground level across all 180 quadrats was 44% (compare this with 5.2% mean cover for *D. glaucophylla*). Significant differences between the three disturbance regimes were found for this variable, with slashed/fire ≥ 14 years ago areas, quite expectedly having lower % cover of associated vegetation than the other two disturbance regimes (Figure 4.1). Comparing areas within the same fire regime (≥ 14 years ago), mean percentage cover of associated vegetation was significantly lower in the slashed than unslashed areas (Table 4.1 & Figure 4.1). There was also a significant difference between macroplots for this variable (Table 4.1 & Figure 4.2). However, no significant interaction was found between slashing and macroplots. Within the unslashed areas, different fire regimes had no significant effect on the percentage cover of associated vegetation (Table 4.2 & Figure 4.1). However, there were significant differences between the macroplots (Figure 4.2) with Girrakool unslashed having the lowest % cover of associated vegetation.

4.3.2 Mean maximum height of vegetation

Mean maximum height of vegetation across all quadrats (n=180) was 1.7 m, but ranged from 0.15 m to 10 m. Differences with respect to mean maximum vegetation height were significant between the three disturbance regimes (F= 73.7, df=2, p<0.0001) with vegetation under a unslashed/fire ≥ 14 years ago regime being significantly taller than the other two regimes (Figure 4.3). Mean maximum height of vegetation was significantly affected by both slashing and macroplot (Table 4.1, Figure 4.3 & 4.4). Within the slashed/fire ≥ 14 years ago macroplots, vegetation height at Girrakool was significantly greater than at Popran (first two columns in Figure 4.4). No interaction was found between these two factors (Table 4.1). Among those
unslashed sites with different fire regimes, a significant difference was detected (Figure 4.3, 4.4 & Table 4.2) with maximum vegetation height being lower under the ≤ 5 years ago fire regime. There were no significant effects at the macroplot level (last four columns in Figure 4.4).

Figure 4.1: Mean % cover of associated vegetation (≤ 1m from ground) within disturbance regimes. Columns with the same letter are not significantly different from one another (at the 0.05 level). Vertical lines represent ± 1 standard error of the mean (n=60 per regime). The effects of slashing can be seen by comparing columns 1 and 3 (orthogonal design) while the effects of fire can be seen by comparing column 2 and 3 (nested design).
Figure 4.2: Mean % cover of associated vegetation (≤ 1m from ground) for macroplots. Columns with the same letter are not significantly different from one another (at the 0.05 level). Vertical lines on bars represent +/- 1 standard error of the mean (n=30 per macroplot). L to R: Columns 1 and 2 are macroplots with a slashed/fire ≥14 years ago disturbance regime, columns 3 and 4 are macroplots with an unslashed/fire ≤5 years ago disturbance regime and columns 5 and 6 are macroplots with an unslashed/fire ≥14 years ago disturbance regime.

Table 4.1: Summary of ANOVA results for the effect of slashing (under an orthogonal design) on environmental variables. Analysis was performed on untransformed data (Cochran’s test was not significant) for all variables except for % cover associated vegetation, PAR and EC. Data for these were transformed using arcsin % or ln (x). Analysis was performed on transformed data (Cochran’s was not significant for transformed data). df = degrees of freedom. *p<0.05, **p<0.01, ***p<0.001.

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<th>% cover associated vegetation at ≤ 1m</th>
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Table 4.2: Summary of ANOVA results for the **effect of fire** regimes (under a nested design) on environmental variables. Analysis was performed on untransformed data (Cochran’s test was not significant) for all variables except for % cover associated vegetation, PAR and EC. Data for these were transformed using arcsin % or ln (x). Analysis was performed on transformed data (Cochran’s was not significant for transformed data). df = degrees of freedom. *p<0.05, **p<0.01, ***p<0.001.

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Figure 4.3: Mean maximum height of vegetation within disturbance regimes. Columns with the same letter are not significantly different from one another (at the 0.05 level). Vertical lines represent ± 1 standard error of the mean (n=60 per regime). The effects of slashing can be seen by comparing columns 1 and 3 (orthogonal design) while the effects of fire can be seen by comparing column 2 and 3 (nested design).

Figure 4.4: Mean maximum height of vegetation within macroplots. Columns with the same letter are not significantly different from one another (at the 0.05 level). Vertical lines on bars represent +/- 1 standard error of the mean (n=30 per macroplot). L to R: Columns 1 and 2 are macroplots with a slashed/fire≥14years ago disturbance regime, columns 3 and 4 are macroplots with an unslashed/fire≤5years ago disturbance regime and columns 5 and 6 are macroplots with an unslashed/fire≥14years ago disturbance regime.
4.3.3 Photosynthetically active radiation (PAR)

PAR differed significantly between disturbance regimes (F= 164.1, df= 2, p <0.0001 ) (Figure 4.5) with macroplots that were unslashed and burnt ≥14 years ago having considerably lower PAR values than other areas (Figures 4.5 & 4.6). Mean PAR across all quadrats was 1823 µmol/sec/m² but ranged between 120 and 2700 µmol/sec/m² (SD = 764). There was a significant interaction between the effect of slashing/not slashing and macroplot for PAR (Table 4.1, Figure 4.6). This was most likely due to differences between the unslashed macroplots at Girrakool and Popran (see last two columns in Figure 4.6). The unslashed macroplot at Popran had the lower mean PAR values. PAR was significantly affected by different fire regimes and by macroplots nested within these fire regimes (Table 4.2). Among the unslashed macroplots, mean PAR was lower in areas burnt ≥14 years ago compared to those burnt ≤ 5 years ago (Figure 4.5). The effect of macroplot was due to PAR being similar at the two unslashed/fire ≤ 5 years ago macroplots (Lyre Trig and Rifle range), while the two unslashed/fire ≥ 14 years ago macroplots (Girrakool and Popran) were significantly different from one another (Figure 4.6, last two columns).
Figure 4.5: Mean PAR for disturbance regimes. Columns with the same letter are not significantly different from one another (at the 0.05 level). Vertical lines represent ± 1 standard error of the mean (n=60 per regime). The effects of slashing can be seen by comparing columns 1 and 3 (orthogonal design) while the effects of fire can be seen by comparing column 2 and 3 (nested design).

