Soil Seed Banks and Vegetation Dynamics in an *Acacia papyrocarpa* Open Woodland




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Cover images: *Acacia papyrocarpa* (LHS), *Brachyscome ciliaris* var*. ciliaris* and *Cephalipterum drummondii* (top RHS), *Maireana turbinata* (middle RHS) and *Rhodanthe floribunda* (bottom RHS). Photos: E Steggles and L Pound.

CONTENTS

Declaration

I, Emma Steggles, certify that this work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

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Thesis Summary

This PhD formed part of a research partnership between the University of Adelaide, Botanic Gardens of Adelaide and Iluka Resources Ltd. The project commenced in early 2008 and focused on improving our ecological understanding of *Acacia papyrocarpa* (Western Myall) open woodlands. The study site had no or little history of stock grazing and whilst there are similar studies in this type of ecosystem, they have all been conducted in areas subject to domestic stock grazing. Mining activities are increasing in arid parts of South Australia, and mid-way through the project a mineral sands mine commenced adjacent to the study site. Research outcomes were therefore also aimed at informing post-mine restoration efforts.

Three key research areas were studied:

1. Spatial and temporal patterns within the germinable soil seed bank;
2. Effects of the biological soil crust (BSC) on seedling emergence;
3. Seed germination of selected species under salinity and water stress conditions.

Soil seed banks are key components of arid ecosystems as they buffer plant populations against stochastic events such as prolonged droughts, and they play an important role in shaping the dynamics of a plant community. Annual plant species exist predominantly as stored seeds within the soil seed bank and are dependent on specific environmental cues to overcome dormancy and germinate. Relationships between seed dispersal, seed accumulation in the soil, microenvironment properties and seedling establishment are poorly understood, yet such information is needed to inform the management and restoration of arid ecosystems.

To investigate spatial and temporal patterns within the germinable soil seed bank, soil samples were collected seasonally from four different patch types over a two year period. Samples were collected beneath the canopies of *A. papyrocarpa* (Western Myall), *Atriplex vesicaria* ssp. *variabilis* (Bladder Salt Bush) and *Maireana sedifolia* (Pearl Blue Bush), as well as in open inter-shrub areas. Nomenclature is according to Jessop and Toelken (1986). As episodic rainfall and flooding can stimulate seedling emergence in a range of arid zone plants, the soil was treated experimentally with two watering regimes (moist and submerged) to study effects of water saturation on the composition of seedlings. To supplement the research, soil nutrient, soil moisture, soil temperature and in situ seedling emergence data were collected from each of the four patch types.

Research results show evidence of both spatial and temporal variation in the germinable soil seed bank. Soil samples beneath the canopies of *A. vesicaria* ssp. *variabilis* produced highest seedling numbers and diversity when compared with most other patch types, indicating potential differences in the way seeds accumulate spatially within the soil. Submerging soil samples did not affect seedling composition; however, the study only tested responses to the high end of the water availability spectrum. Total seedling numbers and taxa were

highest in summer and autumn collections, reflecting seasonal influences on seed accumulation as well as seed dormancy and germination cues. There was a strong indication that canopy presence may provide suitable conditions to enable seeds to overcome dormancy in seasons other than autumn, thus expanding opportunities for seedling emergence throughout the year.

Investigating the BSC and its influence on seedling emergence is important to understand ecosystem processes within *A. papyrocarpa* open woodlands. Crusts are important components of arid ecosystems in that they influence nutrient cycling, water infiltration, surface evaporation and moisture storage, as well as protect soils against erosion. As such, the BSC helps determine landscape structure and function. Very little is known about BSC effects on seedling emergence, particularly within relatively undisturbed *A. papyrocarpa* open woodlands. Such information is pertinent for managing and restoring ecosystems where the BSC has been removed or disturbed through activities such as mining.

A series of experiments were designed to study interactions between BSC and seedling emergence. Initially, quadrats were established in open inter-shrub areas at the study site, where soil crusts predominantly occur. Seedling emergence was compared between areas originally with or without crust as well as between crust/surfaces left intact or shallowly and lightly scratched to simulate disturbance. Seedling abundance, diversity and composition were recorded towards the end of winter (August) when seedling emergence commences and in late spring (November) after peak seedling emergence. This experiment was also replicated in a glasshouse using intact soil cores. Seeds of selected species were later inserted into fresh cores through pre-drilled side holes in order to study their species-specific response to the various surfaces.

Results from the quadrat experiment indicate that seedling emergence and species richness were higher in inter-shrub areas where BSC was absent. Disturbance did not affect seedling emergence in either surface type. Seed extraction and seed germination experiments were then used to determine whether the variability was due to differences in seed accumulation or whether the BSC actually inhibited seedling emergence through allelopathic effects (i.e. chemical leachates from organisms making up the crust). Results from these experiments showed that late successional stage BSC negatively influences seed accumulation and that leachate effects on seed germination are species specific and depend on the successional stage of the crust.

The final area of research was to investigate the germination response of a selection of arid plant species to salinity and water stress. Groundwater is highly saline and its use in mining operations results in the accumulation of saline waste materials beneath reconstructed soil profiles. There was concern that salts may rise through the soil profile by capillary action, potentially inhibiting seed germination, seedling establishment and the long-term survival of plants. In addition to the toxicity effects of salts such as sodium chloride (NaCl), salinity affects seed germination by reducing soil water potential. Species vary in their ability to

germinate under saline conditions and have critical water content requirements for seed germination.

This research investigated germination responses of six plant species that grow in *A. papyrocarpa* open woodland: *Enneapogon cylindricus* (Gramineae), *Eriochiton sclerolaenoides* (Chenopodiaceae), *Eucalyptus oleosa* ssp*. ampliata* (Myrtaceae), *Lepidium phlebopetalum* (Cruciferae), *Maireana trichoptera* (Chenopodiaceae) and *Rhodanthe floribunda* (Compositae). Species were selected to represent a range of plant families and life forms that were locally abundant at the study site.

Preliminary experiments were needed to ascertain methods for overcoming physiological dormancy and promoting seed germination. A series of pre-treatments were investigated, including different concentrations of gibberellic acid (GA_3) and potassium nitrate (KNO_3) as well as dry heat treatments. From these preliminary experiments, the six species listed above were identified as suitable for studying the effects of salinity and water stress. Different concentrations of sodium chloride (NaCl) and polyethylene glycol (PEG-8000) were then used to produce a range of water potentials to assess germination responses.

Results from this component of the research showed that species vary in their germination response to salinity and water stress, displaying high to medium levels of tolerance or extreme sensitivity. Depending on the species, seed germination was affected through high toxicity levels, low water potentials or a combination of these two factors. Salinity and water stress was found to affect the temperature range over which some species (*E. cylindricus*, *L. phlebopetalum* and *M. trichoptera*) were capable of germinating, and low water potentials caused delays in the seed germination of others (*E. sclerolaenoides* and *R. floribunda*). Two species (*E. sclerolaenoides* and *M. trichoptera*) showed evidence of the selective accumulation of ions.

Overall, this research provides important insight into a selection of key ecological processes in a remote and intact arid ecosystem. In particular, the results add to our understanding of ecological processes within *A. papyrocarpa* open woodland, highlighting the importance of trees and shrubs in patterning and conserving understorey plant diversity and demonstrating the role of BSCs in influencing patterns of seed accumulation and seedling emergence. The research was undertaken within the context of increasing human activities within Australian arid zones, in particular mining, which was reflected in the seed germination component of the project.

Acknowledgements

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List of Figures

List of Tables

Chapter 1
Introduction

A summer storm approaching the study site, 2007. Photo E Steggles.

1.1 Project Background

This PhD formed part of a research partnership between the University of Adelaide, Botanic Gardens of Adelaide and Iluka Resources Ltd. The project commenced in early 2008 and focused on improving our ecological understanding of *Acacia papyrocarpa* Benth. (Western Myall) open woodlands. The study site was adjacent to a mineral sands mine that currently operates within Yellabinna Regional Reserve in South Australia, and subsequently findings from our research were used to inform post-mine restoration efforts.

Acacia papyrocarpa open woodland is one of 23 vegetation communities classified within Yellabinna Regional Reserve, which is a large and remote reserve in the far west of South Australia. The study site is in near pristine condition, with only minimal weed invasion and without a recent grazing history. As such, this research project was a unique opportunity to improve our understanding of ecological processes within this remote and relatively undisturbed vegetation community.

The key focus of this study was to investigate spatial and temporal patterns within the soil seed bank. Soil seed banks are key components of arid ecosystems. Many perennial plants rely solely on the soil seed bank to re-establish populations after long periods of drought, whilst annual and short-lived species exist for much of the year or over many years, as stored seeds within the soil seed bank. The restoration of arid ecosystems, which are disturbed through activities such as mining, is often heavily dependent on seedling establishment from re-spread topsoil. Therefore, understanding the spatial and temporal dynamics of the pre-disturbed soil seed bank is critical information for long-term restoration success.

The second component of this project was to investigate the role of the biological soil crust (BSC) and its influence on seedling emergence and seed accumulation. Biological soil crusts play a major role in determining the structure and function of arid landscapes, with spatial heterogeneity of arid ecosystems greatly attributable to the presence of BSCs. Such information is also pertinent for managing biodiversity in areas where the BSC is lost or disturbed.

The final component of this research was to undertake preliminary investigations into the germination response of selected plant species to salinity and water stress. Most arid zone plants have evolved to cope with naturally high salinity gradients. However, mining activities can alter the salinity level of soils and this has important implications for the restoration of mine-affected areas. Information from this component of the research revealed how common plant species in *A. papyrocarpa* open woodland may respond to the use of saline groundwater in mining activities.

1.2 Thesis Outline and Research Aims

The overall goal of this research was to improve our ecological understanding of *A. papyrocarpa* open woodlands. This project had three key research areas:

1. The spatial and temporal composition of the germinable soil seed bank;
2. Interactions between biological soil crust (BSC) and seedling emergence;
3. Seed germination under salinity and water stress conditions.

Chapter 2 provides the reader with background information and research principles pertaining to *A. papyrocarpa* open woodlands and each of the three research themes.

Chapter 3 describes the study site and provides information on key environmental conditions. The chapter includes maps, photographs and results from a series of four data collections on soil nutrient content, soil moisture, soil temperature and seedling emergence.

Chapter 4 presents findings from the soil seed bank component of the research. To investigate spatial and temporal patterns within the germinable soil seed bank, soil samples were collected from four different patch types and studied seasonally over two years within a glasshouse. Samples were collected beneath the canopies of *A. papyrocarpa* (Western Myall), *Atriplex vesicaria* ssp. *variabilis* (Bladder Salt Bush) and *Maireana sedifolia* (Pearl Blue Bush) and in open inter-shrub areas. Nomenclature is according to Jessop and Toelken (1986). Episodic rainfall and flooding can stimulate seedling emergence in a range of arid plant species and consequently soil was treated experimentally with two watering regimes, moist and submerged, to study effects of water saturation on the composition of seedlings.

Aims:

1. Study differences between germinable soil seed banks beneath canopies of *A. papyrocarpa*, *A. vesicaria* ssp. *variabilis* and *M. sedifolia* and in open areas;
2. Determine seasonal differences in seedling emergence between the four patch types;
3. Investigate whether flooding soil samples in different seasons affects seedling composition.

In **Chapter 5** a series of field, glasshouse and laboratory experiments were conducted to study the influence of BSCs on seedling emergence and seed accumulation.

Aims:

1. Examine whether the presence of late successional stage BSC influences seedling emergence and seed accumulation;
2. Establish whether disturbing late stage BSC improves seedling emergence;
3. Determine whether early successional stage BSC facilitates seed germination in some species.

Chapters 6, 7 and 8 form the seed germination component of this research. Preliminary experiments were required to investigate methods to overcome dormancy and promote maximum seed germination in a selection of plant species. These results are presented in Chapters 6 and 7. Seed germination under salinity and water stress conditions is examined in Chapter 8. A more detailed description of each chapter follows.

Chapter 6 presents a preliminary investigation of physiological dormancy (PD) in seeds of three grass species: *Enneapogon avenaceus*, *E. caerulescens* var. *caerulescens* and *E. cylindricus*, using gibberellic acid (GA_3), KNO_3 and dry heat pulse treatments.

Aims:

1. Determine which species have PD and are likely to persist within the soil seed bank;
2. Examine whether temperature affects seed germination;
3. Establish whether pulse treatments could be used to overcome PD in order to maximise germination for further seed germination research.

Chapter 7 presents findings from an investigation of seed germination in ten arid plant species. Optimising the application of GA_3 to overcome PD in seeds was investigated, in particular pulse and chronic applications of pH neutral and acidic GA_3. The information from this research was also used to maximise germination for subsequent salinity and water stress experiments.

Aims:

1. Identify which species have PD and are therefore likely to persist within the soil seed bank;
2. Determine which species respond to pulse GA_3 treatments;
3. Investigate whether the pH value of GA_3 affects its ability to overcome PD in pulse and chronic treatments.

Chapter 8 is the final research chapter that assesses germination responses of six plant species to salinity and water stress. A series of laboratory experiments used different concentrations of sodium chloride (NaCl) and Polyethylene glycol (PEG_{8000}) to produce a range of water potentials for examination.

Aims:

1. Study the germination response of six arid zone plant species to salinity and water stress;
2. Establish whether salinity and water stress affects seed germination at different temperatures;
3. Determine whether germination is affected by toxicity, osmotic effect or the combination of both.

Chapter 9 provides an overall discussion of the research results and a summary of the new information that has been gained during the course of the project. Several of the research outcomes have implications for ecological restoration in arid ecosystems, and these are discussed in this chapter. A list of recommendations for further study is included, to direct future research in soil seed bank ecology, BSC-plant interactions and seed germination studies in *A. papyrocarpa* open woodlands.

Notes on Chapter Style

Research chapters (Chapters 4 to 8) have been written in a style suitable for publication in a scientific journal. Chapters have been designed as stand-alone papers to be read independently of each other. Supervisor comments have been incorporated into each chapter, which is reflected by multiple authors listed beneath title headings. Graphs are presented in colour to assist the reader to quickly discern differences between treatments. All graphs will be converted to black and white symbols prior to their submission for publication. Some reformatting of chapters will also be required, including the separation of figures and graphs from text.

Chapter 2
Background to Key Research Themes

Acacia papyrocarpa open woodland, Yellabinna Reserve, 2010. Photo E Steggles.

2.1 *Acacia papyrocarpa* (Western Myall) Open Woodland

Acacia papyrocarpa Benth. (Leguminosae) is a long-lived tree to 10 m high, often with multi-stems and a rounded canopy that spreads outwards with age (Maslin 2001; Kutsche and Lay 2003). The species is restricted to semi-arid and arid regions in southern Australia and west of the Flinders Ranges (Whibley and Symon 1992) where it occurs on a variety of soil types including brown calcareous earths, neutral red duplex or texture contrast soils and sandy-loam soils of swales, sand plains and watercourses (Johnson and Burrows 2001; Maslin 2001; Kutsche and Lay 2003).

Flowering time is irregular and mainly occurs from August to November (Whibley and Symon 1992). Reproduction is only achieved through seed germination, with large scale recruitment occurring after exceptional rainfall events (Lange and Purdie 1976). Growth is very slow, taking approximately 75 years for individuals to reach maturity, and their lifespan exceeds 250 years (Lange and Purdie 1976). Younger trees have erect habits and as they age their limbs spread outward and eventually meet the ground where they become procumbent or poly-procumbent. The typical life stage of *A. papyrocarpa* trees at the study site is stage IV (Appendix 1) as per the classification system developed by Lange and Purdie (1976) and refined by Ireland (1997).

Acacia papyrocarpa open woodlands occur along a 200-250 mm rainfall belt to the northwest of Port Augusta and Whyalla in South Australia (Whibley and Symon 1992) (Figure 2-1). These woodlands extend into Western Australia as a narrow band fringing the Nullarbor Plain (Johnson and Burrows 2001). In western South Australia, including areas in Yellabinna Regional Reserve, the canopy is very open and often lower than 10 m (Johnson and Burrows 2001).

Tall shrubs and small trees associated with this vegetation include *Senna* spp. and *Alectryon oleifolius* ssp. *canescens* (Johnson and Burrows 2001). Dominant plant families include Chenopodiaceae (e.g. *Maireana, Sclerolaena, Atriplex*), Santalaceae (e.g. *Santalum*), Compositae (e.g. *Cephalipterum, Minuria, Rhodanthe*) and *Zygophyllaceae* (e.g. *Zygophyllum* sp.). The shrub understorey is dominated by two chenopod species, *Atriplex vesicaria* ssp. *variabilis* (Bladder saltbush) and *Maireana sedifolia* (Pearl bluebush) (Johnson and Burrows 2001). Nomenclature is according to Jessop and Toelken (1986). *Atriplex vesicaria* ssp. *variabilis* is a fast growing shrub that recruits relatively often and lives for around 30 years, whilst *M. sedifolia* is slow growing, reproduces infrequently and lives for more than 150 years (Crisp 1978). A diverse array of forbs and grasses form the annual component of this vegetation community (Appendix 2).