Figure 4.6: Mean PAR for macroplots. Columns with the same letter are not significantly different from one another (at the 0.05 level). Vertical lines on bars represent +/- 1 standard error of the mean (n=30 per macroplot). L to R: Columns 1 and 2 are macroplots with a slashed/fire ≥ 14 years ago disturbance regime, columns 3 and 4 are macroplots with an unslashed/fire ≤ 5 years ago disturbance regime and columns 5 and 6 are macroplots with an unslashed/fire ≥ 14 years ago disturbance regime.
4.3.4 Soil variables

Of the suite of soil variables investigated, most showed significant differences between disturbance regimes and/or macroplots, but no interactions were found between these two factors (Tables 4.1 & 4.2). Mean soil pH across the 30 quadrats in this study was 4.9 (acidic). ANOVA demonstrated there was a significant difference in pH between disturbance regimes (Figure 4.7) (F= 7.05, d f= 2, p=0.0037). Considering those macroplots with the same fire regime, pH was significantly higher in the slashed macroplots compared to the unslashed plots (Table 4.1 & Figure 4.8). No significant difference in soil pH was detected between unslashed plots with different fire regimes or between macroplots (Table 4.2, Figures 4.7 & 4.8)

![Figure 4.7: Mean pH for disturbance regimes. Columns with the same letter are not significantly different from one another (at the 0.05 level). Vertical lines represent ± 1 standard error of the mean (n=60 per regime). The effects of slashing can be seen by comparing columns 1 and 3 (orthogonal design) while the effects of fire can be seen by comparing column 2 and 3 (nested design).]
Mean electrical conductivity (EC) of soil solution (1:5, soil:water) was 168.4 µS/cm/sec across all quadrats investigated (n=30). Distribution of data for EC was skewed and the variances were heterogeneous. Consequently a non-parametric Kruskal-Wallis test was conducted and this showed that soil EC was significantly different between disturbance regimes ($\chi^2 = 8.15$, df=2, p=0.017) (Figure 4.9). Clearly this difference is due to lower mean EC values recorded at slashed areas burnt $\geq$14 years ago (Figure 4.9).

Tests for differences in mean soil EC among those macroplots with the same fire regime revealed a significant effect due to slashing, where slashed sites reported lower mean soil EC values compared with their unslashed counterparts. A significant difference was also found between macroplots (Table 4.1. Figure 4.10) with the Popran slashed site contributing most to this. No interaction was found between slashing and macroplot (Table 4.1). For unslashed
sites, there was no significant effect due to different fire regimes or between macroplots for EC of soil (Table 4.2, Figures 4.9 & 4.10).

![Figure 4.9: Mean EC (uS/cm/sec) for disturbance regimes. Columns with the same letter are not significantly different from one another (at the 0.05 level). Vertical lines represent ± 1 standard error of the mean (n=60 per regime). The effects of slashing can be seen by comparing columns 1 and 3 (orthogonal design) while the effects of fire can be seen by comparing column 2 and 3 (nested design).]
Figure 4.10: Mean EC ($\mu$S/cm/sec) for macroplots. Columns with the same letter are not significantly different from one another (at the 0.05 level). Vertical lines on bars represent +/- 1 standard error of the mean (n=30 per macroplot). L to R: Columns 1 and 2 are macroplots with a slashed/fire $\geq$ 14 years ago disturbance regime, columns 3 and 4 are macroplots with an unslashed/fire $\leq$ 5 years ago disturbance regime and columns 5 and 6 are macroplots with an unslashed/fire $\geq$ 14 years ago disturbance regime.

Mean percentage field moisture of soil across the quadrats investigated (n= 30) was 4%.

Significant differences in this soil variable were found among the three disturbance regimes (Figure 4.11) ($F= 4.5$, df =2, $p = 0.021$). For macroplots under the same fire regime, there was no significant difference in mean percentage field moisture of soil among slashed and unslashed macroplots (Table 4.1). However, there was a significant difference between macroplots (Table 4.1), with the slashed Popran macroplot having higher percentage soil moisture than the slashed Girrakool macroplot (Figure 4.12). It is worth noting here that the Popran macroplots had higher percentage soil moisture than Girrakool, irrespective of slashing regime (Figure 4.12). There was no significant interaction between slashing and macroplot for percentage field moisture of soil (Table 4.1).

For unslashed macroplots, fire regime had a significant effect on percentage soil moisture (Table 4.2 & Figure 4.11) with those areas burnt $\leq$ 5 years ago reporting lower soil moisture
compared with macroplots burnt ≥ 14 years ago. No significant interactions between fire regime and macroplot were found (Table 4.2, Figure 4.12).

Figure 4.11: Mean % moisture (field) for disturbance regimes. Columns with the same letter are not significantly different from one another (at the 0.05 level). Vertical lines represent ± 1 standard error of the mean (n=60 per regime). The effects of slashing can be seen by comparing columns 1 and 3 (orthogonal design) while the effects of fire can be seen by comparing column 2 and 3 (nested design).
Nitrogen (NH$_4^+$, NO$_3^-$) concentrations ranged from 230 to 880 mg/kg with a mean concentration of 470 mg/kg (within the same order of magnitude for Australian heath, Siddiqui & Carolin, 1975; McLaughlin, 1996). Significant differences (F=9.7, df =2, p=0.0008) in nitrogen levels were detected between the three disturbance regimes (Figure 4.13), with highest mean nitrogen values (700mg/kg) recorded from those areas having experienced a fire ≤ 5 years ago (Figure 4.13). For macroplots with the same fire regime, slashed plots had significantly lower nitrogen levels than unslashed plots (Table 4.1 & Figure 4.13). For unslashed macroplots, fire regime had an effect on soil nitrogen concentration that was close to significance (Table 4.2, p=0.052). No significant differences were found to exist between macroplots nor was there an interaction between slashing and macroplots found (Table 4.1 & Table 4.2, Figure 4.14).
slashing can be seen by comparing columns 1 and 3 (orthogonal design) while the effects of fire can be seen by comparing column 2 and 3 (nested design).

![Graph showing mean nitrogen concentration equivalent for macroplots](image)

Figure 4.14: Mean nitrogen (mg/kg) concentration equivalent for macroplots. Columns with the same letter are not significantly different from one another (at the 0.05 level). Vertical lines on bars represent +/- 1 standard error of the mean (n=30 per macroplot). L to R: Columns 1 and 2 are macroplots with a slashed/fire≥14 years ago disturbance regime, columns 3 and 4 are macroplots with an unslashed/fire≤5 years ago disturbance regime and columns 5 and 6 are macroplots with an unslashed/fire≥14 years ago disturbance regime.

Phosphorous levels were very similar between disturbance regimes (Figure 4.15), having a mean value of 62 mg/kg with no significant difference detected (F=1.3, df=2, p=0.289). There was no significant effect of slashing (among macroplots under the same fire regime) (Table 4.1) nor was there a significant effect of fire regime among unslashed macroplots (Figure
4.15). However, a significant difference in soil phosphorous did exist between macroplots (Table 4.2). This was probably due to the low values recorded at the Rifle range (Figure 4.16).

![Disturbance regimes](image)

**Figure 4.15:** Mean phosphorous (mg/kg) for regimes. Columns with the same letter are not significantly different from one another (at the 0.05 level). Vertical lines represent ± 1 standard error of the mean (n=60 per regime). The effects of slashing can be seen by comparing columns 1 and 3 (orthogonal design) while the effects of fire can be seen by comparing column 2 and 3 (nested design).

![Macroplots](image)

**Figure 4.16:** Mean phosphorous (mg/kg) for macroplots. Columns with the same letter are not significantly different from one another (at the 0.05 level). Vertical lines on bars represent +/- 1 standard error of the mean (n=30 per macroplot). L to R: Columns 1 and 2 are macroplots with a slashed/fire≥14years ago disturbance regime, columns 3 and 4 are macroplots with an
unslashed/fire≤5years ago disturbance regime and columns 5 and 6 are macroplots with an
unslashed/fire≥14years ago disturbance regime. Significant differences in mean percentage organic matter of soil between the three disturbance
regimes were detected (F = 4.4, df = 2, p=0.022 ), with the lowest values recorded within
slashed/fire≥14years ago macroplots (Figure 4.17). For macroplots with the same fire regime,
the slashed macroplots had significantly lower soil organic matter compared with soil sampled
from unslashed macroplots (Table 4.1, Figure 4.17). For unslashed macroplots, fire regime had
no significant effect on mean percentage soil organic matter (Table 4.2). There was however a
significant difference in soil organic matter between the two unslashed and more recently burnt
macroplots (Table 4.2), with Lyre Trig having significantly higher levels than Rifle Range
(Figure 4.18).

Figure 4.17: Mean percentage (%) soil organic matter for disturbance regimes. Columns with
the same letter are not significantly different from one another (at the 0.05 level). Vertical lines
represent ± 1 standard error of the mean (n=60 per regime). The effects of slashing can be seen
by comparing columns 1 and 3 (orthogonal design) while the effects of fire can be seen by
comparing column 2 and 3 (nested design).
Figure 4.18: Mean percentage (%) soil organic matter for macroplots. Columns with the same letter are not significantly different from one another (at the 0.05 level). Vertical lines on bars represent +/- 1 standard error of the mean (n=30 per macroplot). L to R: Columns 1 and 2 are macroplots with a slashed/fire≥14 years ago disturbance regime, columns 3 and 4 are macroplots with an unslashed/fire≤5 years ago disturbance regime and columns 5 and 6 are macroplots with an unslashed/fire≥14 years ago disturbance regime.

*Darwinia glaucophylla* was found to be mycorrhizal with a mean percent colonization of 22% across four macroplots. Both ectomycorrhiza and vesicular arbuscular mycorrhizal (VAM) fungi were found in association with the roots of *D. glaucophylla* (Plate 4.1 & 4.2). The highest percentage mycorrhizal colonization occurred at Popran (32%) and the lowest (10%) at Lyre trig (Figure 4.19). It was not possible to examine the effects of time since last fire or slashing, as the unslashed/fire≥14 years ago macroplots were not sampled due to low abundance of *D. glaucophylla*. Chi-squared ($\chi^2$) tests showed that percentage mycorrhizal colonization of those plots sampled was significantly ($\chi^2 = 11.5$, df=1, $p=0.001$) higher in the slashed/fire≥14 years ago regime compared to the unslashed/fire≤5 years ago regime. There was also a significant difference between macroplots ($\chi^2 = 12.3$, df =3, $p=0.007$), which was most likely attributable to low mycorrhizal colonisation at Lyre Trig.
Figure 4.19: Percentage mycorrhizal colonisation for the two slashed/fire ≥ 14 years macroplots (Girrakool and Popran) and for the two unslashed/fire ≤ 5 years macroplots (Lyre Trig & Rifle Range). See text above for significant differences.

Plate 4.1: Ectomycorrhiza hyphae on *D. glaucophylla* roots (40x magnification)
4.3.5 Correlations between investigated variables

Table 4.3 shows only the significant correlations between variables investigated in chapters 3 and 4 (see Appendix D for full correlation table). Several variables were significantly correlated with quadrats containing *D. glaucophylla* and with percentage cover of the species (Table 4.3). Mean maximum height of vegetation, percentage cover of associated vegetation, soil moisture content and soil EC were all associated with fewer quadrats containing *D. glaucophylla* and with lower percentage cover of the species (Table 4.3). Conversely, significant positive correlations existed between percentage cover of *D. glaucophylla* and PAR, apical growth, presence of *D. glaucophylla* seedlings, percentage mycorrhizal colonistaion and pH. Although some of these correlations were significant, some correlation co-efficients were small, indicating that the relationship between some of the variables was weak (e.g. quadrats containing seedlings and average apical growth) (Table 4.3).
Mean apical growth of *D. glaucophylla* was significantly positively correlated with PAR and negatively correlated with height of vegetation (Table 4.3). The greater the percentage cover of the species, the greater was mean apical growth. This was most likely due to the fact that the presence of more mature/established (thriving) plants favoured apical growth. None of the soil variables were significantly correlated with apical growth.

The presence of *D. glaucophylla* seedlings was significantly and positively correlated with soil EC, N, P and percentage organic matter (Table 4.3). The presence of *D. glaucophylla* seedlings was negatively correlated with degree of mycorrhizal associations.

Low seed viability was significantly correlated with higher percentage mycorrhizal colonisation and with greater mean apical growth of *D. glaucophylla* (Table 4.3). High seed viability was also significantly associated with greater mean maximum vegetation height, greater cover of associated vegetation and higher total nitrogen. The positive correlation of seed viability with mean maximum height may be due to the fact that mean maximum height in the slashed/fire≥14years areas was 0.7 m and 1.2 m in the unslashed/fire≤5years areas. That is, seed viability is higher were fire has been more recent. The tallest mean vegetation height (3 m) was not included for reasons previously mentioned.