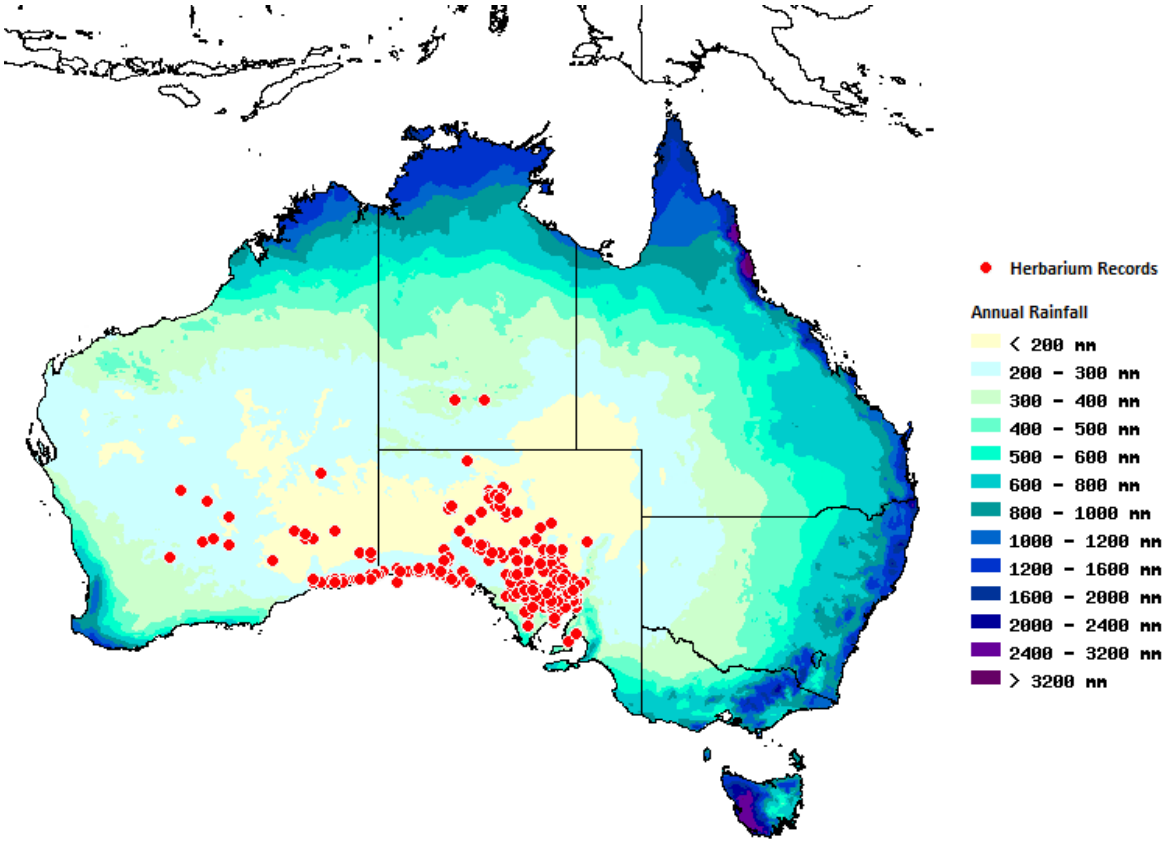


Figure 2-1 Distribution of *Acacia papyrocarpa* based on Australian herbaria collections. Sourced: 10 August 2011 from http://chah.gov.au/avh/.

2.2 Soil Seed Banks

Soil Seed Bank Classification

Soil seed banks are reserves of viable non-germinated seeds that accumulate within the soil profile following dispersal (Baskin and Baskin 1998; Fenner and Thompson 2005). At the population level, two broad categories of soil seed banks are commonly distinguished: 1) transient and 2) persistent. The two types are determined on the basis of seed longevity, as well as seed dormancy and germination strategies (Csontos and Tamas 2003). Thompson and Grime (1979) recognise four types of soil seed banks and this system is the most commonly used in contemporary plant ecology (Fenner and Thompson 2005).

Transient soil seed banks occur within the soil profile for only a short period of time. Soil seed banks that germinate immediately after dispersal from the parent plant are classified as Type 1 when they germinate in autumn and Type 2 when they germinate in spring (Thompson and Grime 1979). Annual and perennial grasses in disturbed habitats commonly display Type 1 transient soil seed banks. In cold climates, annual and perennial herbs that

colonise gaps in vegetation during spring demonstrate Type 2 transient soil seed banks. Both strategies take advantage of seasonal opportunities to germinate and establish between mortality events (Thompson and Grime 1979).

Persistent soil seed banks reside within the soil profile for more than one year or germination cycle. Baskin and Baskin (1998) define short-term persistent seed banks as at least one year but less than five years old, and long-term persistent soil seed banks are at least five years old. According to Thompson and Grime (1979), Type 3 persistent soil seed banks occur when most seed germinates soon after dispersal, yet a small reserve of viable seeds remain in the soil. Type 4 persistent soil seed banks occur when a small number of seeds germinate immediately after dispersal, leaving a large reserve of viable seeds within the soil. Germination may occur at any time in Type 3 and Type 4 persistent soil seed banks but it generally occurs in autumn (Thompson and Grime 1979; Baskin and Baskin 1998).

Persistent soil seed banks act as buffers against stochastic events, such as fire and persistent drought, which may otherwise cause long-term decline or even local extinction of plant populations. They also act as a store of genetic diversity to protect plant species against genetic drift (Honnay,Bossuyt et al. 2008). The dominant strategy used by plant species in *A. papyrocarpa* open woodland at Yellabinna Regional Reserve is to store seeds in persistent soil seed banks. *Eucalyptus oleosa* ssp. *ampliata* is the only species within the study area to store seeds by serotiny (i.e. aerial seed bank) and this species is generally restricted to scattered sandy ridges and creek lines (pers. obs. 2008) (Enright,Mosner et al. 2007).

Past research in *A. papyrocarpa* open woodland (also referred to in the literature as chenopod shrubland with sparse overstorey of *A. papyrocarpa*) have studied relationships between seed dispersal and seed accumulation in the soil, microenvironment properties and seedling establishment (Ireland 1992; Ireland 1997; Meissner and Facelli 1999; Facelli and Brock 2000; Facelli and Temby 2002; Weedon and Facelli 2008). Such studies have been conducted in sites with prolonged histories of sheep grazing near Whyalla, South Australia. As grazing can substantially change the spatial distribution of seeds (Emmerson,Facelli et al. 2010), very little information is available on the spatial and temporal distribution of soil seed banks in undisturbed *A. papyrocarpa* open woodland.

Seed Accumulation in the Soil Profile

Many factors influence the way in which seeds disperse and accumulate within the soil profile. Seeds may disperse through a number of mechanisms including wind, water run-off and animal vectors. Seeds of some species (i.e. Leguminosae) have arils to assist their dispersal through myrmecochory, with seed dispersal by ants particularly common in *A. papyrocarpa* open woodlands (Ireland 1992). Physical characteristics of the seed or dispersal unit, such as size and shape, will determine how far a seed is capable of dispersing from the parent plant, as well as how deep into the soil profile it is capable of travelling (Venable and Brown 1988; Leishman and Westoby 1994; Bekker,Bakker et al. 1998). Soil particle size can influence horizontal and vertical seed dispersal, as small seeds are more

likely to be trapped by small soil particles and large seeds are more likely trapped by large soil particles (Baskin and Baskin 1998). Some seeds have mucilaginous seed coats that enable them to anchor to the soil upon contact with water (Gutterman,Gendler et al. 2007). This strategy is used by a range of species at the study site, including members of the Cruciferae family: *Lepidium phlebopetalum, Stenopetalum lineare and Carrichtera annua*.

Features within an ecosystem also determine how seeds disperse and accumulate within the landscape. Trees and shrubs influence the composition of vegetation beneath their canopies (Facelli and Brock 2000) and create microenvironments that trap seeds differently from open areas (Facelli,Chesson et al. 2005). Some microhabitats provide 'safe sites' for seeds to accumulate and persist, subsequently enabling germination and seedling establishment (Kinloch and Friedel 2005). As such, the depth to which seeds accumulate in the soil profile varies between different ecosystems. In arid environments, the majority of the soil seed bank is known to occur within the top 3 cm of the soil profile (Pake and Venable 1996; Guo,Rundel et al. 1998).

Soil Seed Bank Dynamics in Arid Ecosystems

Annual and short-lived species exist for much of the year as stored seeds within the soil seed bank (Kinloch and Friedel 2005). Relationships between soil seed banks and their standing vegetation is both multifaceted and complex. Soil seed banks influence spatial and temporal distributions of vegetation and plant communities through patterns of seed dispersal and accumulation within the soil (Chambers and Macmahon 1994), regeneration succession (Skoglund 1992), variations in germination requirements and strategies of individual species (Facelli,Chesson et al. 2005), as well as different requirements for seedling establishment and survival (Kinloch and Friedel 2005).

Conversely, the spatial distribution of vegetation may facilitate, delay or inhibit seed accumulation, germination and seedling establishment (Facelli and Temby 2002). The requirements for seed germination are species-specific and the composition of a plant community may vary from year to year depending on environmental and microenvironmental conditions. Recruitment in annual plant communities may result from the germination of only a fraction of the seeds present in the soil seed bank once environmental conditions have become suitable (Facelli,Chesson et al. 2005).

Methods for Soil Seed Bank Assessment

Two methods commonly used to characterise soil seed banks are seed extraction and seedling emergence. The limitations and restricted applications of each method have been explored in Appendix 4. Seed extraction can be used to assess seed abundance; however, it can often be time consuming and impractical for large-scale studies. It has also been reported that seed extraction may overlook small seeds and seed viability can be difficult to determine in propagules that have been stored in soil over time (Capon and Brock 2006).

The seedling emergence method involves spreading soil samples in trays kept in a greenhouse or a glasshouse and applying water to promote seedling emergence. Although seedling emergence cannot detect seeds with dormancy, it can be used to assess the composition of the germinable component of the soil seed bank, which makes it an appropriate method for detecting spatial and temporal trends within ecosystems. Seedling emergence is generally considered the more practical method for large scale studies (Baskin and Baskin 1998).

2.3 Biological Soil Crust and Seedling Emergence

Biological soil crusts (BSCs) form dominant surface features in arid ecosystems (Metting 1991). These crusts are thin layers on the soil surface that consist of abiotic and biological components, including mosses, lichens, fungi, algae and bacteria (Lange,Kidron et al. 1992; Eldridge,Zaady et al. 2000; George,Roundy et al. 2003). Biological soil crusts are often the final stage of succession in open inter-shrub areas when water availability is a limiting factor for plant cover (Belnap,Budel et al. 2001). Studies from Koonamore in South Australia showed that the establishment of chenopod shrubs, *Atriplex vesicaria* and *A. stipitata*, were precursors to the development of a lichen-dominated BSC (Eldridge and Greene 1994). Crusts are important components of arid ecosystems in that they influence nutrient cycling, water infiltration, surface evaporation and moisture storage, and overall they protect the soil against erosion (Lange,Kidron et al. 1992; Eldridge and Leys 2003).

Despite the important role that BSCs play in arid ecosystems, often little is known about specific functions and taxonomic compositions (Lange,Kidron et al. 1992; George,Roundy et al. 2003). It is difficult to generalise about the effects of BSCs on seed germination and seedling emergence within a given system, as past research has produced varied results (Prasse and Bornkamm 2000). Crusts can have neutral, inhibitory or facilitative effects on seedling emergence (Johansen 1993; Zaady,Gutterman et al. 1997; Facelli and Springbett 2009), as the influence of BSCs on soil and ecological processes varies between ecosystems (Eldridge & Greene 1994) and can be species-specific within an ecosystem (Facelli & Springbett 2009).

This variability in BSC and vascular plant interactions may be attributable to variations in the composition of the crust and the life history stages of both micro-organisms and plants. In general, the successional sequence of BSC commences with the establishment of cyanobacteria such as *Microcoleus* spp. followed by algae, which stabilises the soil surface and enables the establishment of lichens and mosses (Belnap and Eldridge 2001). Biological soil crusts are often categorised into four successional stages: very early, early, mid and late (Belnap and Eldridge 2001). Positive BSC-plant relationships frequently occur during early successional stages of the crust (Eldridge and Greene 1994), and although lichen-dominated BSCs can inhibit seed germination, the degree of impact is also heavily dependent on crust composition (Deines,Rosentreter et al. 2007).

2.4 Seed Dormancy Classification

The function of seed dormancy is to enable seeds to maximise survivorship in optimal condition after they have matured until environmental conditions are able to support their germination and growth (Fenner and Thompson 2005; Turner,Pearce et al. 2006). A system developed by Nikolaeva (1969) and modified by Baskin & Baskin (2004), classifies seed dormancy into classes, levels and types, recognising five classes of seed dormancy: physiological, morphological, physical, morphophysiological and combinational dormancy.

To break physiological dormancy, a chemical change is required to take place within the seed. Morphological dormancy requires a period of growth and differentiation of the immature embryo before germination can occur, whilst physical dormancy involves the exclusion of water by an impermeable testa or pericarp that leaves the embryo dry until the seed coat is ruptured. Morphophysiological dormancy occurs in seeds when both morphological and physiological characters are present, and combinational dormancy occurs when both physical and physiological dormancy characters are exhibited (Baskin and Baskin 2004; Fenner and Thompson 2005).

Dormancy is an internal characteristic of the seed and external cues that break dormancy do not necessarily promote germination. Seeds may remain ungerminated in the soil even after dormancy has been broken. Therefore, seeds do not necessarily need to be dormant in order for them to persist in a soil seed bank, although non-dormant seeds tend to be less persistent (Thompson,Ceriani et al. 2003; Merritt,Turner et al. 2007; Ooi,Auld et al. 2007).

Past research has shown that seeds of many arid plant species have cyclic dormancy, whereby dormancy can only be broken at certain times of the year (Venable and Brown 1988; Baskin and Baskin 1998; Facelli,Chesson et al. 2005; Facelli and Chesson 2008; Pound,Facelli et al. 2009). Should the necessary conditions for germination not occur at this time, then the seed re-enters dormancy until the next period of suitable conditions. Viable seeds may, therefore, persist in soil seed banks through multiple germination cycles (i.e. more than one year) until the right combination of external cues occur to break dormancy and trigger germination. Natural germination cues include consistent rainfall, temperature fluctuations and suitable light conditions (Fenner and Thompson 2005).

2.5 Salinity and Water Stress

Salinity is a major environmental factor in Australia, both because of the nature of the environment and because of increased salinity caused by the removal of perennial plants through land clearance, over-grazing and mining activities. Most arid zone plants have evolved to cope with naturally high salinity gradients; however, species vary in their germination response to salinity and water stress, with each species thought to have critical water content requirements for germination (Baskin and Baskin 1998; Fenner and Thompson 2005).

Salinity can affect seed germination, seedling establishment and the long-term survival of plants (Baskin and Baskin 1998), with the germination stage of a plant's life cycle being the most sensitive to increased levels of salinity and water stress (Choudhuri 1968). Seed germination occurs after a seed imbibes water, which then activates the developmental processes and growth of the radicle. Imbibition rates depend on the permeability of the seed coat, the size of the contact area between the seed and the substrate, and differences in water potential (ψ) between the seed and soil water (Fenner and Thompson 2005).

Salinity may have toxic and/or osmotic effects on seed germination (Romo and Haferkamp 1987; Baskin and Baskin 1998). Saline ground water contains dissolved salts, in particular sodium chloride (NaCl) but also sodium sulphate (NaSO) and potassium chloride (KCl). High concentrations of dissolved salts can be toxic and thus have a direct impact on the ability of seeds to germinate (Baskin and Baskin 1998). Salinity also reduces water potential, which is a measure of the water free energy. Water moves from higher to lower water potentials, entering plant cells and tissues when the surrounding water potential is higher than that in the plant compartments. Consequently, water potential is often used to assess levels of water stress (Allaby 1998; Baskin and Baskin 1998). Dissolved ions in the water reduce water potential and may bring it to levels close or below those in seed tissues, limiting the movement of water to the seed and thus preventing germination.