Percentage mycorrhizal colonisation was negatively correlated with both height of vegetation and percentage cover of associated vegetation. Soil pH conditions were positively correlated while EC, nitrogen and percentage organic matter of soil were all negatively correlated with percentage mycorrhizal colonisation.
Among the soil variables, several significant correlations exist (Table 4.3). pH was negatively correlated with soil moisture, nitrogen and organic matter. Conversely, EC was positively correlated with soil moisture, nitrogen and organic matter.
Table 4.3: Correlations of significance across some of the variables from chapters 3 and 4. Flowering was not included in these correlations because 58 out of 59 quadrats containing *D. glaucophylla* presented with flowers.

Presence/absence and relative abundance of the species against other variables were considered the more important correlations (For full correlation table see Appendix D) *p<0.05, **p<0.01, ***p<0.001.

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<td>**</td>
</tr>
<tr>
<td>quadrats containing <em>D. glaucophylla</em> seedlings % cover of <em>D. glaucophylla</em></td>
<td>0.1783</td>
<td>*</td>
</tr>
<tr>
<td>quadrats containing <em>D. glaucophylla</em> seedlings Average apical growth (cm)</td>
<td>0.1506</td>
<td>*</td>
</tr>
<tr>
<td>% mycorrhiza colonisation quadrats containing <em>D. glaucophylla</em></td>
<td>0.1866</td>
<td>*</td>
</tr>
<tr>
<td>% mycorrhiza colonisation % cover of <em>D. glaucophylla</em></td>
<td>0.2854</td>
<td>**</td>
</tr>
<tr>
<td>% mycorrhiza colonisation Mean max. height of vegetation (m)</td>
<td>-0.5256</td>
<td>***</td>
</tr>
<tr>
<td>% mycorrhiza colonisation % cover associated vegetation</td>
<td>-0.4272</td>
<td>***</td>
</tr>
<tr>
<td>% mycorrhiza colonisation quadrats containing <em>D. glaucophylla</em> seedlings</td>
<td>-0.2798</td>
<td>**</td>
</tr>
<tr>
<td>% viable seeds Mean max. height of vegetation (m)</td>
<td>0.6365</td>
<td>***</td>
</tr>
<tr>
<td>% viable seeds % cover associated vegetation</td>
<td>0.2893</td>
<td>**</td>
</tr>
<tr>
<td>% viable seeds Average apical growth (cm)</td>
<td>-0.3162</td>
<td>***</td>
</tr>
<tr>
<td>% viable seeds % mycorrhiza colonisation</td>
<td>-0.6325</td>
<td>***</td>
</tr>
<tr>
<td>pH quadrats containing <em>D. glaucophylla</em></td>
<td>0.4110</td>
<td>*</td>
</tr>
<tr>
<td>pH % cover of <em>D. glaucophylla</em></td>
<td>0.5578</td>
<td>**</td>
</tr>
<tr>
<td>pH Mean max. height of vegetation (m)</td>
<td>-0.4420</td>
<td>*</td>
</tr>
<tr>
<td>pH % mycorrhiza colonisation</td>
<td>0.6561</td>
<td>**</td>
</tr>
<tr>
<td>EC (µS/cm) % cover of <em>D. glaucophylla</em></td>
<td>-0.4112</td>
<td>*</td>
</tr>
<tr>
<td>EC (µS/cm) quadrats containing <em>D. glaucophylla</em> seedlings</td>
<td>0.4218</td>
<td>*</td>
</tr>
<tr>
<td>EC (µS/cm) % mycorrhiza colonisation</td>
<td>-0.6947</td>
<td>***</td>
</tr>
<tr>
<td>EC (µS/cm) pH</td>
<td>-0.5740</td>
<td>**</td>
</tr>
<tr>
<td>% moisture (field) quadrats containing <em>D. glaucophylla</em></td>
<td>-0.3746</td>
<td>*</td>
</tr>
<tr>
<td>% moisture (field) pH</td>
<td>-0.3850</td>
<td>*</td>
</tr>
<tr>
<td>% moisture (field) EC (µS/cm)</td>
<td>0.4848</td>
<td>**</td>
</tr>
<tr>
<td>% moisture (field) % mycorrhiza colonisation</td>
<td>0.4661</td>
<td>*</td>
</tr>
<tr>
<td>% moisture (field) % viable seeds</td>
<td>0.4802</td>
<td>*</td>
</tr>
<tr>
<td>% moisture (field) N (mg/kg)</td>
<td>0.5872</td>
<td>**</td>
</tr>
<tr>
<td>N (mg/kg) quadrats containing <em>D. glaucophylla</em> seedlings</td>
<td>0.4661</td>
<td>*</td>
</tr>
<tr>
<td>N (mg/kg) % mycorrhiza colonisation</td>
<td>-0.8255</td>
<td>***</td>
</tr>
<tr>
<td>N (mg/kg) % viable seeds</td>
<td>0.4802</td>
<td>*</td>
</tr>
<tr>
<td>N (mg/kg) pH</td>
<td>-0.5189</td>
<td>**</td>
</tr>
<tr>
<td>N (mg/kg) EC (µS/cm)</td>
<td>0.6253</td>
<td>***</td>
</tr>
<tr>
<td>N (mg/kg) % moisture (field)</td>
<td>0.5872</td>
<td>**</td>
</tr>
<tr>
<td>P (mg/kg) quadrats containing <em>D. glaucophylla</em> seedlings</td>
<td>0.3941</td>
<td>*</td>
</tr>
<tr>
<td>% OM quadrats containing <em>D. glaucophylla</em> seedlings</td>
<td>0.4081</td>
<td>*</td>
</tr>
<tr>
<td>% OM % mycorrhiza colonisation</td>
<td>-0.7276</td>
<td>***</td>
</tr>
<tr>
<td>% OM pH</td>
<td>-0.3790</td>
<td>*</td>
</tr>
<tr>
<td>% OM % mycorrhiza colonisation</td>
<td>0.6102</td>
<td>**</td>
</tr>
<tr>
<td>% OM % moisture (field)</td>
<td>0.6781</td>
<td>***</td>
</tr>
<tr>
<td>% OM N (mg/kg)</td>
<td>0.8882</td>
<td>***</td>
</tr>
</tbody>
</table>
4.4 Discussion

The results obtained from the variables investigated in this chapter serve to illustrate how different types of disturbance, namely fire or slashing have altered the heath habitat of *D. glaucophylla* in different ways. These changes are, in turn, influential in the life cycle of *D. glaucophylla*.

Slashing has resulted in a habitat with less cover, vegetation of lower stature, greater light penetration, denser mycorrhizal colonisation and soil substrate properties having lower EC, lower nitrogen, lower organic matter and lower moisture levels when compared with unslashed areas. Unslashed areas, however, have been subject to fire at different times in the past. Surprisingly, different times since last fire have resulted in habitats with fewer differences between them when compared to slashed areas. However, those areas burnt less than five years ago resemble slashed habitats 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. It is likely that a taller canopy of relatively broader foliage, supplies leaf litter to the ground which, combined with less sun exposure, provides a mulching effect to retain soil moisture in unslashed areas burnt more than a decade ago.

It is not surprising that percentage cover of vegetation associated with *D. glaucophylla* was lowest at slashed macroplots given that the primary objective of slashing is to reduce the amount of vegetation growing above the pipeline for a number of reasons (utilities access, fuel reduction, aerial survey). Among the unslashed macroplots, difference in time (5 years or 14 years) since last fire had less of an impact on percentage cover of associated vegetation compared to slashing. Among the unslashed macroplots burnt over a decade ago, Popran had
significantly greater percentage cover of associated vegetation in the understorey compared with the Girrakool macroplot. This may be explained by the presence of thickets of *Gahnia* spp. growing along the creek line within the Popran macroplot.

In Australian heath biomes, a post-fire environment is conducive to high rates of growth at the ground level in the absence of overstorey competition (Pickett & White, 1985; Hobbs, 2002 in Bradstock *et al.*, 2002). As secondary succession progresses, overstorey shading limits light penetration, suppressing the understorey (Specht & Morgan, 1981). Consistent with this trend, the present study found that lower PAR values (as with overstorey shading) were associated with taller mean heights of vegetation. An interesting result at the unslashed Popran macroplot revealed that while mean maximum height of vegetation was not the tallest recorded this plot did record the lowest mean PAR values in the study. This could be related again to the presence of *Gahnia* thickets and thus serves to illustrate the influence of floristic composition in creating habitat mosaics.

The soil variables investigated in this study are all inter-related and therefore it is difficult to isolate the importance of these in terms of favourable habitat for *D. glaucophylla*. However, some points of interest regarding soil variables are noted here. Soil pH values were within the range typically found in heath on Hawkesbury sandstone (Murphy, 1993). Slashed macroplots reported significantly higher soil pH values and lower EC values compared to their unslashed counterparts. The soil of slashed macroplots also had lower nitrogen levels and less organic matter compared with unslashed macroplots. The results obtained in this study showed no significant difference in soil nitrogen levels between unslashed macroplots under different fire regimes. This may be the result of post-fire plant uptake or perhaps even leaching, as these soils are very sandy.
The soil properties of slashed macroplots described above are likely to be the result of removal of slashed biomass from the pipeline easement which reduces re-entry of nutrients into soils within these areas. Removing slashed plant matter minimises fire hazards and improves visibility of the easement. It was noted at the Girrakool site, that small lopped trees from the slashed portion of the site were pushed to the edges of the easement. Further, this site has a service track running through it, no doubt with the dual purpose of servicing overhead powerlines and to access a gas/oil pipeline viewing station within the vicinity. From time to time, such a track would also require grading. This action, combined with vehicular access would contribute to soil compaction and more bare ground exposed to insolation, both factors contributing to a reduction in soil moisture content. While some of the lowest mean soil moisture values were recorded at the slashed Girrakool macroplot, this was not the case for the other slashed macroplot at Popran. The presence of a creek at the Popran site may contribute toward an explanation for this result. Overall, the soil conditions at slashed macroplots are inter-related in that lower moisture and nutrient levels do not provide the necessary medium for movement of ions, resulting in the lower EC values recorded at these macroplots.

The mycorrhizal status of this species had not previously been investigated and the observations recorded in this study provide preliminary data for future investigation of fungal associations with *D. glaucophylla*. An understanding of these associations may contribute to the preservation and success of the species both in situ and in areas to which the species may be translocated.
The presence of mycorrhizal fungi in heath habitat is expected, as such associations tend to form in low nutrient soils with a pH range of between 4 to 7 (Alex et al., 1996). Mycorrhiza colonisation was greatest in slashed macroplots and lowest in unslashed macroplots burnt within the last 5 years (unslashed/fire ≥ 14 years ago macroplots could not be sampled for mycorrhiza). The higher frequency and greater mean percentage cover of *D. glaucophylla* in slashed macroplots could explain higher mycorrhizal colonisation compared with more recently burnt macroplots. Light availability promotes photosynthesis in the host plant, thus providing heterotrophic fungi with adequate carbohydrate reserves which contributes to successful and higher colonisation levels (Furlan & Fortin, 1977; McGee, 1990 & Alex et al., 1996) in slashed macroplots compared with unslashed plots.

Fire adversely affects density of mycorrhizal fungi (Miranda et al., 1989) and post-fire re-colonisation of plant roots takes time as fungal spores from neighbouring stands arrive to recolonise burnt stands or as soil surface fungal densities are re-instated from subsurface stocks (Alex et al., 1996; Pattinson et al., 1999). An apparent contradiction here is that the recently burnt macroplot (Lyre Trig) was the only macroplot to contain seedlings of *D. glaucophylla* yet the presence of mycorrhiza fungi is reported to be a key factor in the successful establishment of juveniles in many species (Alex et al, 1996). The post-fire flush of nitrates and phosphates may be more influential in germination (Bell et al., 1999), seedling establishment and regrowth of *D. glaucophylla* than mycorrhizal associations. Other studies (Furlan & Fortin, 1977; Miranda et al.; 1989) have shown that higher nutrient levels (particularly of phosphates) are not commensurate with the establishment of mycorrhizal fungi and that these fungal associations may only benefit the plant host some time after a fire disturbance.
Chapter 5: Discussion

5.1 Overall discussion and conclusions

*Darwinia glaucophylla* is a vulnerable Central Coast endemic having high habitat specificity and currently covering a small geographic range (Dept of Environment, Climate Change and Water, 2009). The species is of low stature, is an obligate-seeder with a low seed dispersal range and requires fire to stimulate germination (Auld & Ooi, 2009) and recruit new individuals into existing disjunct populations. This set of attributes, is likely to have contributed to the vulnerable status currently afforded the species. However, plant traits alone do not contribute to the rarity of species. Environmental factors, particularly catastrophic events and anthropogenic disturbances are influential in the demise of many species (Kirkpatrick, 1999; Lindenmayer & Fisher, 2006) particularly when such disturbances are also threatening processes (Dept of Environment, Climate Change and Water, 2009).