Seed size affects the frequency with which water requirements can be met. Large seeds have a relatively smaller surface-to-volume ratio and consequently will have a greater absolute water requirement (Fenner and Thompson 2005). Small seeds attain their maximum water uptake quicker than large seeds and they are more likely to fall into microsites that promote water uptake and minimise desiccation. Although high levels of soil salinity may lead to the reduction or delay of germination in plants within a natural system, rainfall events may temporarily increase soil water potential and enable successful seed germination to occur (Stevens,Barrett-Lennard et al. 2006). Rainfall not only reduces osmotic stress but also dilutes the concentration of ions especially at the soil surface. Osmotic or ionic conditions then become suitable for germination following winter rains (Romo and Haferkamp 1987).

Sodium chloride (NaCl) and polyethylene glycol 8000 (PEG-8000), formerly PEG-6000, are commonly used to investigate salinity and water stress in ex situ germination experiments (Michel 1983). Polyethylene glycol is an aqueous solution that can be used to produce different water potentials and is relatively non-toxic to seeds in short-term experiments, thus any differences that arise between seed germination rates are considered to be from osmotic effects (Kebreab and Murdoch 2000). As a precaution, it is often best to minimise contact between seeds and PEG (Facelli and Chesson 2008) and avoid concentration build up from the reapplication of solutions. By comparing NaCl and PEG at identical water potentials, toxicity effects can be isolated from the effects of water stress.

2.6 Restoration Ecology

Since the 1980s, restoration ecologists have focussed their attentions on exploring and developing new methods and techniques for restoring degraded ecosystems and habitats. Each site and plant community presents different challenges and requires different revegetation strategies (Anderson and Ostler 2002). The importance of microsites and the role that succession, facilitation and competition can play in initiating and accelerating the recovery of ecosystems is increasingly being recognised in ecological restoration (Elmarsdottir,Aradottir et al. 2003; Brooker,Maestre et al. 2008). Key themes in the restoration of arid ecosystems include soil seed bank ecology, the function and composition of BSCs and plant germination strategies.

Researching soil seed bank ecology prior to disturbance is important to understand the natural dynamics of a plant community. Information on spatial and temporal patterns in the soil seed bank can be used to inform and direct the management and restoration of a system where the soil seed bank has been modified by activities such as grazing and mining. Mining often involves the removal rather than the modification of the system, and thus requires a different restoration approach to grazed areas. Generally the soil seed bank is removed and stored in top soil stockpiles, and when the hole is refilled, the top soil is respread over the surface. Successful mine-site restoration depends heavily on the ability of seeds to germinate and seedlings to establish from re-spread topsoil (Doudle 2010). The respreading of stockpiled topsoil (i.e. the soil seed bank) targets the re-establishment of a broad range of species and is practical for restoring large areas. Information on seed diversity, density and spatial patterns can help determine how much topsoil is needed for restoration projects (Li,Liu et al. 2008).

Improving the understanding of BSC and plant interactions is also important for the management and restoration of ecosystems where the BSC is modified through activities such as grazing and mining. Biological soil crusts have the capacity to facilitate plant succession to later seres (Bowker 2007) and cyanobacteria provide the cohesive quality of BSCs that enables soil surfaces to withstand erosion (Belnap,Budel et al. 2001). Biological soil crusts are often underexploited as a resource for restoration projects, yet they are critical for re-establishing a functioning ecosystem in the long-term (Bowker 2007; Doudle 2010).

Understanding seed biology is vital to ensure the establishment of seedlings in restoration projects (Commander,Merritt et al. 2009). In particular, knowledge of dormancy and germination requirements is essential to maximise the return of recalcitrant species to restored areas. Increased soil salinity resulting from activities such as grazing and mining, can affect seed germination, seedling establishment and the long-term survival of plants (Baskin and Baskin 1998). Knowledge of individual species responses to increased salinity levels and water stress, particularly at the seed germination stage, is therefore very important for improving restoration outcomes in the long-term.

Chapter 3
Study Site and Environmental Conditions

Soil temperature and soil moisture data loggers installed at the study site. Photo: E Steggles

3.1 Introduction

Location of Study Site

The study site (30°50'17.99"S and 132°12'10.37"E) was located in Yellabinna Regional Reserve, which covers 25,227 km^2 and extends north and north-west of the coastal town of Ceduna in South Australia (Figure 3-2). The reserve is bordered to the west by the Nullarbor Regional Reserve, to the north by the Trans Australia Railway and the Maralinga Tjarutja Lands and to the south by Yumbarra Conservation Park and Pureba Conservation Park.

Climate

The weather station situated 220 km east of the study site at Tarcoola, provides the best indication of climatic patterns for the reserve (Figure 3-1). Mean monthly maximum temperatures recorded between 1922 and 1999 range between 18°C in July and 35°C in January, and mean monthly minimum temperatures range between 4°C in July and 18°C in January (BOM 2012). Rainfall is generally consistent during winter months, however, large summer rainfall events that often produce floods, occur during La Niña years (Chesterfield and Parsons 1985; Sinclair 2005; Facelli and Chesson 2008). The highest mean monthly rainfall is therefore recorded in the month of February. The mean annual rainfall calculated from data collected between 1904 and 1999 at Tarcoola is approximately 174 mm (BOM 2012).

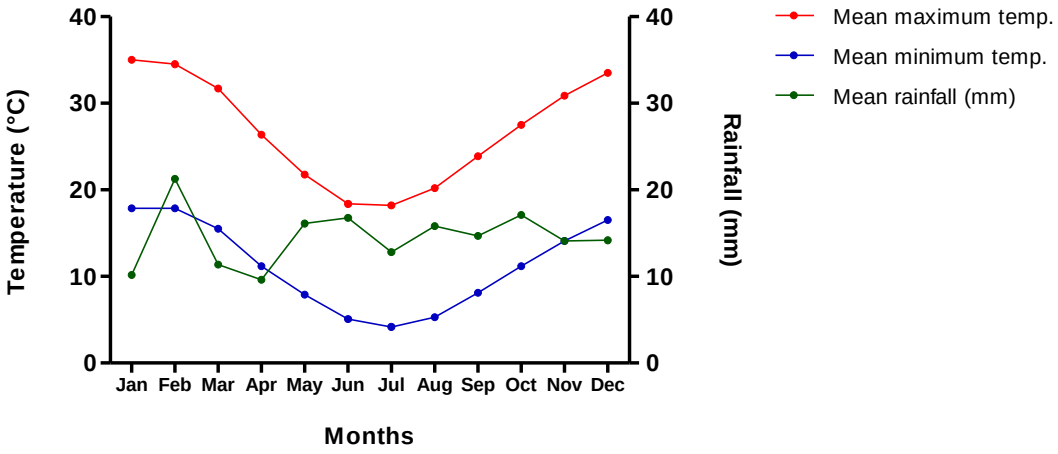


Figure 3-1 Mean monthly maximum and minimum temperatures and mean monthly rainfall recorded at Tarkoola weather station, 220 km east of the study site. Source: Australian Government Bureau of Meteorology (BOM 2012).

Figure 3-2 Location map showing Yellabinna Regional Reserve and the position of the study site. Adapted from a map supplied by Iluka Resources Ltd. (Data source: Geosciences Australia, DENR, PIRSA and Iluka).

Soils

Soils at the study site are deep calcareous sandy loam soils consisting of a thick layer of brown sandy loam (average 4m) overlying calcrete (Specht 1972; Pratt 2008; Doudle 2010). Beneath the calcrete layer, red sandy loam extends to a depth of approximately 10 m and overlies white sand (Pratt 2008). Deep calcareous yellow sand is associated with 2-7 m deep dunal ridges (Pratt 2008) that are spaced throughout the open woodland. These sandy ridges are relicts of ancient longitudinal dune fields, which are generally orientated northwest-southwest.

Vegetation

A biological survey of the Yellabinna region undertaken in 1987 (Copley and Kemper 1992) identified 686 plant species (51 introduced) and classified 23 different vegetation communities, including *Acacia papyrocarpa* (Western Myall) open woodland (also referred to as sparse low woodland), chenopod low shrubland (Nullarbor low shrubland) and *A. papyrocarpa* (minor) and mallee woodland. The plant families best represented in the area of the study site are Leguminosae (e.g. *Acacia, Senna*), Chenopodiaceae (e.g. *Maireana, Sclerolaena, Atriplex*), Santalaceae (e.g. *Santalum*), Compositae (e.g. *Cephalipterum, Minuria, Rhodanthe*) and Zygophyllaceae (e.g. Zygophyllum sp.) (Appendix 2). Sandalwood (*Santalum spicatum*) is currently the only plant species recorded at the site with a conservation rating. This species is a small semi-parasitic tree in the Santalaceae family and is listed as vulnerable under the South Australian National Parks and Wildlife Act 1972 (NP&WA 1972; Kutsche and Lay 2003).

***Acacia papyrocarpa* Benth. (Western Myall)**

The typical growth stage of *A. papyrocarpa* trees at the study site is classified as stage IV (Figure 3-4 a, Appendix 1) as per the classification system developed by Lange and Purdie (1976) and later refined by Ireland (1997). Individuals generally range between four and seven metres high and have three to four main branches. Trees have an upright habit with approximately 35% canopy cover (pers. obs. 2008). Other age classes are rarely represented by scattered stage II and stage V specimens (pers. obs. 2008). Consistency of ages suggests a single recruitment episode took place between 75 and 100 years ago, which was likely driven by an exceptional rainfall event or a series of consecutive events (Lange and Purdie 1976).

Dominant Shrubs

Two dominant shrubs associated with *A. papyrocarpa* open woodlands are *Atriplex vesicaria* ssp. *variabilis* and *Maireana sedifolia* (Figure 3-4 b & c). Nomenclature is according to Jessop and Toelken (1986). *Atriplex vesicaria* ssp. *variabilis* is a relatively fast growing shrub that recruits frequently and lives for around 30 years, whilst *M. sedifolia* is a slow growing shrub that reproduces infrequently and lives for more than 150 years (Crisp 1978). The canopy of *A. vesicaria* is less dense than that of *M. sedifolia* (pers. obs. 2008) and the former is the

smaller of the two shrubs. Both shrubs occur beneath the canopies of *A. papyrocarpa* and in open spaces, and both species were observed to trap litter and propagules at the base of their stems, where the accumulation of wind dispersed matter and sand forms raised areas (Figure 3-5a). Facelli and Temby (2002) also report differences in the soil properties (i.e. nutrients) and the composition of the soil seed bank beneath the canopies of these two shrubs. Annual and short-lived perennial forbs and grasses were observed growing beneath the canopies of both shrubs (Figure 3-5 b).

Annual Diversity

A diverse array of forbs and grasses form the annual component of the plant community. Many annual species germinated and established over vast areas during spring 2007, following substantial autumn and winter rains (Figure 3-6 a). Three common species that were present in this emergent event were *Brachyscome ciliaris* var. *ciliaris*, *Cephalipterum drummondii* and *Rhodanthe floribunda* (Figure 3-7 a, b & c). This scale of emergence was not repeated at the study site for the remainder of the project. The propagule and seed morphology of both annual and perennial species is highly diverse and some of the smallest-seeded annual species recorded at the study site were *Calandrinia eremaea* and *Crassula colorata* var. *colorata* (Figure 3-9 a, b & c).

Biological Soil Crust

The biological soil crust (BSC) occurs in open inter-shrub areas, with patchy and less frequent occurrences beneath canopies of *A. papyrocarpa* (pers. obs. 2008). The crust is generally absent beneath shrub canopies (Figure 3-10). The BSC associated with the study site has recently been classified by Doudle (2010) into four successional stage types based on Budel, Darienko et al.(2009). Type 1 (light) forms a hard soil surface with slight discolouration. Type 2 (early-mid) forms a thin, light, irregular covering that lacks species diversity. Type 3 (mid-late) is dark, of medium thickness, forms dense and extensive areas and has medium-level species diversity. Type 4 (late) is dark, thick, species diverse and lichen dominated. Refer to Appendix 3 for a list of species that were identified in the BSC.

Mallee Ridges

Acacia papyrocarpa trees are often excluded from sandy ridges that are widely spaced throughout the open woodland. These sandy ridges are relicts of ancient longitudinal dune fields, which are generally orientated northwest-southwest. Each sandy ridge varies in width and can be relatively narrow in parts, with some only 30 metres wide. *Eucalyptus oleosa* ssp. *ampliata* is a dominant mallee tree in these areas (Figure 3-6 b). Certain understorey species may be associated with this community more than with *A. papyrocarpa* open woodland, i.e. *Zygophyllum apiculatum* and *Z. aurantiacum* ssp. *aurantiacum* (pers. obs. 2009); however, such correlations need to be fully researched.

Mining in *Acacia Papyrocarpa* Open Woodland

Acacia papyrocarpa open woodland, with its understorey of perennial chenopod shrubs and annual species, is the dominant vegetation community affected by activities at the Jacinth-Ambrosia heavy mineral sands mine in Yellabinna Regional Reserve (Figure 3-3). The mine commenced operations in 2009 and is the first mine in South Australia to be developed in a Regional Reserve and has an expected lifespan of between 10 and 15 years. The two deposits, Jacinth and Ambrosia, cover an area of 340 hectares, and the ore will be excavated to a depth of approximately 45 m (Doudle 2010).

The development and management of the mine is governed by a mining and rehabilitation plan (MARP) (Iluka 2009), which details procedures for closure and restoration of areas post-mining. Soil will initially be removed and stockpiled during the first two years of the mine. Then a process of direct return will be employed to rehabilitate areas, whereby topsoil, subsoil and overburden soils from newly cleared areas will be placed directly onto reconstructed soil profiles. Iluka Resources Ltd. aims to rehabilitate approximately 70% of the mining footprint using direct return (Doudle 2010).

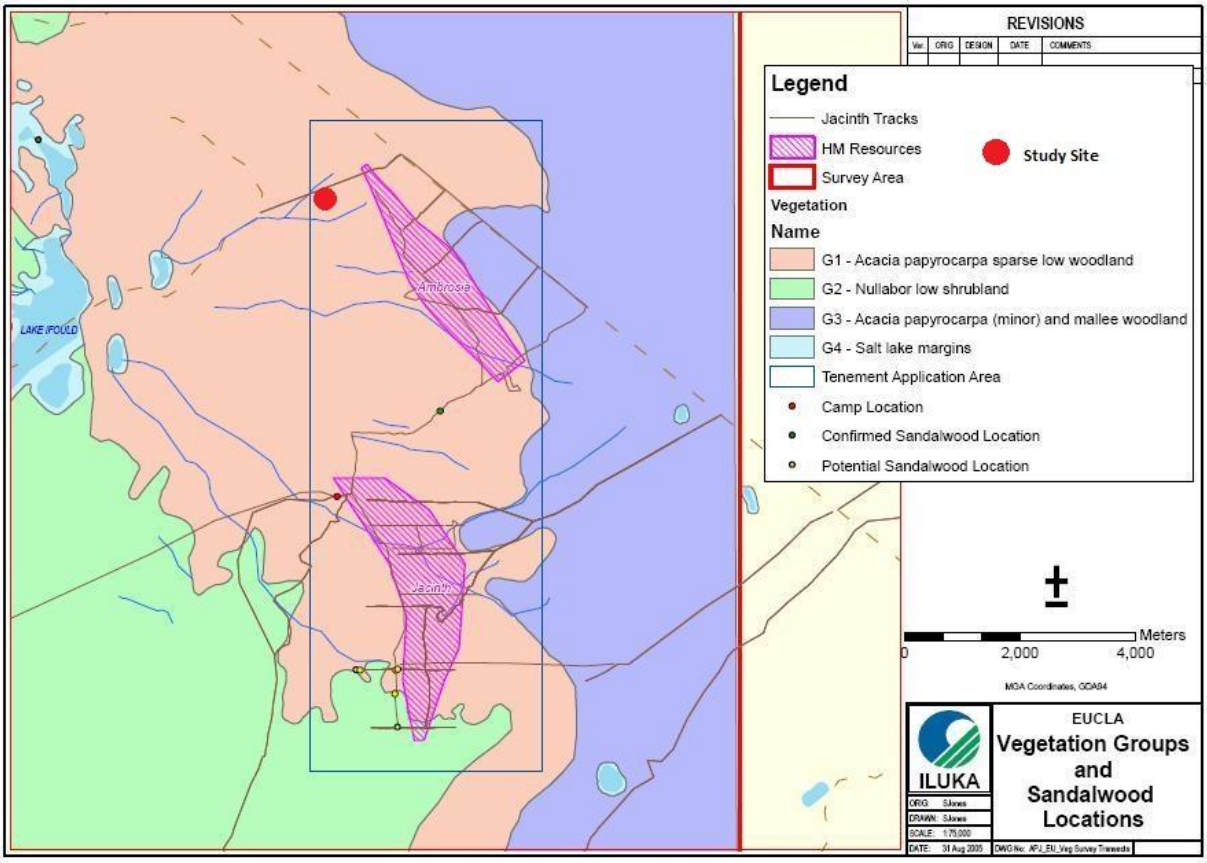


Figure 3-3 Vegetation groups in and adjacent to the study site at Jacinth-Ambrosia mineral sand mine, Yellabinna Regional Reserve, South Australia. Source: Iluka Resources Ltd 2007.