Coates et al. (1999) investigated reasons for rarity of two Tasmanian shrubs. They concluded that rarity was not related to limited habitat, but rather to the set of life history strategies possessed by the two species. One of the species is an obligate seeder with low seed dispersal, low tolerance for fire and for tall overstorey vegetation. This set of attributes is similar to that of *D. glaucophylla*. The other Tasmanian species investigated by Coates et al. (1999), also performed unfavourably in the presence of a dense overstorey, even though it is a post-fire resprouter rather than an obligate-seeder.

Plants may be directly or indirectly affected by disturbances. One of the disturbances to which *D. glaucophylla* is currently indirectly exposed is that of slashing of overstorey vegetation
along the Sydney to Newcastle gas/oil pipeline. Slashing may emulate conditions similar to those around rocky platforms where the species occurs naturally and where competition is low and drainage is good. Slashing or mowing under powerlines 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 the case where biomass is removed, this adversely affects the nutrient status of the substrate and may account for low organic matter and soil nitrogen levels along the pipeline as recorded in this current study. Of possible greater influence to soil properties along the easement in this study may be the initial laying of the pipeline in 1978 (32 years ago). Given the fragility of Hawkesbury sandstone, soil disturbance of this magnitude may take many decades to recover to pre-disturbance conditions, if at all.

The effects of removal of vegetation cover on seedling emergence were demonstrated by Reader (1993) who showed that in plots from which cover had been deliberately removed, recruitment of seedlings was significantly greater, when compared with uncleared plots. Williams et al. (2005) found that seedling emergence in monitored Australian savanna mostly occurred when rain followed a fire event or with the removal of biomass. Investigations of coastal heath by Siddiqi et al. (1972) not only illustrated the effect of substrate (particularly depth of water table) on floristics, but also the effect of competition/inhibition on shorter stature vegetation due to the presence of a taller over-storey.

Unfortunately traditional slashing practices result in increased edge effects and reduce habitat for a host of species. A possible solution for managers of such areas is provided by Clarke et al. (2006) who suggest that allowing some degree of succession within easements provides structural variation in the landscape which is favourable to small mammals. In the current study, variety in structural complexity under powerlines and along the pipeline easement was
noted. Managers of these areas may be aware of the value of retaining some vegetation along easements.

Some populations of *D. glaucophylla* (notably those in slashed areas and where the last fire occurred 14 years ago) may not be self-sustaining in the long term because current management practices do not promote conditions conducive to recruitment of the seed bank and the longevity of the seedbank is unknown. Current slashing practices along the Sydney to Newcastle gas/oil pipeline, while promoting growth of existing plants, are unlikely to provide conditions necessary for germination that accompany fire. Those areas where fire has occurred more recently saw a greater density of seedlings compared with unburnt sites. However, further aspects of the fire regime, other than merely time since last fire, need to be investigated to ascertain an appropriate long term fire regime.

Disturbances of fire and slashing appear to have some positive effects on *D. glaucophylla* (Table 5.1). While *D. glaucophylla* is a vulnerable species, it is also a disturbance-dependent species where disturbance to the plant (or parts of it such as heat required to germinate seeds) and its surrounds are required for its success. The very low frequency of *D. glaucophylla* in unslashed macroplots with a long time since last fire serves to confirm its need for disturbance. Tall overstorey vegetation that reduces light levels to the understorey in these macroplots is one of the limiting factors contributing to the low representation of the species in these locations.

Habitat preference could account for the rapid apical growth observed on plants exposed to open sky such as in slashed areas, those burnt more recently and those around rocky shelves. Apical growth appears to be seasonal and occurs in response to excess moisture, which when
combined with warm summer temperatures, sees the species exploit the abundance of these resources to expand its range in favoured habitat.

Table 5.1: Summary of biotic and abiotic conditions within the different disturbance regimes to which *D. glaucophylla* is exposed. Note the terms ‘high’ or ‘low’, as used here, are relative measures derived from an average of the two macroplots representing each disturbance regime.

* This result is higher than expected as is likely due to the influence of a creek at the Popran site.

<table>
<thead>
<tr>
<th>Slashed/fire ≥14 years ago</th>
<th>Unslashed/fire ≤ 5 years ago</th>
<th>Unslashed/fire ≥ 14 years ago</th>
</tr>
</thead>
<tbody>
<tr>
<td>- High frequency of quadrats containing <em>D. glaucophylla</em></td>
<td>- Low frequency of quadrats containing <em>D. glaucophylla</em></td>
<td>- Low frequency of quadrats containing <em>D. glaucophylla</em></td>
</tr>
<tr>
<td>- High % cover <em>D. glaucophylla</em></td>
<td>- Low % cover <em>D. glaucophylla</em></td>
<td>- Low % cover <em>D. glaucophylla</em></td>
</tr>
<tr>
<td>- High apical growth</td>
<td>- High apical growth</td>
<td>- No apical growth</td>
</tr>
<tr>
<td>- No seedlings</td>
<td>- Seedings present</td>
<td>- No seedlings</td>
</tr>
<tr>
<td>- Similar flowering density</td>
<td>- Similar flowering density</td>
<td>- Similar flowering density</td>
</tr>
<tr>
<td>- Low seed viability</td>
<td>- Higher seed viability</td>
<td>- No seeds collected</td>
</tr>
<tr>
<td>- Low % cover veg in understory</td>
<td>- High % cover veg in understory</td>
<td>- High % cover veg in understory</td>
</tr>
<tr>
<td>- No overstorey</td>
<td>- No overstorey</td>
<td>- Overstorey present</td>
</tr>
<tr>
<td>- Low stature vegetation</td>
<td>- Low stature vegetation</td>
<td>- Tall stature vegetation</td>
</tr>
<tr>
<td>- High PAR</td>
<td>- High PAR</td>
<td>- Low PAR</td>
</tr>
<tr>
<td>- Mean pH 5.1</td>
<td>- Mean pH 4.9</td>
<td>- Mean pH 4.8</td>
</tr>
<tr>
<td>- Low EC</td>
<td>- High EC</td>
<td>- High EC</td>
</tr>
<tr>
<td>- Medium soil moisture (field)*</td>
<td>- Low soil moisture (field)</td>
<td>- High soil moisture (field)</td>
</tr>
<tr>
<td>- Low N</td>
<td>- High N</td>
<td>- High N</td>
</tr>
<tr>
<td>- Similar P</td>
<td>- Similar P</td>
<td>- Similar P</td>
</tr>
<tr>
<td>- High % OM</td>
<td>- High % OM</td>
<td>- High % OM</td>
</tr>
<tr>
<td>- Lower mycorrhiza colonisation</td>
<td>- Insects observed</td>
<td>- Mycorrhiza not sampled</td>
</tr>
<tr>
<td>- Insects observed</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Disturbance regime had a significant effect on both the attributes of *D. glaucophylla* and its habitat. Table 5.1 shows that there were more similarities in both plant characteristics and habitat features under the two unslashed disturbance regimes than between the slashed and unslashed regimes. By far, slashing resulted in the greatest frequency and percentage cover of the species than unslashed areas, despite the different times since last fire represented in this study. *Darwinia glaucophylla* possesses characteristics consistent with shade intolerance. Its relative success along the slashed pipeline easement and on edges of rocky platforms suggests adaptations to well drained sites, reduced soil organic matter, relatively low nitrogen levels, lower soil moisture levels and higher mycorrhiza colonisation. Marginally higher seed viability in unslashed areas burnt relatively recently, may be a due to the influence of fire, but may also
be related to lower soil moisture levels. The soft-coated fruits of *D. glaucophylla* may be more susceptible to decay in moist environments. Greater mean nitrogen levels in recently burnt areas may contribute to more successful establishment of seedlings in these locations (Bell *et al*., 1999).

Some of the abiotic traits of soil differed significantly across disturbance regimes, while others did not. Most notably, phosphorous levels were consistent across regimes and macroplots. Phosphorus plays a pivotal role in determining plant distribution (Beadle, 1962). However, the result here suggests that phosphorous status of the soil may not play as significant a role in abundance of *D. glaucophylla* as other factors such as disturbance regimes and the microclimatic conditions that arise from these. This is exemplified by the soil condition at slashed sites in this study where EC, N and OM levels were all lower when compared with unslashed sites burnt either 5 or 14 years ago. These three soil variables are clearly linked. Removal of biomass reduces organic matter in soil, which also reduces available nitrate and ammonium ions, contributing to lower electrical conductivity of soil water (1:5).

The irony in this study is that despite the seemingly disadvantageous conditions created by the effects of putting in a pipeline and the subsequent management practise of slashing, *D. glaucophylla* appears to thrive in such locations. Its preference for reduced light competition and its ability to exploit the niche created by slashing has perhaps been its saviour. This is particularly significant given the limited sites of ‘natural’ habitat on the edges of rocky platforms where it occurs. However, the long term sustainability of these pipeline populations is an issue that requires further investigation for reasons previously discussed.
Disturbance regimes significantly impact populations of *D. glaucophylla* on the Central Coast of NSW with slashing rather than time since last fire (for those regimes presented here) contributing to greater cover in the short term. Slashed areas and those burnt relatively recently present less vegetation competition and more light resources for potentially emerging sub-shrubs such as *D. glaucophylla*. *Darwinia glaucophylla* appears to be shade and moisture intolerant. It was also found to be mycorrhizal. Seeds that are dispersed to, and spend a short time on the soil surface are of poor quality with very few containing any material, let alone potentially viable material. The reason for this is unknown but ants may selectively remove viable seeds; the fate of which is unknown. Mature/established *D. glaucophylla* plants exhibit a seasonal period of rapid growth, while seedlings grow very slowly. Seedlings only appear in more recently burnt plots. Flowering numbers are huge but follow a similar pattern over time despite different disturbance regimes and locations.

### 5.2 Management implications and recommendations

The aim of NPWS is, through research-based management, to have self-sustaining populations of *D. glaucophylla*. To achieve this, it is suggested that the three recommendations outlined below should be considered. Ideally, items for future research included in this chapter would precede implementation of these recommendations. However, should time and resources be limited, the following recommendations may still be considered. An additional goal of having the species expand into suitable habitat across its range may also follow from the use of the management activities suggested here. Implementation of these and other measures will hopefully result in the removal of the species from the ROTAP list and away from vulnerable status on the NSW TSC Act.
1) Burn the Rifle Range site some time within the next 2-3 years. The last fire at this site occurred during the 2002/2003 fire season and was unprescribed. This action will be in keeping with the 5-10 year minimum interval between fires as suggested by Auld & Scott (1995) for the species. Permanent quadrats should be re-examined post-fire to monitor impacts before subjecting other areas to a similar regime. Fire intensity should be monitored in light of the germination requirements of the species. Fire intensities that result in soil temperatures outside of the 80 – 100 ° C range are ineffective as germination cues (Auld & Ooi, 2009).

2) Reinstate an appropriate fire regime along sections of and adjacent to the pipeline. The Popran site has not experienced a fire event in 20 years and with a life-span of around 20 - 30 years (Auld & Scott, 1995) for this species, a fire recruitment event is imperative. A staged approach should be considered so that post-fire monitoring can be assessed prior to burning larger tracts of habitat. Collaboration with the managers of the gas/oil pipeline would be required to establish the feasibility risks associated with burning in proximity of the pipeline. A possible alternative to fire could be the use of smoke, although this would have to be investigated and trialled at small-scales in the field. The Girrakool pipeline has low-hanging overhead powerlines and consequently, the use of fire at this location may be limited by this infrastructure.

3) In their assessment of the biodiversity along the Sydney to Newcastle pipeline Monsted & McMillan (2007) concluded that current slashing practices (hand-held cutter 30 cm above ground) in habitats containing *D. glaucophylla* are not adversely affecting the species. The findings of this project confer with the recommendations of Monsted & McMillan (2007) who suggest that widening the easement to standardise the width along the length of the pipeline is feasible if done so in the same manner currently practised. Further slashing to widen the pipeline easement would reduce shading by overstorey vegetation and possibly promote
expansion of existing stands of *D. glaucophylla*, as apical growth as observed in this study was greater at slashed compared with unslashed sites. Slashing may be a short to medium term solution to retain current populations of *D. glaucophylla*, but all the evidence points to fire (and/or smoke) being required for recruitment. This is consistent with the presence of seedlings only at the most recently burnt Lyre Trig site. Perhaps pipeline sites would benefit from occasional, controlled burning (provided it is safe) rather than slashing in order to restore soil nutrients and provide necessary cues for germination of the soil seed bank. Close post-fire monitoring would confirm any benefits.