Spatial Heterogeneity

Trees and shrubs contribute to small-scale heterogeneity within arid ecosystems and this has been identified as an important process in *A. papyrocarpa* open woodlands. The modifications trees and shrubs make to accumulation and establishment conditions influences the plant community beneath their canopies. Their presence may facilitate, delay or inhibit seed accumulation, seed germination and seedling establishment (Meissner and Facelli 1999; Facelli and Temby 2002; Weedon and Facelli 2008).

Acacia papyrocarpa trees accumulate litter beneath their canopies, including wind dispersed propagules (pers. obs. 2009). These propagules are then exposed to increased nutrient availability from the breakdown of litter and high water availability from increased soil water retention properties (Facelli and Brock 2000). Such conditions may favour some species and disadvantage others, thus contributing to spatial heterogeneity, which in turn influences species diversity beneath canopies (Warnock,Westbrooke et al. 2007).

Shrubs also play important roles in structuring soil seed banks and plant communities, and it is common for annual plants to be more abundant beneath shrub canopies than in open areas (Went 1942; Emmerson and Facelli 1996). *Atriplex vesicaria* ssp. *variabilis* and *Maireana sedifolia* trap propagules and seeds (pers. obs. 2008) and provide microenvironments that differ from open areas (Facelli and Temby 2002). Such factors may increase seed abundance and composition, as well as provide environmental cues for seeds to overcome dormancy and germinate. This was observed in a similar grazed system, where the germinable soil seed bank was found to be higher beneath both shrub canopies than in open areas (Facelli and Temby 2002). Yet, even though seeds may be ready to germinate, canopy presence may reduce the ability of certain species to establish and grow. This was detected through the experimental removal of *A. vesicaria*, which resulted in increased annual plant densities (Weedon and Facelli 2008).

No past studies have characterised heterogeneity concurrently between *A. papyrocarpa*, *A. vesicaria* ssp*. variabilis*, *M. sedifolia* and open areas. Facelli and Brock (2000) compared *A. papyrocarpa* and open spaces, Facelli and Temby (2002) studied *A. vesicaria*, *M. sedifolia* and open spaces, and Weedon and Facelli (2008) investigated *M. sedifolia* and open spaces. All previous studies were conducted in sites with prolonged histories of sheep grazing, which is known to substantially change the spatial distribution of seeds (Emmerson,Facelli et al. 2010). There have also been no previous studies that measured soil nutrient content, soil temperature and soil moisture content together for the different microenvironments.


(a)


(b)


(c)

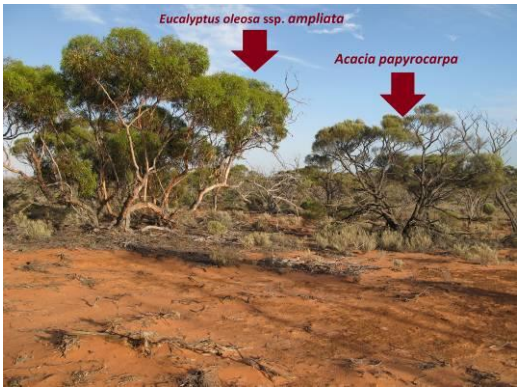
Figure 3-4 Example of the typical life stage of *Acacia papyrocarpa* (a), *Atriplex vesicaria* ssp. *variabilis* (b) and *Maireana sedifolia* (c) at the study site.


(a)


(b)

Figure 3-5 *Atriplex vesicaria* ssp. *variabilis* shrub trapping litter and *Austrostipa nitida* seeds beneath its canopy (a) and five different species of seedlings beneath the canopy of a *M. sedifolia* shrub.


(a)



(b)

Figure 3-6 Annual daisies, *Cephalipterum drummondii*, carpeting open areas at the study site (a) and *Eucalyptus oleosa* ssp. *ampliata* (mallee) on sandy ridges (b).


(a)

(b)

(c)

Figure 3-7 Annual daisies: Brachyscome *ciliaris* var*. ciliaris* (a); *Cephalipterum drummondii* (b); and *Rhodanthe floribunda* (c). Photos: L Pound.


(a)

(b)

(c)

Figure 3-8 Seedling emergence: *Sida spodochroma* (a); *Zygophyllum* sp. (b) and *Maireana sedifolia* (c).

(a)


(b)


(c)

Figure 3-9 *Calandrinia eremaea* seeds (a), *Crassula colorata* var*. colorata* follicles (b) and seeds (c).


(a)

(b)

(c)

Figure 3-10 Extent of the biological soil crust (a), its absence beneath shrub canopies (b) and cracks in the crust and bare patches (c).

3.2 Methods

To provide background information for the research on soil seed banks and vegetation dynamics of *A. papyrocarpa* open woodland, a series of three data collections were made comparing four different patch types. Information on soil nutrient content, soil temperature, soil moisture and seedling emergence was collected beneath the canopies of *A. papyrocarpa*, *A. vesicaria* ssp. *variabilis* and *M. sedifolia* as well as in open inter-shrub areas.

Soil Nutrient Analysis

Three replicate soil samples were collected from the four types of patches as listed above. Each sample consisted of three small cores (10 cm x 9 cm x 3 cm deep) pooled into one 810 cm^3 sample. In arid ecosystems, most of the nutrients and the soil seed bank are contained within the top 3 cm of the soil profile (Pake and Venable 1996; Guo,Rundel et al. 1998). Samples were retrieved towards the end of February 2009 and sent to CSBP Limited (Western Australia) for analyses: pH, soil electrical conductivity, organic carbon, total nitrogen, ammonium, nitrate, phosphorous, sulphur, calcium, potassium, magnesium, and sodium.

Soil Temperature and Moisture Data

Tiny Tag® soil temperature and soil moisture data loggers were installed at the study site to collect information from each of the four different patch types. A set of loggers were installed beneath one *A. papyrocarpa* tree, one *A. vesicaria* ssp. *variabilis* and one *M. sedifolia* shrub and in an open inter-shrub area. Data were logged at a depth of 3 cm. Soil temperature (C°) and volumetric soil water content (% mineral) was recorded every hour for the duration of this study i.e. March 2008 through to February 2010.

Monitoring Seedling Emergence

Seedling emergence was recorded in the four patch types at the end of July (mid-winter), September (early spring) and November (late spring) 2009 and at the end of February (late summer) 2010. Five replicates for each patch type were selected along a 100 m transect. Each replicate consisted of one *A. papyrocarpa*, five *A. vesicaria* ssp. *variabilis* and five *M. sedifolia* shrubs and one open inter-shrub area approximately 5 m x 5 m. Seedlings were defined as young plants that had not reached reproductive maturity (Figure 3-8).

Braun-Blanquet Cover-Abundance Scale was used to estimate quantities of seedlings according to taxa. This technique uses scale values to estimate numbers, which can then be analysed statistically (Mueller-Dombois and Ellenberg 1974; Wikum and Shanholtzer 1978) (Table 3-1).

Table 3-1 Braun-Blanquet Cover-Abundance Scale with midpoint of cover range.

Scale	Range of Cover (%)	Midpoint of cover range (%)
r	Solitary, with small cover	0.1
+	Few, with small cover	0.5
1	Numerous or scattered with cover ≤ 5%	2.5
2	Any number with cover 5% - 25%	15.0
3	Any number with cover 25% - 50%	37.5
4	Any number with cover 50% - 75%	62.5
5	Any number with cover > 75%	87.5

Statistical Analyses

One-way Analysis of Variance was used for the soil nutrient data, with alpha 0.05 used to determine significance (PASW version 18 2010, formerly SPSS). Indicator Species Analyses (Dufrene and Legendre 1997) were conducted on final seedling emergence data to determine relationships between sampling areas and season for each taxa (PCORD version 5). Midpoint of cover range percentages were used for analyses (Table 3-1).

3.3 Results

Soil Nutrient Analysis

Soil nutrient content often varied between the four patch types (Figure 3-11). Organic carbon contents were higher in soils beneath *A. papyrocarpa* than soils from open areas (*P* = 0.047). Phosphorous levels were higher beneath *A. papyrocarpa* when compared with both shrubs (*P* values = 0.011), yet the same as in open areas (*P* = 0.122). Potassium was higher beneath *A. vesicaria* ssp. *variabilis* (P = 0.009) and *M. sedifolia* (*P* = 0.003) than in open areas, yet the same as *A. papyrocarpa* (*P* = 0.109).

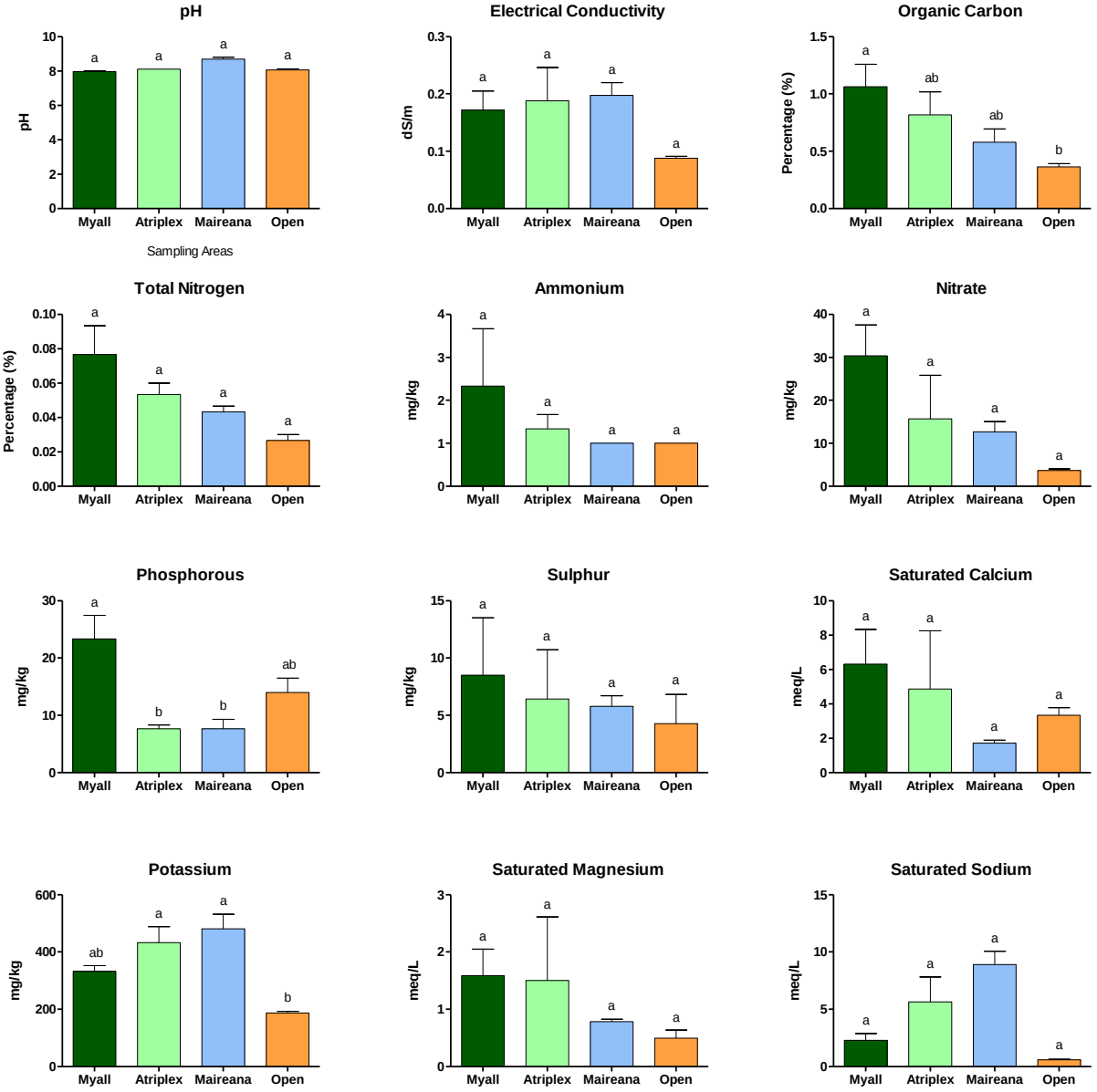


Figure 3-11 Soil nutrient analyses from four different patch types: *A. papyrocarpa*, *A. vesicaria* ssp. *variabilis, M. sedifolia* and open inter-shrub areas.

Soil Temperature and Moisture Data

Soil moisture and soil temperature graphs show seasonal patterns of cool wet winters (May, June and July) and hot dry summers (December, January and February)(Figure 3-12). In general, soil moisture was found to be lower beneath the canopy of *M. sedifolia* when compared with other patch types. Soil temperatures are consistently one to two degrees higher in open areas than beneath *A. papyrocarpa* and both shrub canopies.

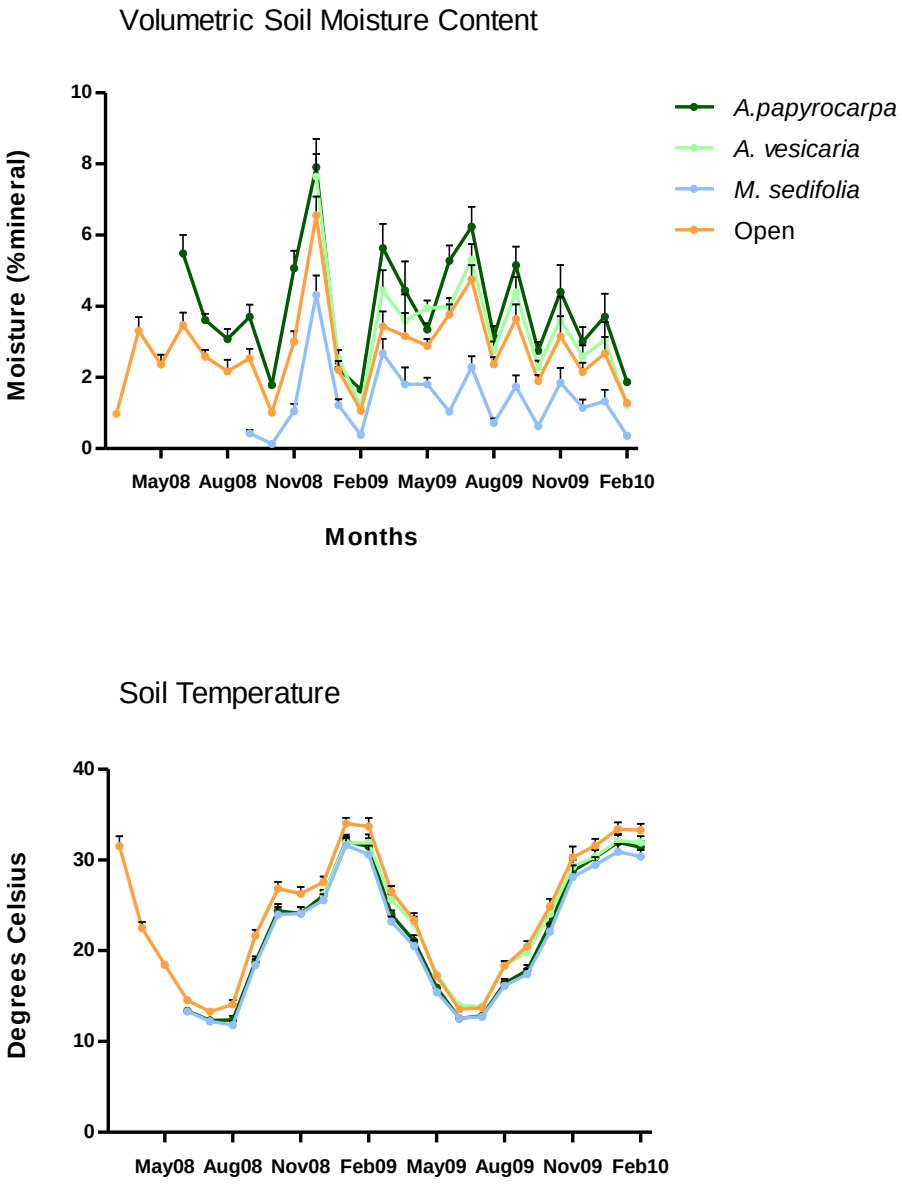


Figure 3-12 A comparison of mean monthly volumetric soil water content and mean monthly soil temperature between four different patch types (*A. papyrocarpa*, *A. vesicaria* ssp. *variabilis, M. sedifolia* and open inter-shrub areas) over 28 months.

In December 2008, heavy summer rains caused some areas to flood and fill creek lines (pers. obs. 2008). A closer inspection of this period shows that soil moisture quickly dissipated within days after each rainfall event (Figure 3-13).