5.3 Future research

It seems that a study of this sort often results in more questions asked at its conclusion than were addressed at its inception. A prioritised set of possible avenues for future research is suggested below, the answers to which will better inform decisions concerning management and conservation of *D. glaucophylla*. The suggestions serve to illustrate much work yet to be done in an effort to understand the ecological interactions between this species and its ever-changing surrounds. It is unlikely that all of the ideas below will be investigated in a time frame commensurate with the pragmatic concerns of conservation. This point alone poignantly illustrates the challenges facing conservationists the world over.

The low seed viability observed in this study is of concern given the species is reportedly an obligate-seeder (Briggs, 1962; Auld & Scott, 1995; Kubiak, 2009) and recovers solely from a soil seedbank after fire disturbances. Results arising from the current study differ from those of Auld & Ooi (2009) who achieved good germination of *D. glaucophylla* seeds when these were exposed to temperatures in the 80° - 100° C range. The method of fruit collection used by Auld & Ooi (2009) differed to that used in the current study and this may have contributed to
the difference in results. Whether the results of the present study are due to methodological, seasonal, site, pollinator or myrmecochoric factors, this potential problem needs further investigation in the immediate future. A lack of viable seed supply to the soil seedbank is of concern, especially for a vulnerable species.

While Auld (2009) established heat as a cue to stimulate germination of *D. glaucophylla*, the effect of smoke on the species also needs to be researched. The germination trial in the present study was designed to test for this, but poor quality seed failed to provide evidence regarding the role of smoke in the germination of *D. glaucophylla* seed. Sometimes fire is inappropriate to use in some landscapes (such as under power lines or close to housing) but a product of fire, namely smoke, may achieve favourable ecological outcomes by promoting germination (Dixon *et al.*, 1995). Future research may include the use of smoke water in laboratory trials and/or the use of pelletised smoke in the field.

One possible reason for the absence of seed in many of fruits in the present study, could be related to ineffective and/or inappropriate pollinators. This is a particular problem for fragmented plant populations (Gross, 2001). A project that aimed to quantify the number and types of pollinators and their relative effectiveness by means of an exclusion experiment, while difficult, could prove useful in ascertaining possible pollinator contributions toward low seed production in *D. glaucophylla* fruits.

The Rifle Range site represents an area that will soon require re-burning, under the regime suggested by Auld & Scott (1995). Associated vegetation at this site has reached tall enough heights to negatively affect the presence and percentage cover of *D. glaucophylla*, except around rocky ledges. A monitoring project at this site could record several important post-fire
parameters, namely the emergence of seedlings of *D. glaucophylla* and subsequent rates of growth of these, patterns of regrowth in rocky versus non-rocky areas (particularly any non-seeding growth responses), changing features of soil chemistry and rates of mycorrhiza re-colonisation.

Apart from the viability of *D. glaucophylla* in the soil seedbank, investigation of seedbank size and longevity also need to be undertaken. This is especially of interest in those areas adjacent to the slashed pipeline easement (i.e. unslashed sites) where low light, high moisture and a long time since the last fire has most likely significantly reduced both size and longevity of the soil seedbank in these areas. Extracting soil from unslashed sites adjacent to the pipeline (such as the Popran and Girrakool sites in this study) and germinating the contents would provide preliminary data concerning the soil seedbank at such sites. Doing likewise in slashed and recently burnt areas would provide comparative results across different disturbance regimes after the pattern in this study.

The fate of *D. glaucophylla* seeds by ant harvesters would be a fascinating study. If seeds are eaten at the rate at which they are removed, this would leave very few seeds to replenish the soil seed bank. If seeds are stored underground at depths to which the effects of heat from fire can not reach them (Baskin & Baskin, 1998), these will not germinate, limiting the potential size and genetic variation of a given population. An invertebrate study of this nature would be difficult to execute, but would represent the first of its kind on Central Coastal heath in Australia.

While *D. glaucophylla* is largely protected from slashing (by virtue of its low stature) in those areas where this action occurs, of interest would be the effect of slashing the plant itself (i.e. can it tolerate pruning?). Such an investigation may reveal some vegetative responses of the
plant. Preliminary observation of a mowed patch of *D. glaucophylla* near the Rifle Range site, suggests that the species may tolerate pruning. This may be an important management technique to improve vigour of existing stands when seed regeneration appears deficient.

### 5.4 Limitations of the study

Even the best planned projects encounter challenges and after-thoughts as they progress. This project has been no different.

The method of seed collection was inadequate because of rapid and possibly selective harvesting by ants. Future collection will require bagging of branches to capture seeds before ant predation can occur. Fecundity could not be ascertained due to the large numbers of flowers that developed over time. More accurate counts could be gained from sub-sampling quadrats and possibly sampling more frequently.

Percentage cover rather than density was used to measure relative abundance of *D. glaucophylla*. While this method is a recognised method, it is subjective and therefore consistency between different observers is not as accurate as counting number of individuals per unit area. Density may be a better measure of success as it provides a measure of the number of individuals that have been recruited, rather than expansion of existing individuals, but this is simply not possible with this species. Despite this, the photographic record of cover collected in this study will provide some baseline data with which to compare abundance or decline in future years.

Few seedlings were available to monitor in this project. A more intensive and extensive search for seedlings across the range of *D. glaucophylla*, followed by a longer monitoring period,
would serve to improve the data set on this part of the life cycle of *D. glaucophylla* both in terms of relative abundance of seedlings and rates of growth in different habitats.

Fire regime traditionally includes fire frequency, fire intensity and season of burn (Gill, 1975). However, fire regime in this study could only take time since last fire into account. Further to this point, the limited combination of disturbance regimes available for inclusion in the study, limited the experimental design and resulted in an unbalanced design. For example, a slashed/fire ≤ 5 years ago regime did not exist. This stands to reason as both fire and slashing provide the same outcome from a biomass reduction perspective, but are significantly different in terms of ecological management. There is another type of habitat in which *D. glaucophylla* exist, but was not included in the study. These are higher rocky platforms that represented very patchy distribution and occur in less accessible sites.

Measurements of soil variables was limited to a subset of quadrats (due to budget constraints) in which the cover of *D. glaucophylla* was investigated. This may have resulted in weaker correlations among soil variables and between soil and plant variables.

Despite the limitations of this study, it has achieved its intended objectives. That is, to ascertain the range of effects of fire and slashing on the species and on its environment. The observations and data collected herein will be a valuable resource as managers implement actions to conserve *D. glaucophylla*. 
References:


Environment Protection and Biodiversity Conservation Act (1999)