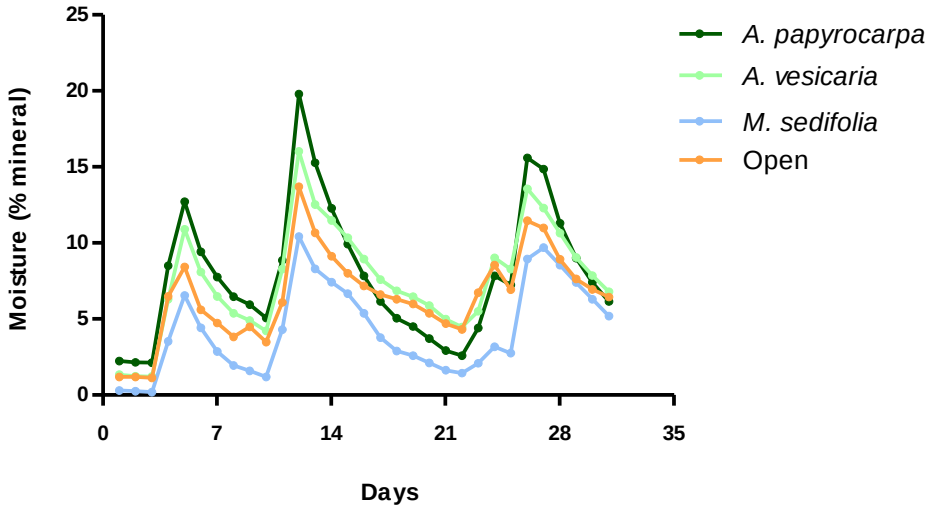


Figure 3-13 Volumetric soil water content data showing heavy rainfall events during December 2008 between four different patch types (*A. papyrocarpa*, *A. vesicaria* ssp. *variabilis, M. sedifolia* and open inter-shrub areas).

Monitoring Seedling Emergence

Two species, *A. vesicaria* ssp*. variabilis* and *S. lineare,* were significantly associated with the canopies of *A. papyrocarpa* and Gramineae spp. were associated with open areas (Table 3-2).

Table 3-2 Results from Indicator Species Analyses on seedling emergence data collected from four different patch types (*A. papyrocarpa, A. vesicaria*, *M. sedifolia and* open areas). Bold type indicates significant associations ($P < 0.01$).

Species or Species Groups	Max. Group*	Observed Indicator Value (IV)	Mean	S.Dev	*P**
Atriplex vesicaria* ssp. *variabilis	*A. papyrocarpa*	**36.5**	**32.7**	**1.15**	**0.0030**
Crassula spp.[1]	*A. papyrocarpa*	38.8	41.2	3.81	1.0000
Chenopodium curvispicatum	*A. papyrocarpa*	28.6	26.3	0.77	0.0132
Euphorbia tannensis ssp. *eremophila*	*A. papyrocarpa*	26.5	26.1	0.79	0.6053
Maireana erioclada	*A. papyrocarpa*	53.9	37.8	6.39	0.0110
M. radiata	*A. papyrocarpa*	54.5	39.1	7.95	0.0142
M. sedifolia	*A. papyrocarpa*	26.8	25.9	0.63	0.0186
M. trichoptera	*A. papyrocarpa*	31.1	32.5	4.04	0.4337
Salsola australis	*A. papyrocarpa*	55.5	45.6	5.15	0.0380
Santalum acuminatum	*A. papyrocarpa*	25.9	25.9	0.37	1.0000
Sclerolaena obliquicuspis	*A. papyrocarpa*	32.9	45.7	10.99	0.8430
Sonchus oleraceus	*A. papyrocarpa*	25.9	25.9	0.37	1.0000
Stenopetalum lineare	***A. papyrocarpa***	**60.0**	**38.7**	**6.94**	**0.0014**
Zygophyllum spp.	*A. papyrocarpa*	62.2	45.3	9.61	0.0746
Calotis hispidula	*A. vesicaria*	32.6	33.1	0.80	0.9938
Cephalipterum drummondii	*A. vesicaria*	56.3	61.8	5.32	0.8116
Erodium spp.	*A. vesicaria*	25.6	25.9	0.64	1.0000
Rhodanthe floribunda	*A. vesicaria*	25.9	25.9	0.37	1.0000
Sida spodochroma	*A. vesicaria*	38.5	40.9	3.74	1.0000
Vittadinia eremaea	*A. vesicaria*	33.0	33.2	0.66	0.9852
Chenopod spp.[2]	*M. sedifolia*	32.4	32.8	3.95	0.4169
Eriochiton sclerolaenoides	*M. sedifolia*	67.1	58.0	5.19	0.0648
Chamaesyce drummondii	Open	31.3	42.4	9.07	0.9666
***Grass* spp.**	**Open**	**62.2**	**37.8**	**6.42**	**0.0038**
Sclerolaena spp.	Open	64.2	67.3	4.39	0.4889

* *P* value is significant when < 0.01, also highlighted in bold.

[1]*Crassula colligata* ssp. *lamprosperma* and *Crassula colorata* var. *colorata* seedlings combined.

[2]Chenopodiaceae seedlings that could not be identified to genus, includes *Enchylaena tomentosa*, *Eriochiton sclerolaenoides, Maireana* spp., and *Sclerolaena* spp.

Six seedling taxa were associated with the month of July (mid-winter) (Table 3-3). The emergence of two annuals (*C. drummondii* and *Crassula* spp.) was significantly associated with this month, as well as four perennial taxa (Chenopod spp., *Santalum acuminatum*, *Sclerolaena* spp. and *Sida spodochroma*).

Table 3-3 Results from Indicator Species Analyses on seedling emergence data collected at different time periods (July 2009; Sept 2009; Nov 2009; Feb 2010). Bold type indicates significant associations ($P < 0.01$).

Taxa	Max. Group*	Observed Indicator Value (IV)	Mean	S.Dev	*P**
Calotis hispidula	July 2009	27.4	26.3	0.76	0.1880
Cephalipterum drummondii	July 2009	25.9	25.9	0.37	1.0000
Chamaesyce drummondii	July 2009	**59.4**	**38.7**	**6.85**	**0.0020**
***Chenopod* spp.**[2]	July 2009	**48.7**	**41.0**	**3.75**	**0.0096**
***Crassula* spp.**[1]	July 2009	**80.3**	**61.7**	**5.27**	**0.0002**
Eriochiton sclerolaenoides	July 2009	25.9	25.9	0.37	1.0000
Euphorbia tannensis ssp. *eremophila*	July 2009	49.8	42.5	8.94	0.1296
Grass spp.	July 2009	34.1	33.2	0.67	0.2394
M. radiata	July 2009	34.8	33.1	0.80	0.0350
M. trichoptera	July 2009	48.3	41.1	3.75	0.0552
Maireana erioclada	July 2009	26.5	26.1	0.79	0.6179
Salsola australis	July 2009	25.6	25.9	0.64	1.0000
Santalum acuminatum	July 2009	**57.5**	**37.6**	**6.28**	**0.0056**
***Sclerolaena* spp.**	July 2009	**90.9**	**45.5**	**9.87**	**0.0002**
Sida spodochroma	July 2009	**84.3**	**45.5**	**11.05**	**0.0002**
Vittadinia eremaea	July 2009	77.4	67.4	4.50	0.0610
Zygophyllum spp.	July 2009	29.1	32.5	4.01	0.9952
Chenopodium curvispicatum	Sept 2009	59.6	58.1	5.23	0.3759
Erodium spp.	Sept 2009	44.3	45.6	5.20	0.5935
Sclerolaena obliquicuspis	Sept 2009	35.8	37.8	6.28	0.5315
Sonchus oleraceus	Sept 2009	34.8	39.2	7.96	0.7606
M. sedifolia	Nov 2009	37.2	32.9	3.98	0.1112
Rhodanthe floribunda	Nov 2009	25.9	25.9	0.37	1.0000
Stenopetalum lineare	Nov 2009	25.6	25.9	0.63	1.0000
Atriplex vesicaria ssp. *variabilis*	Feb 2010	31.2	32.8	1.16	0.9998

* *P* value is significant when < 0.01, also highlighted in bold.

[1] *Crassula colligata* ssp. *lamprosperma* and *Crassula colorata* var. *colorata* seedlings combined.

[2] Chenopodiaceae seedlings that could not be identified to genus, includes *Enchylaena tomentosa, Eriochiton sclerolaenoides, Maireana* spp. and *Sclerolaena* spp.

3.4 Discussion

Tree and shrub presence often creates microenvironments that influence the establishment of other plant species, and this is particularly relevant in arid ecosystems where water and nutrients are strong limiting factors for plant establishment (Facelli and Brock 2000; Facelli and Temby 2002). Trees and shrubs often accumulate debris and organic matter, which may cause soil nutrient levels to increase beneath canopies (Garner and Steinberger 1989; Callaway,Nadkarni et al. 1991; Belsky and Canham 1994; Facelli and Brock 2000). Variations in soil nutrients were observed between the four patch types. Organic carbon levels were higher in soils beneath *A. papyrocarpa* than open areas, possibly reflecting increased accumulation of organic matter beneath these trees. This finding is similar to other research in a similar grazed system (Facelli and Brock 2000). In contrast to our research, however, Facelli and Temby (2002) found that total nitrogen (N) was higher in soils beneath *A. vesicaria* ssp. *variabilis* than in open areas. This difference may be attributable to the effects of grazing patterns.

Phosphorous (P) levels were higher in soils beneath *A. papyrocarpa* than both chenopod shrubs, yet levels were similar between *A. papyrocarpa* and open areas, as well as shrubs and open areas. In contrast, Facelli and Temby (2002) found that levels of P were lower in soils beneath *M. sedifolia* than open areas in a similar grazed system. The primary source of P is weathering of minerals in parent rock material, whereby P is formed from primary apatite minerals that are released into the soil solution where they may be leached, taken up by plants and microorganisms or transformed into secondary minerals (Lajtha and Schlesinger 1988). Both horizontal and vertical gradients occur in the distribution of soil nutrients, (Charley and West 1975) and the depth at which *A. papyrocarpa* trees access P compared with *A. vesicaria* ssp. *variabilis* and *M. sedifolia*, may explain differences in P content between the patch types. Both chenopod shrubs have short root systems and would therefore utilise P closer to the soil surface, whereas *A. papyrocarpa* has a deep root system that would allow access to P to greater depths. The uptake of available P would be consistent beneath shrubs, whereas the uptake by microorganisms and annual plants in open areas would fluctuate from year to year.

There were no significant differences between potassium (K) content in soils beneath *A. papyrocarpa* and open areas, which is similar to previous findings by Facelli and Brock (2000). However, levels of K were higher in soils beneath *A. vesicaria* and *M. sedifolia* than in open areas. Similarly, research undertaken in Sierra Nevada, Spain showed that shrubs significantly increased available K in soils beneath their canopies (Gomez-Aparicio,Gomez et al. 2005). Potassium is generally associated with inorganic deposition, where particulate matter collects in plant canopies and are deposited beneath (DeSoyza,Whitford et al. 1997). Increased potassium content may improve seedling survival beneath canopies (Gomez-Aparicio,Gomez et al. 2005), as K produces higher water-use efficiency in plants through osmotic adjustments that reduce transpiration rates (Bradbury and Malcom 1977; Egilla,Davies et al. 2001).

Trees and shrubs influence light and radiation levels beneath their canopies, which affects both soil temperature and soil moisture (Valiente-banuet and Ezcurra 1991). The soil temperature was consistently one to two degrees higher in open areas than beneath tree and shrub canopies. The soil temperature beneath *M. sedifolia* was also consistently lower than *A. papyrocarpa* and *A. vesicaria* by up to half a degree, possibly reflecting differences in canopy density and structure. Soil moisture was higher beneath *A. papyrocarpa* and *A. vesicaria* when compared with open areas, which is likely due to lower soil temperatures increasing the capacity of soils to retain water (Jordan and Nobel 1979) and increased water availability through hydraulic lift (Dawson 1993; Burgess,Adams et al. 1998; Facelli and Temby 2002). The soil moisture was consistently lower beneath *M. sedifolia* than all other patch types. This finding is similar to a previous study (J.M. Facelli, unpublished data) and possibly reflects higher water uptake by the shrub.

Such variations in soil nutrient contents, soil temperature and soil moisture influence the dormancy-breaking cues and germination conditions that seeds receive in the soil seed bank. Interactions between environmental variables and germination, emergence and growth are dynamic and complex. Facelli and Brock (2000) demonstrate that seedlings do not occur preferentially in high fertility patches. A good site for emergence does not necessarily equate to a good site for reproductive output. For example, better conditions for germination may result in more seedling recruitment, and consequently increased seedling density, combined with higher resource availability leads to stronger competition.

Although episodic rainfall events such as flooding can stimulate seedling emergence in a range of arid zone plants (Chesterfield & Parsons 1985; Sinclair 2005), timing of the event is critical. Heavy summer rains occurred during December 2008 (Figure 3-13) and were observed to promote new growth and flowering in perennial plants, such as *A. vesicaria* ssp. *variabilis* and *M. sedifolia* (Figure 3-4 b & c). These events did not, however, stimulate the emergence of annual species (pers. obs. 2008). This suggests that seeds may have re-entered dormancy and that episodic rainfall events during summer months may not always provide the right conditions for seeds to overcome dormancy and germinate.

Past research has shown that many arid zone plants have cyclic dormancy, whereby dormancy can only be broken at certain times of the year (Venable and Brown 1988; Baskin and Baskin 1998; Facelli,Chesson et al. 2005; Facelli and Chesson 2008; Pound,Facelli et al. 2009). Should the necessary conditions for germination not occur at this time, then the seed re-enters dormancy until the next period of suitable conditions the following year. This strategy reduces the risk of seedling mortality from desiccation as summer rainfall events are unreliable. In some arid systems, such as those in the SW of the United States of America, there is a distinct winter and summer annual flora (Baskin,Chesson et al. 1993). Some plants at the study site, mainly grass species (i.e. *Enneapogon* spp.) are known to respond strongly to summer rains (Kutsche and Lay 2003).

Plant densities beneath trees and shrubs may be higher than open areas due to seeds becoming trapped beneath their canopies (Reichman 1984; Chambers and Macmahon 1994;

Facelli and Brock 2000; Facelli and Temby 2002). In a shrubland of the Negev, productivity and species richness of annual plants in open inter-shrub areas were found to be limited by the absence of structures stopping the outflow of water, soil and seeds (Boeken and Orenstein 2001). We observed the accumulation of litter and propagules beneath *A. papyrocarpa* and both shrubs at the study site (pers. obs. 2008) and this is likely to have contributed to patterns in seedling emergence. Several seedling taxa were associated with mid-winter, likely reflecting the cooler temperatures and increased moisture levels required for seeds to overcome dormancy and germinate. Only grass emergence was found to be significantly higher in open areas, which was supported by field observations made of adult plant distribution patterns (pers. obs. 2009). Similarly, Boeken, Ariza et al. (2004) observed that densities of *Stipa capensis* in the Negev were higher in inter-shrub areas than beneath shrubs. They theorised that temperature and light conditions may have favoured germination of this species in the exposed patches.

A key difference between this study site and a similar grazed system was the relative abundance of perennial seedlings, which contrasts to the mostly annual species recorded by Facelli and Temby (2002). There are two potential non-exclusive explanations for this variation between the two systems. Firstly, that grazing can substantially change the spatial distribution of seeds and patterns in the deposition of detritus (Emmerson,Facelli et al. 2010) as well as the resources available for plant production (Golodets and Boeken 2006). Secondly, that different age stages of *A. papyrocarpa* trees can contribute to variation in the composition of seedlings. At the study site, *A. papyrocarpa* trees are younger (stage IV) than those in the grazed system (stages IV, V and VI) and lack the procumbent (and sometimes polyprocumbent) canopies often represented there. These variations in canopy structure can have different influences on the spatial distribution of seeds and patterns in the deposition of litter. This research may, therefore, add further weight to the importance of age structure and population dynamics of woody perennial species for ecosystem and community dynamics in arid lands (Facelli and Brock 2000).

Through combining field observations with information from soil analyses, data loggers and records of seedling emergence, there is clear evidence that *A. papyrocarpa*, *A. vesicaria* ssp. *variabilis* and *M. sedifolia* influence the environmental conditions beneath their canopies. This in turn, directly determines the composition of soil seed banks, seed dormancy and seed germination cues, as well as conditions for seedling establishment and survival beneath their canopies.

Chapter 4
Spatial and Temporal Variation in the Soil Seed Bank of an *Acacia papyrocarpa* Open Woodland

Standing water after heavy rains in *Acacia papyrocarpa* open woodland. Photo: J M Facelli.

Spatial and temporal variation in the soil seed bank of an *Acacia papyrocarpa* Open Woodland

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Abstract

Understanding the ecology of soil seed banks, particularly their spatial and temporal patterns, is essential for managing, protecting and restoring arid ecosystems. In this study, seedling emergence was used to investigate spatial and temporal patterns within the germinable soil seed bank of Acacia *papyrocarpa* Benth. open woodland. Whilst there are similar studies in this type of ecosystem, these were all conducted in areas subject to domestic stock grazing. The study site used here has no or little history of stock grazing. Soil samples were collected seasonally for two years beneath the canopies of *A. papyrocarpa*, *Atriplex vesicaria* ssp. *variabilis* and *Maireana sedifolia* as well as open inter-shrub areas. The samples were transferred to trays in a glasshouse and seedling emergence was assessed over time. Episodic rainfall and flooding can stimulate seedling emergence in a range of arid zone plant species. Therefore, soil was treated experimentally with two watering regimes (moist and submerged) to study effects of water saturation on the composition of seedlings.

In general, soil collected beneath canopies of *A. vesicaria* ssp. *variabilis* and *M. sedifolia* produced similar patterns of seedling emergence. A notable exception was the emergence of *Crassula* spp., which were consistently higher in samples collected beneath *A. vesicaria* ssp. *variabilis* canopies than all other sampling areas. Seedling emergence in *A. papyrocarpa* soils was often similar to soils from open areas, possibly reflecting the young life stage of trees at the study site. Submerging soil samples did not affect seedling emergence; however, this study only tested responses to the high end of the water availability spectrum.

Strong seasonal patterns were observed in the germinable soil seed bank. Seedling emergence was highest in autumn soil collections, indicating that by the end of autumn many seeds had received the necessary conditions required to overcome dormancy. The relatively high levels of seedling emergence in summer collections suggest that the flora in this system may have a trend towards winter and summer partitioning. In open areas, 68% of taxa were associated with autumn soil collections compared with ≤ 50% of taxa beneath trees and shrubs. This suggests that canopy presence may provide conditions that enable seeds to overcome dormancy in seasons other than autumn, thus expanding opportunities for seedling emergence throughout the year.

4.1 Introduction

Soil seed banks are key components of arid ecosystems as they play an important role in shaping the dynamics of the plant community. Soil seed banks provide a buffer against stochastic events, such as fire or prolonged drought, which may otherwise cause long-term decline or even local extinction of plant populations. They also act as stores of genetic diversity to protect plant species against genetic drift (Honnay,Bossuyt et al. 2008). In arid ecosystems, many perennial plants rely solely on the soil seed bank to re-establish populations after long periods of drought, whilst annual and short-lived species exist for much of the year, and often for many years, as stored seeds in the soil seed bank (Kinloch and Friedel 2005).

Soil seed banks influence spatial and temporal distribution of vegetation through a range of mechanisms. Spatial distribution reflects seed dispersal and accumulation within the soil, which is responsible for patterning many plant communities (Chambers and Macmahon 1994). Variations in seed germination requirements and strategies of individual species (Facelli,Chesson et al. 2005), as well as requirements for seedling establishment and survival (Kinloch and Friedel 2005), contribute to the temporal patterning of plant communities and may play important roles in facilitating species coexistence (Chesson,Gebauer et al. 2004; Facelli,Chesson et al. 2005).

Although non-dormant seeds tend to be less persistent, dormancy is not necessary for the accumulation of persistent soil seed banks (Thompson,Ceriani et al. 2003). In arid ecosystems, species often show evidence of having cyclic dormancy, whereby viable seeds re-enter dormancy when environmental conditions are not suitable for germination. Seeds are able to persist in this manner, through multiple germination cycles (i.e. more than one year) until the right combination of external cues occur to trigger germination (Baskin,Chesson et al. 1993; Baskin and Baskin 1998). Requirements for seed germination are species-specific and subsequently the composition of standing plant communities may vary from year to year depending on environmental conditions. Recruitment of annual plant communities may also result from the germination of only a fraction of seeds present in the soil seed bank (Facelli,Chesson et al. 2005).

Past ecological research has been undertaken in *Acacia papyrocarpa* open woodland (also referred to as chenopod shrubland with sparse overstorey of *A. papyrocarpa*). These include studies of relationships between seed dispersal and seed accumulation in the soil, microenvironment properties and seedling establishment (Ireland 1992; Ireland 1997; Meissner and Facelli 1999; Facelli and Brock 2000; Facelli and Temby 2002; Weedon and Facelli 2008). Such studies, however, have all been conducted in sites with prolonged histories of sheep grazing. Grazing can substantially change the spatial distribution of seeds (Emmerson,Facelli et al. 2010) and therefore no previous research was available on seed accumulation patterns within this type of ecosystem under natural grazing regimes.

Canopies of trees and shrubs contribute to small-scale heterogeneity within arid ecosystems. Their presence may facilitate, delay or inhibit seed accumulation, seed germination and seedling establishment (Meissner and Facelli 1999; Facelli and Temby 2002; Weedon and Facelli 2008). The modifications they make to accumulation and establishment conditions influences the plant community beneath their canopies. *Acacia papyrocarpa* trees accumulate litter and wind or water dispersed propagules beneath their canopies (pers. obs. 2009). These propagules are then exposed to increased nutrient availability from the breakdown of litter and high water availability from increased soil water retention and reduced evaporation (Facelli and Brock 2000). Such conditions may favour some species and disadvantage others, thus contributing to spatial heterogeneity, which in turn influences species diversity beneath canopies (Warnock,Westbrooke et al. 2007).

Shrubs also play important roles in structuring soil seed banks and plant communities, and it is common for annual plants to be more abundant beneath shrub canopies than in open areas (Went 1942; Emmerson and Facelli 1996). The two dominant shrub species that occur in *A. papyrocarpa* open woodland, *Atriplex vesicaria* ssp. *variabilis* and *Maireana sedifolia,* trap propagules (pers. obs. 2008) and provide microhabitat conditions that differ from open areas (Facelli and Temby 2002)(refer to Chapter 3 of this thesis). Such factors may increase seed abundance and composition, as well as influence environmental conditions needed for seeds to overcome dormancy and germinate.

This was observed in a similar grazed system, where the germinable soil seed bank was found to be higher beneath *A. vesicaria* and *M. sedifolia* canopies than in open areas (Facelli and Temby 2002). Yet, even though seeds may be ready to germinate, canopy presence may reduce the ability of certain species to establish and grow. This was detected through the experimental removal of *A. vesicaria*, which resulted in increased annual plant densities (Weedon and Facelli 2008). It is possible that the removal of the shrub produced conditions that broke seed dormancy and/or provided conditions that were suitable for germination. Although dormancy in a seed is either a yes or no state, in seed populations there is substantial variation in dormancy and germination ability.

Knowledge of soil seed bank ecology is important to understand the natural dynamics of arid lands and to inform and direct management and restoration of ecosystems where the soil seed bank has been modified by activities such as grazing and mining. Successful mine-site restoration depends heavily on the ability of seeds to germinate and seedlings to establish from re-spread topsoil (Doudle 2010). In this study, we used seedling emergence to investigate spatial and temporal patterns within the soil seed bank of *A. papyrocarpa* open woodland at Yellabinna Regional Reserve, South Australia. Seedling emergence assesses the germinable soil seed bank i.e. the potential plant community, which makes it an appropriate method for detecting spatial trends within ecosystems.

Reliable rains generally occur during winter months at the study site, however, large rainfall events including summer floods may occur at other times of the year (Facelli and Chesson 2008). Episodic rainfall events such as flooding can stimulate seedling emergence in a range

of arid zone plants (Chesterfield & Parsons 1985; Sinclair 2005). Therefore, this research aimed to answer three key questions: (1) Do germinable soil seed banks differ between four patch types: *A. papyrocarpa*, *A. vesicaria* ssp. *variabilis*, *M. sedifolia* and open areas? (2) How does simulating a large flooding event influence seedling emergence in different seasons? (3) Do tree and shrub canopies affect seedling emergence differently in different seasons?

4.2 Methods

Study Site

The study site (30°50'17.99"S and 132°12'10.37"E) was located in Yellabinna Regional Reserve, which covers 25,227 km^2 and extends north and north-west of the coastal town of Ceduna in South Australia (Figure 3-2). Mean monthly maximum temperatures recorded between 1922 and 1999 range between 18°C in July and 35°C in January, and mean monthly minimum temperatures range between 4°C in July and 18°C in January (Figure 3-1). Overall rainfall is low and generally consistent during winter months, however, large summer rainfall events that often produce floods, occur during La Niña years (Chesterfield and Parsons 1985; Sinclair 2005; Facelli and Chesson 2008). The mean annual rainfall calculated from data collected between 1904 and 1999 at Tarcoola is approximately 174 mm (BOM 2012).

Experimental Design

Seedling emergence under glasshouse conditions was used to assess the composition of the germinable soil seed bank. Fieldwork for this research commenced in May 2008 when soil samples were collected for the first glasshouse trial. In order to investigate spatial and temporal patterns within the soil seed bank, soil samples were collected and studied seasonally from four different patch types:

1. Beneath the canopy of the *A. papyrocarpa* (Western Myall);
2. Beneath the canopy of the *A. vesicaria* ssp. *variabilis* (Bladder Salt Bush);
3. Beneath the canopy of the *M. sedifolia* (Pearl Blue Bush);
4. In open inter-shrub areas.

Collections were made during the last week of each season over a two-year period in: May, August and November 2008; February, May, August and November 2009; and February 2010. The soil samples were treated experimentally with two watering regimes (moist and submerged) to study the effects of water saturation on the composition of the seedling community.

Fifteen replicate sites were established at the beginning of the study, each containing one *A. papyrocarpa* and eight *A. vesicaria* ssp. *variabilis* and *M. sedifolia* shrubs. Eight shrubs of each species were needed to sample seasonally for two years, as each shrub could only be sampled once. Care was taken to select *A. papyrocarpa* trees of similar height (i.e. 5 - 8 metres) canopy spread (i.e. 6 - 8m) and canopy intactness. Selected trees were stage IV (Figure 3-4 a, Appendix 1) as per the classification system developed by Lange and Purdie

(1976) and later refined by Ireland (1997). Similarly, only A. *vesicaria* ssp. *variabilis* and *M. sedifolia* shrubs of similar canopy size and intactness were selected. All trees and shrubs were required to be a minimum distance of five metres from surrounding tree canopies. The aspect of shrubs in relation to the *A. papyrocarpa* tree was unrestricted and varied between replicate sites.

Random compass points were used to select core sites beneath the tree canopy at the mid-way point between the trunk and canopy edge. After sampling, holes were filled in with soil from nearby areas to prevent them from becoming seed traps and subsequently marked to ensure they were not re-sampled. A random number generator was used to choose the pair of shrubs to be sampled at a given time. The core sites in open areas were chosen at a random point adjacent to the pair of shrubs.

In most arid ecosystems, the soil seed bank is contained within the top 3 cm of the soil profile (Pake and Venable 1996; Guo,Rundel et al. 1998) (J.M. Facelli pers. obs.). To ensure samples were representative, three small cores (10 cm x 9 cm x 3 cm deep) were pooled into one 810 cm^3 sample per spatial area and replicate, with a total of 60 samples collected each season. Composted litter on the ground surface was included in the samples. However, large litter including leaves, bark and obvious surface-lying seeds/fruits found generally beneath *A. papyrocarpa* and shrubs were removed prior to sampling. This material does not generally contain any seeds (J.M. Facelli, unpublished data).

Preparation of Soil Samples

Soil samples were placed into plastic bags and transported to a drying room where they were stored for one week at 20 ± 5ºC and ambient relative humidity. Plastic bags were opened to allow evaporation and to stop seeds from germinating in the bags and to minimise the loss of seed viability through seed ageing and fungal growth.

Each soil sample was then sieved to remove large stones and break up aggregated matter. A riffle splitter (Gerlach,Dobb et al. 2002) (Figure 4-1 a) was used to divide samples into two even sub-samples of approximately 405 cm^3 (i.e. a total of 120 sub-samples). Different watering treatments (moist and submerged) were applied to sub-samples (Figure 4-1 b). Soil was spread over sand to a depth no greater than 1 cm to ensure that seeds capable of germinating could do so (Gurnell,Boitsidis et al. 2006). Rectangular Pohlman™ ½ trays (32 cm x 13 cm) were used in glasshouse trials, each with a capacity of holding 416 cm^3 of soil spread to less than 1 cm thickness. White sand was used as a substrate into which seedlings could grow. Sand was sterilised using an autoclave for 20 minutes at 120°C and a pressure of 120 kPa and then spread to a depth of approximately 2 cm across seedling trays. Shade cloth (UV protection 90%) lined all seedling trays to retain sand and enable free drainage. Seedling trays were arranged along bench tops inside a glasshouse (Figure 4-1 c).


(a)

(b)

(c)

Figure 4-1 Riffle splitter for dividing soil samples into two even sub-samples (a); sub-samples in submerged treatment (b); and layout of seedling trays in the glasshouse (c).

Glasshouse Conditions and Treatments

A weekly record was kept of maximum and minimum temperatures within the glasshouse. The main objective was not to replicate field conditions, but rather to provide temperature and humidity conditions that would maximise seed germination. Ten additional seedling trays containing sterilised sand were placed randomly within the glasshouse as controls.

Sub-samples received one of two watering treatments for a total duration of 6 weeks:

1. *Moist treatment*
   Automatic overhead sprayers twice a day for 10 minutes.

2. *Submerged treatment*
   Initially, Pohlman™ ½ trays were placed fully submerged into large water-tight trays filled with water and irrigated from automatic overhead sprayers twice a day for 10 minutes. The large trays containing water were removed after 7 days and the sub-samples were allowed to drain. Irrigation continued as per moist treatment conditions.

Seedling emergence was scored once a week for a total period of six weeks. Seedling trays were rotated every week to minimise position effects within the glasshouse. Seedlings were flagged with toothpicks to ensure they were not recorded twice or missed due to deaths. All seedlings were transferred to potting tubes containing Australian native seed raising mix, photographed at various stages of their development and where possible identified to species level. Nomenclature followed Jessop and Toelken (1986).

Statistical Analyses

Non-parametric permutational multivariate analysis of variance test, or PerMANOVA (Anderson 2007), was used to analyse final data, with alpha set at 0.05 (PerMANOVA version 1.6). Data were transformed to fourth root and analysis based on Euclidean distances. Pair-wise *a-posteriori* comparisons were conducted to identify differences between treatments. As the initial analysis did not show any significant differences between watering treatments, both data sets were combined to increase the sample size from 15 to 30 replicates and the analysis was repeated. The most dominant taxa (*Crassula* spp.), which constituted 85% of all

seedlings counted, was compared separately to avoid skewing the data. Indicator Species Analysis (Dufrene and Legendre 1997) was conducted on final data to determine associations between seedling emergence and area and season for each taxa, as well as differences in seedling composition between watering treatments (PCORD version 5). Chi-Square Goodness-of-Fit Test was used to determine whether sampling time affected the distribution of taxa in each patch type (Bluman 2001).

4.3 Results

A total of thirty-two species representing sixteen plant families were identified from soil samples collected over eight seasons (Appendix 5). One taxon could not be grouped. Plants identified included annual forbs (i.e. *Cephalipterum drummondii* and *Stenopetalum lineare*) and grasses (i.e. *Austrostipa nitida*), short-lived perennials and perennial shrubs (i.e. *Eriochiton sclerolaenoides* and *Sida spodochroma*) and long-lived trees (i.e. *Casuarina pauper* and *Eucalyptus* sp.). Seasonal patterns of emergence were observed, with peak seedling emergence recorded in autumn collections. Declines in seedling numbers were most notable in spring collections (Figure 4-2). No significant differences in number of seedlings (P = 0.8018, df = 1, SS = 203.6765) and number of taxa (P = 0.9606, df = 1, SS = 13.5160) were found between moist and submerged treatments. Seedling composition was also similar between moist and submerged treatments, with only one taxon significantly associated with *A. papyrocarpa* in winter 2 (P = 0.0050) (data not shown).

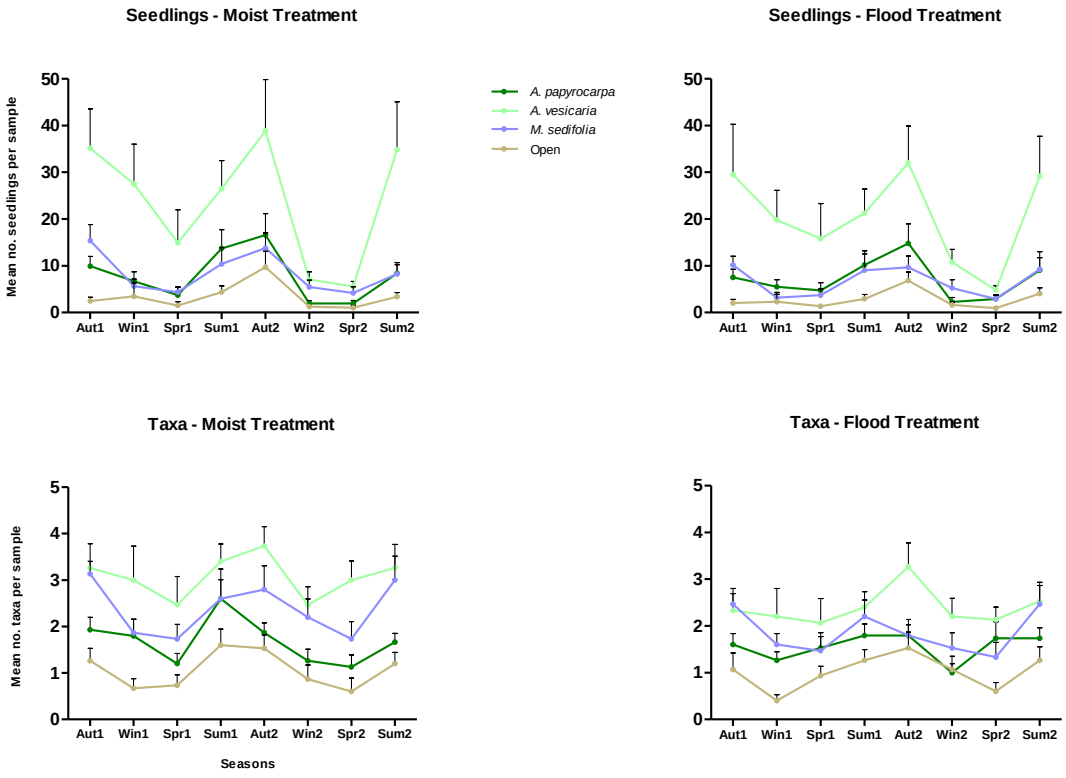

Figure 4-2 Mean number of seedlings and taxa recorded per soil seed bank sample in moist and submerged treatments, from different patch types and different seasons.

Total seedling numbers differed between treatments (P = 0.0001, df = 31, SS = 56.0222). Seedling emergence was significantly higher in all soil samples collected beneath *A. vesicaria* ssp. *variabilis* canopies than open inter-shrub areas (*P* values < 0.05) (Figure 4-3). Similarly, seedling numbers were higher in samples collected beneath *M. sedifolia* canopies than open areas (*P* values < 0.05), excluding summer 1 and Autumn 2 collections (*P* values > 0.05). Emergence was similar between *A. papyrocarpa* and open areas (*P* values > 0.05), except in spring 1, summer 1 and spring 2 collections, when seedling numbers were higher in *A. papyrocarpa* soils (*P* values < 0.05). Seedling numbers were similar between the two chenopod shrubs, with emergence in *A. vesicaria* samples only exceeding those of *M. sedifolia* in spring 2 collection (*P* < 0.05).

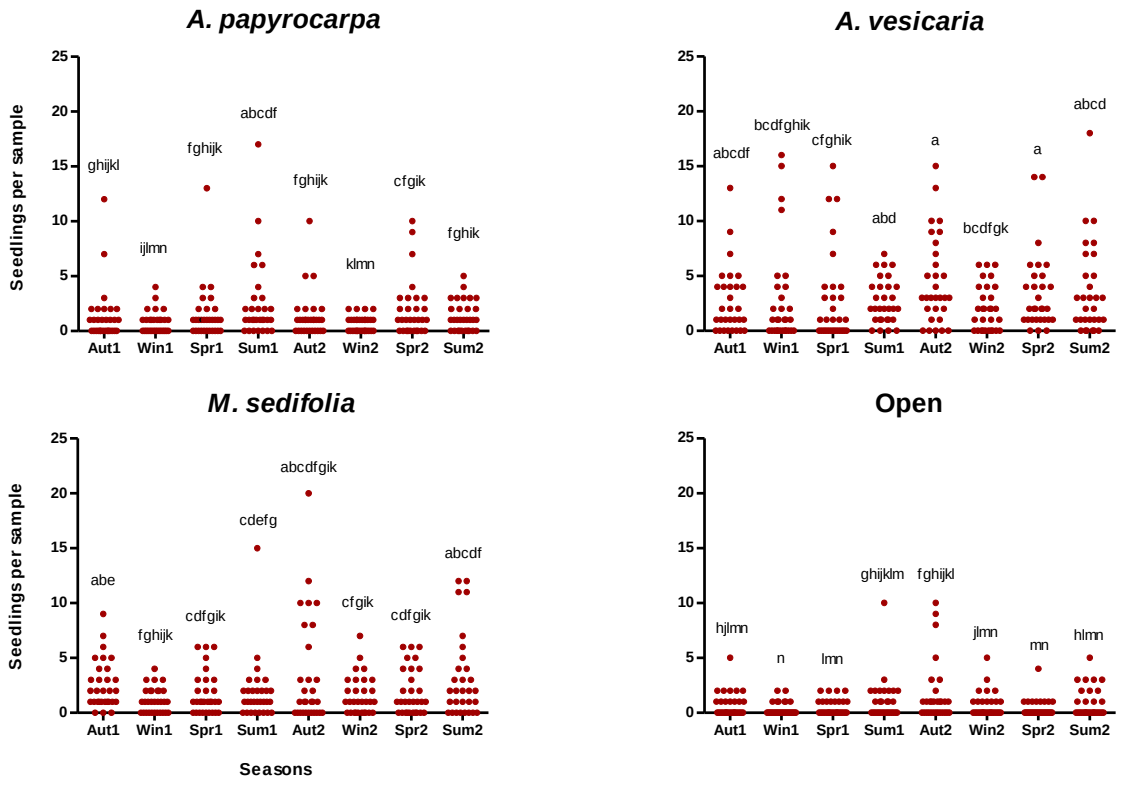

Figure 4-3 Seedling numbers recorded from soil seed bank samples collected from four different patch types and eight different seasons. Different letters indicate significant differences between seedling numbers and statistics are compared between the four patch types. The data excludes *Crassula* spp. (refer to Figure 4-5).

Similar patterns were observed between the number of taxa (*P* = 0.0001, df = 31, SS = 65.4858). Samples collected beneath *A. vesicaria* ssp. *variabilis* and *M. sedifolia* canopies, excluding autumn 2 and winter 2 (*P* values > 0.05), had greater numbers of taxa than open inter-shrub areas collected at the same time (*P* values < 0.05) (Figure 4-4). The taxa recorded in soil beneath *A. papyrocarpa* and open areas were similar (*P* values > 0.05), excluding winter 1, summer 1 and spring 2 collections where numbers of taxa were higher in *A. papyrocarpa* soil samples *(P* values < 0.05).

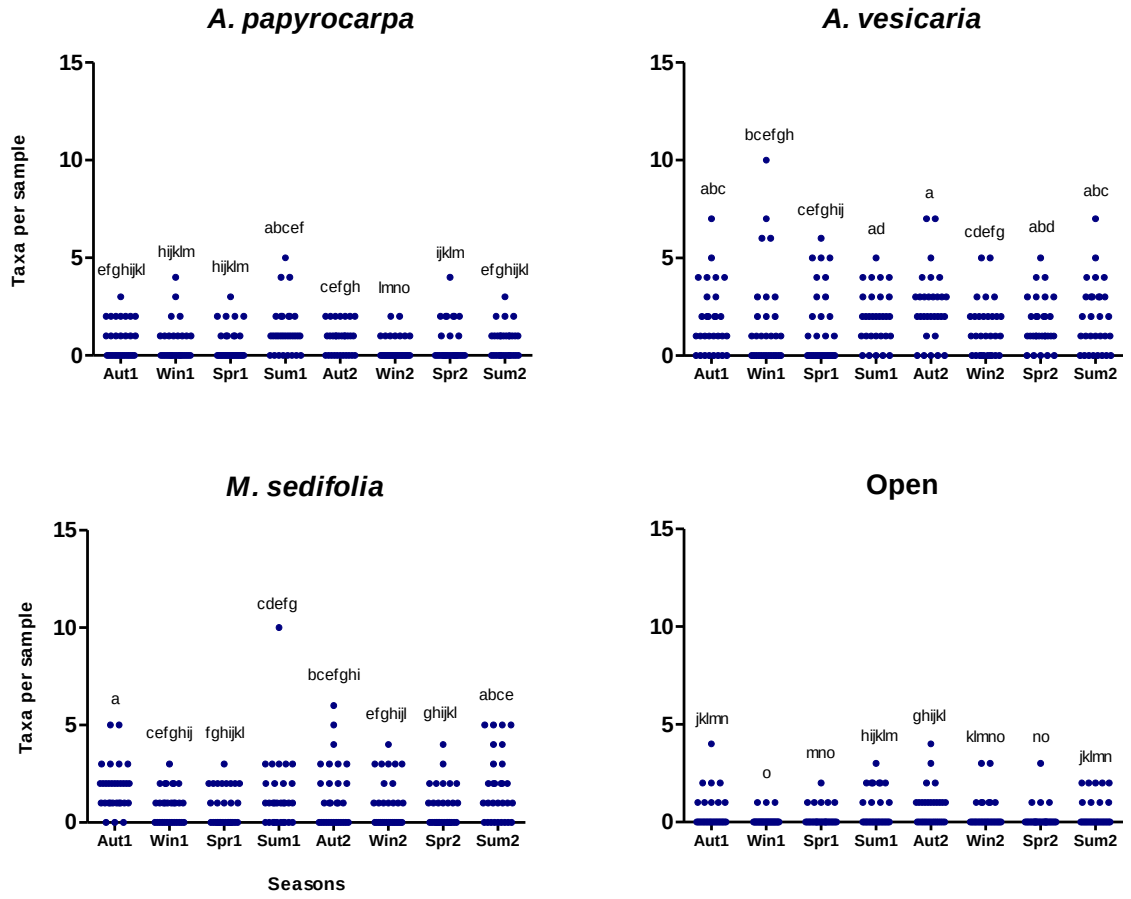

Figure 4-4 Number of taxa recorded from soil seed bank samples collected from four different patch types and eight different seasons. Different letters indicate significant differences between number of taxa and statistics are compared between the four patch types. The data excludes *Crassula* spp. (refer to Figure 4-5).

The most abundant taxa recorded in this study was *Crassula* spp. (85%) and the pattern of emergence was different across the treatments (P = 0.0001, df = 31, SS = 270.5204). Two species were identified, *Crassula colligata* ssp. *lamprosperma* and *Crassula colorata* var. *colorata*. These were pooled together as they were difficult to distinguish in the seedling stage and mortality rates were high. Seedling emergence was significantly higher in samples collected beneath *A. vesicaria* ssp. *variabilis* canopies than all other areas (P values < 0.05) (Figure 4-5). No differences were detected in *Crassula* spp. emergence between soil samples collected beneath *M. sedifolia* and *A. papyrocarpa* (P values > 0.05).

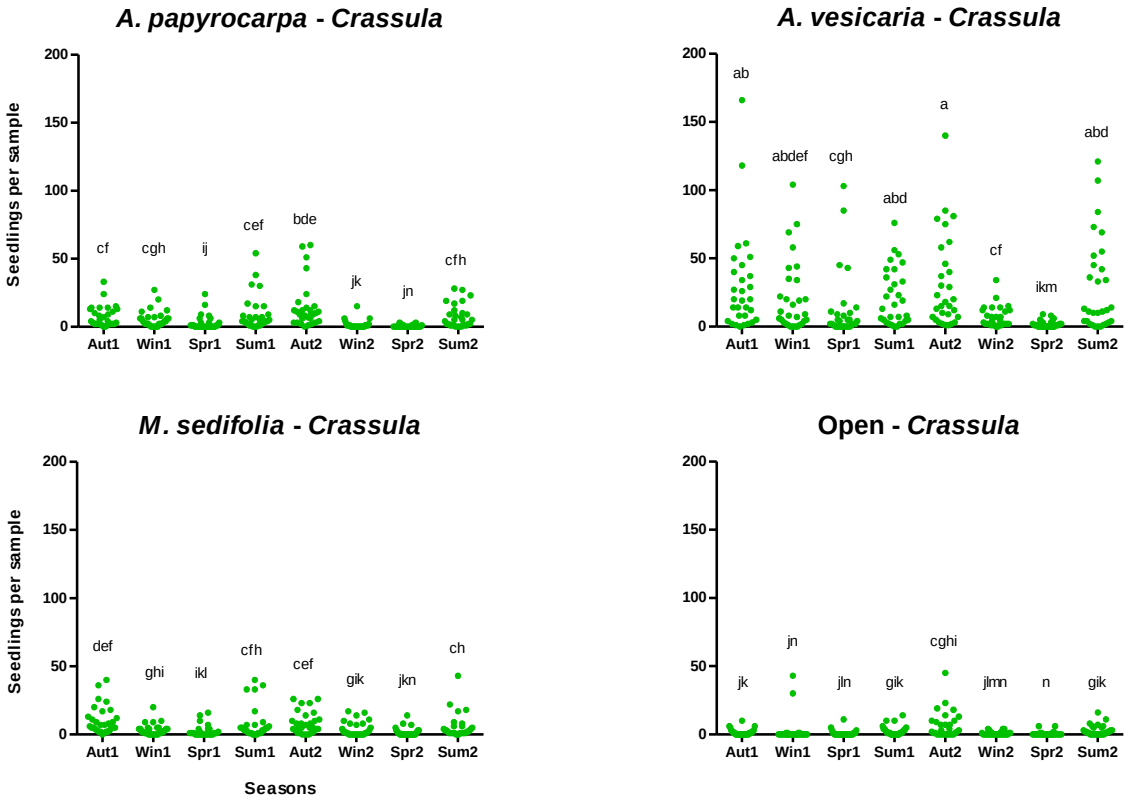

Figure 4-5 Number of *Crassula* spp. seedlings recorded from soil seed bank samples collected from four different patch types and eight different seasons. Different letters indicate significant differences between seedling numbers and statistics are compared between the four patch types.

A small number of seedling taxa were associated with either *A. papyrocarpa* or *A. vesicaria* ssp. *variabilis* canopies at certain collection times (Table 4-1). Nearly all *Crassula* spp. emergence was significantly associated with *A. vesicaria* ssp. *variabilis* canopies, with the exception of Spring 2. Refer to Appendix 6 and Appendix 7 for complete ISA results.

Table 4-1 Summary of significant results ($P < 0.01$) from Indicator Species Analyses, which show seedling taxa collected during different seasons and their associations with patch type. * *P* value is significant when < 0.01.

Taxa	Collection Season	Max Group	IV	Mean	S. Dev	*P**
Calandrinia eremaea	Summer2	*A. papyrocarpa*	59.3	37.6	6.07	0.0029
Cephalipterum drummondii	Autumn2	*A. vesicaria*	60.9	38.8	6.59	0.0028
Chenopod spp.	Autumn2	*A. vesicaria*	51.4	35.7	5.26	0.0099
Chenopod spp.	Winter1	*A. vesicaria*	64.1	38.8	6.01	0.0005
Chenopod spp.	Winter2	*A. vesicaria*	50.0	35.2	4.89	0.0069
Crassula spp.	Summer1	*A. vesicaria*	51.4	32.1	3.53	0.0002
Crassula spp.	Summer2	*A. vesicaria*	64.0	34.2	4.50	0.0001
Crassula spp.	Autumn1	*A. vesicaria*	60.5	34.1	4.26	0.0001
Crassula spp.	Autumn2	*A. vesicaria*	51.2	32.1	3.49	0.0001
Crassula spp.	Winter1	*A. vesicaria*	64.4	35.0	4.86	0.0001
Crassula spp.	Winter2	*A. vesicaria*	52.6	33.1	4.01	0.0004
Crassula spp.	Spring1	*A. vesicaria*	66.8	39.8	7.18	0.0007
Eriochiton sclerolaenoides	Autumn2	*A. vesicaria*	57.1	42.8	6.49	0.0079
Grass spp.	Spring2	*A. vesicaria*	51.8	39.4	4.79	0.0048

The majority of taxa from open areas (68%) recorded peak seedling emergence in samples collected at the end of autumn, of which there was a relatively even mix of annuals and perennial species (Table 4-2). In soils beneath *A. papyrocarpa*, *A. vesicaria* ssp. *variabilis* and *M. sedifolia*, the number of taxa recording peak emergence in Autumn collections was lower, ranging between 41% and 50% of taxa. Of these taxa ≥ 80% were annual species.

Table 4-2 A summary of the seasonal distribution of seedling taxa in four different patch types. For each patch type and collection time, the total percentage is calculated from the total number of taxa recording peak seedling emergence. * *P* value is significant when < 0.01.

Patch Type	Season	No. of Taxa	Total Percentage (%)	Life Form (%)		*Chi-square Results
				Annual	Perennial	
A. papyrocarpa	Autumn	9	40.91	88.89	11.11	χ^2=16.320, 3df (*P*=0.001)
	Winter	5	22.73	60.00	40.00	
	Spring	5	22.73	20.00	80.00	
	Summer	3	13.64	66.67	33.33	
A. vesicaria	Autumn	10	45.45	80.00	20.00	χ^2=26.560, 3df (*P*<0.001)
	Winter	2	9.09	50.00	50.00	
	Spring	5	22.73	40.00	60.00	
	Summer	5	22.73	60.00	40.00	
M. sedifolia	Autumn	11	50.00	90.91	9.09	χ^2=43.12, 3df (*P*<0.001)
	Winter	1	4.55	0.00	100.00	
	Spring	4	18.18	25.00	75.00	
	Summer	6	27.27	50.00	50.00	
Open	Autumn	15	68.18	53.33	46.67	χ^2=99.280, 3df (*P*<0.001)
	Winter	2	9.09	50.00	50.00	
	Spring	2	9.09	100.00	0.00	
	Summer	3	13.64	100.00	0.00	

4.4 Discussion

The results show a strong spatial pattern in the germinable soil seed bank of *A. papyrocarpa* open woodland. Seedling emergence and diversity were generally higher in soil samples collected beneath the canopies of *A. vesicaria* ssp. *variabilis* and *M. sedifolia* when compared with open inter-shrub areas. In particular, seedling emergence in soils collected beneath *A. vesicaria* ssp. *variabilis* was higher than open areas in every season. Two potential explanations for this variance are differences in seed accumulation between patch types and differences in the microenvironmental conditions, such as temperature, water availability, light and nutrients (Facelli and Brock 2000; Facelli and Temby 2002; Warnock,Westbrooke et al. 2007). Information from data loggers showed only subtle differences in soil nutrient content (Figure 3-11), soil moisture and soil temperature conditions (Figure 3-12) between shrub canopies and open areas. This suggests that differences in the way seeds accumulate in the soil rather than seed persistence (which are affected by environmental conditions) is the main mechanism through which heterogeneity in propagule distribution arises. This may be affected by the physical presence of the shrubs trapping seeds and the increased seed deposits into the soil seed bank by local production.

In general, soil collected beneath canopies of *A. vesicaria* ssp. *variabilis* and *M. sedifolia* produced similar patterns of seedling emergence. A notable exception was the emergence of *Crassula* spp., which was consistently higher in samples collected beneath *A. vesicaria* ssp. *variabilis* than all other sampling areas. This finding is consistent with results from a similar yet grazed system, whereby *C. colorata* var. *colorata* seedling emergence was higher in soils beneath *A. vesicaria* than *M. sedifolia* (Facelli and Temby 2002). Seedling emergence in *A. papyrocarpa* soils, including *Crassula* ssp., was often similar to soils from open areas. Soils beneath *A. papyrocarpa* were higher in organic carbon (Figure 3-11); however, plant establishment does not always occur preferentially in high fertility patches (Facelli and Brock 2000). Similarities between *A. papyrocarpa* and open areas are likely due to the life stage of trees at the study site, which are relatively upright and have sparse canopies (Figure 3-4 a) (Appendix 1). It may also be possible that seeds blown or washed towards trees may become trapped at the edge of the patch as soon as the litter starts, and this may warrant further investigation.

Several mechanisms can contribute to the heterogeneity documented here, and their importance would vary for different species and even for the various patch types. *Acacia papyrocarpa*, *A. vesicaria* ssp. *variabilis* and *M. sedifolia* trap seeds differently beneath their canopies. Large propagules of *Sclerolaena obliquicuspis*, *Eriochiton sclerolaenoides*, *Cephalipterum drummondii* and *Austrostipa nitida* were observed accumulating beneath tree and shrub canopies at the study site (pers. obs. 2008) (Figure 3-5 a). As *Crassula* seeds are very small, i.e. less than 0.5mm long (Figure 3-9 c), they would not be expected to disperse over large distances as they would quickly penetrate the soil profile (Bekker,Bakker et al. 1998). It is also possible that the microhabitat created by *A. vesicaria* ssp. *variabilis* provides suitable conditions for *Crassula* spp. to overcome dormancy, germinate, establish

and reproduce. *Crassula* spp. may therefore be continually contributing to the soil seed bank beneath *A. vesicaria* at a greater rate than other sampling areas. Per capita seed contribution is determined by the number of plants germinating (which is also a drain in the soil seed bank), environmental conditions and density dependent effects. A study of the differences in seed abundance (i.e. through seed extraction) and the redistribution of seeds is required to determine whether *Crassula* spp. accumulate differently beneath *A. vesicaria* ssp. *variabilis* when compared with other patch types.

Shading, increased seed bank density and soil moisture are key influences on plant communities beneath canopies (Warnock,Westbrooke et al. 2007). *Crassula colligata* ssp. *lamprosperma* and *C. colorata* var. *colorata* are delicate annual herbs that have relatively high water requirements to protect against desiccation (pers. obs. 2008)(Warnock,Westbrooke et al. 2007). Although soil temperature was found to be similar beneath the two shrubs, lower soil moisture levels were consistently recorded beneath the canopy of *M. sedifolia*. *Atriplex vesicaria* ssp. *variabilis* shrubs are relatively smaller with canopies that are less dense than those of *M. sedifolia* (Facelli and Temby 2002). Variations in plant water use, mound heights and their effect on water infiltration and soil hydrophobicity may explain disparities in *Crassula* ssp. emergence between the two shrubs.

The life forms of plant species identified in this study were highly variable and included annual forbs, annual and perennial grasses, short-lived perennials and perennial shrubs and long-lived trees (Appendix 5). A key difference between this study site and a similar grazed system was the relative abundance of perennial species recorded in the soil seed bank. In contrast, most of the species recorded in the disturbed system were annual species (Facelli and Temby 2002). There are two potential non-exclusive explanations for this variation between the two systems. Firstly, that grazing can substantially change the spatial distribution of seeds and patterns in the deposition of detritus, and transported particles may be substantially different (Emmerson,Facelli et al. 2010). Secondly, different life stages of *A. papyrocarpa* trees can contribute to variation in seedling composition. At the study site, *A. papyrocarpa* trees are younger (stage IV) than those in the grazed system (stages IV, V and VI) and lack the procumbent (and sometimes polyprocumbent) canopies often represented in the latter. These variations in canopy structure can influence the spatial distribution of seeds and patterns in the deposition of litter. This study, therefore, adds further weight to the importance of age structure and population dynamics of woody perennial species for ecosystem and community dynamics of arid lands (Facelli and Brock 2000).

Seedling emergence peaked in soil samples collected at the end of autumn, reflecting seasonal changes, such as cooler temperatures and increased moisture levels, which may be required for seeds to overcome dormancy and germinate. The composition of the soil seed bank includes seeds of different ages that vary in their after-ripening and stratification requirements. The response of the soil seed bank suggests that seeds may receive the

necessary conditions to overcome dormancy by late autumn, even though emergence events generally occur during late winter and early spring.

Seedling emergence was lowest in soil samples collected in late spring. This was not caused by natural depletion of the soil seed bank, as seedling emergence at the study site was both patchy and minimal during 2008 and 2009 (pers. obs. 2008, 2009) and most species are likely to have small germination fractions (Facelli,Chesson et al. 2005). It is more likely that warmer and/or dryer winters failed to provide the necessary conditions for seeds to overcome dormancy. Seeds may have either remained dormant or re-entered dormancy after not receiving the necessary cues for germination. Past research has shown that seeds of many arid zone plants exhibit cyclic dormancy, whereby dormancy can only be broken at certain times of the year (Facelli and Chesson 2008; Pound,Facelli et al. 2009). Should the necessary conditions for breaking dormancy not occur at this time, then the seed re-enters dormancy until suitable conditions arise the following year. As summer rainfall events are unreliable, this strategy reduces the risk of seedling mortality caused by hot summer temperatures and desiccation.

However, relatively high seedling emergence was recorded from soil samples collected at the end of summer, suggesting that the flora in this system may have a trend towards winter and summer partitioning, more similar to the SW of North America (Baskin,Chesson et al. 1993). This strategy was not previously recorded in this system, with Facelli et al. (2005) barely recording any emergence in samples collected in late spring through to late summer. This implies that summer rains may be more predictable at the study site than in similar systems further east in South Australia. Unfortunately, our investigation of water availability and its effects on the composition of the germinable soil seed bank at different times of the year was limited to only one variable. In our research, soil samples were submersed in water for seven days to simulate extreme flooding events and the pooling of water on the soil surface, and this was at the very high end of the water availability spectrum. Water availability has been found to affect seedling emergence in samples from open spaces in a similar grazed system (Facelli,Chesson et al. 2005). Subsequently, a more comprehensive study testing a range of different water contents is needed to confirm summer and winter partitioning of species in this system.

Submerging soil samples for seven days did not affect seedling numbers, diversity or composition when compared with moist treatments. It is possible that shorter or longer durations of submersion may have produced differences in seedling numbers and composition. Overall, Facelli, Chesson et al. (2005) found that constant exposure to medium and high water availability (66% and 100% field water capacity θ_{fc}) produced higher levels of seedling emergence than low water availability (33% θ_{fc}) under simulated summer, autumn/spring and winter temperatures. The emergence of *Tetragonia tetragonoides* was significantly higher in 100% θ_{fc} than both low to medium water treatments under all three temperature conditions, suggesting that some taxon respond to constant high water availability across different seasons.

Submerging soil samples in water can dilute certain chemicals that maintain seed dormancy (i.e. through leaching) and enable seeds to break dormancy and then germinate. However, extended periods of submersion would create anaerobic conditions and species would vary in their thresholds for germination under such a state. In some species, seed germination is inhibited by leaching. For example, germination in *Eriochiton sclerolaenoides* was reduced by the process of leaching after 72 hours (Pound,Facelli et al. 2009) (also refer to Chapter 7 of this thesis). In this study, submerging soil seed bank samples for seven days may have had both positive and negative effects on seed germination, depending on the species, but these effects may not have been detected through measuring seedling emergence. It is important that future research compare a range of exposures times (i.e. one, three and five days of submersion) in order to detect the effects of water availability and flooding on soil seed bank response.

Strong seasonal patterns were observed in the germinable soil seed bank of *A. papyrocarpa* open woodland and three processes are likely contributing to this pattern. Firstly, the degree of cyclic dormancy in the seed population, which is affected by the dynamics of cyclic dormancy and the age structure of the seed population, with younger seeds tending to have more strict requirements for germination. Secondly, the variations in seed production and accumulation in the soil seed bank at different times of the year. Finally, the changes in soil seed bank composition from seed losses due to germination and mortality. These variables were not measured directly; however, numbers of seedlings and taxa were relatively high in soil samples collected at the end of summer, most likely reflecting a soil seed bank at its peak following natural seed dispersal. This suggests that the soil seed bank, and not the state of the seeds, changes over time.

Evidence of seasonal changes in seed numbers (i.e. through seed extraction) occurring in a similar grazed system was found by Facelli, Chesson et al. (2005). No natural seedling emergence was observed during summer (pers. obs. 2008, 2009), yet a range of plant species were shown to be capable of germinating under glasshouse conditions. This result indicates that seeds of many species, including annuals, may be capable of responding to extended rainfall events during summer months. Heavy summer rains occurred in December 2008 (Figure 3-13) and were observed to promote new growth and flowering on perennial plants, such as *A. vesicaria* ssp. *variabilis* and *M. sedifolia* (Figure 3-4 b & c). These events did not, however, stimulate the emergence of annual species (pers. obs. 2008).

A majority of the taxa emerging from soil collected in open areas recorded peak seedling emergence in autumn collections. The taxa consisted of an even mix of annual and perennial plant species. Open areas are generally less protected from the elements i.e. sun and wind, and there is a greater likelihood for seedling establishment and survival if seedling emergence occurs in late winter and early spring. Seedlings would then avoid months where rainfall is less reliable. In contrast, tree and shrub canopies seem to provide conditions that enable seeds of some species to overcome dormancy at other times of the year. Only 41% of taxa in soil samples from *A. papyrocarpa* recorded their highest emergence in autumn,

and of these 89% were annuals. The remaining taxa were relatively evenly distributed across soil samples collected at the end of winter, spring and summer. Similar patterns were also observed in soils beneath *A. vesicaria* ssp. variabilis and *M. sedifolia*. These results show further evidence for the importance of trees and shrubs to annual plant species, as their presence may expand opportunities for seedling emergence when conditions become suitable (Shachak,Boeken et al. 2008).

Conclusions

This research highlights the important role played by arid zone trees and shrubs in shaping the diversity of annual and perennial understorey plants. Understanding these roles has implications for arid land management and in particular, attempts to reinstate maximum biodiversity levels in disturbed and degraded areas. The results also have implications for seed germination and seedling emergence under climate change conditions, as seeds that require lower temperatures to break dormancy and germinate may become restricted to tree and shrub canopies, which remain cooler for longer periods throughout the year.

Chapter 5
Effects of the Biological Soil Crust on Seedling Emergence in an *Acacia papyrocarpa* Open Woodland

Species of lichen *(Psora* sp.) in the biological soil crust, 2010. Photo E Steggles.

Effects of the Biological Soil Crust on Seedling Emergence in an *Acacia papyrocarpa* Open Woodland

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Abstract

Biological soil crusts (BSCs) are key components of arid environments and they play a major role in determining the structure and function of arid landscapes. Investigating the role of BSCs and their influence on seedling emergence is essential to improving our understanding of arid ecosystems. Such information is also pertinent for managing and restoring ecosystems where the BSC is lost or disturbed through activities such as grazing and mining.

Research was undertaken to investigate interactions between BSCs and vascular plants in an *Acacia papyrocarpa* Benth. (Western Myall) open woodland. Field and glasshouse experiments were used to study the effects of late successional stage BSC on seedling emergence, and seed extraction was used to examine differences in seed accumulation between these two areas. Laboratory experiments were used to explore potential allelopathic effects of early and late successional stage BSCs on seed germination.

Results from field and glasshouse experiments showed that BSC physically inhibited seedlings, as emergence increased when the BSC was disturbed in both field and glasshouse experiments. Seeds also accumulated differently between areas with and without BSC, since less propagules were extracted from soil seed banks beneath crust. Propagule size was a critical factor, with differences observed in propagules of medium (0.7-2.0 mm) and large (2.0-6.1 mm) sizes. The crust therefore, plays a pivotal role in influencing patterns of soil seed bank distribution.

Leachates of late successional stage BSC produced allelopathic effects on the germination of three species (*Enneapogon cylindricus*, *Lepidium phlebopetalum* and *Rhodanthe floribunda*) but early successional stage BSC accelerated germination in *Lepidium phlebopetalum* by 7 to 14 days. These results indicate that different successional stage crusts affect seed germination differently. Overall, the interactions between BSC and vascular plants were found to be species-specific and often the result of more than one process.

5.1 Introduction

In arid ecosystems where water availability limits plant cover, biological soil crusts (BSCs) often form the final stage of succession in open inter-shrub areas (Belnap,Budel et al. 2001). Crust communities occur as thin layers in the upper most millimetres of the soil surface and consist of abiotic and biological components, including soil particles, mosses, lichens, fungi, algae and cyanobacteria (Lange,Kidron et al. 1992; Eldridge,Zaady et al. 2000; George,Roundy et al. 2003). They are key components of arid ecosystems since they influence nutrient cycling, water infiltration, surface evaporation and moisture storage, and overall they protect the soil against erosion (Lange,Kidron et al. 1992; Eldridge and Leys 2003). Spatial heterogeneity in water distribution in arid ecosystems is to a great extent attributable to the presence of BSCs (Belnap,Prasse et al. 2001). Crusts also influence seed accumulation in the soil seed bank, seed dormancy and germination cues as well as seedling emergence and survival (Deines,Rosentreter et al. 2007; Facelli and Springbett 2009). As such, BSCs play a major role in determining the structure and function of arid landscapes (Mucher,Chartres et al. 1988; Zaady,Gutterman et al. 1997).

Understanding relationships between BSCs and vascular plants helps to identify factors that cause patchiness and spatial patterns in arid ecosystems (Deines,Rosentreter et al. 2007). Past studies have investigated relationships between BSCs and vascular plants, including the ability of crusts to modify soils and influence seed dispersal, seed germination and seedling establishment (Boeken and Shachak 1994; Eldridge 2001; Boeken,Ariza et al. 2004; Hawkes 2004; Li,Jia et al. 2005). The influence of BSCs on soil, ecological processes and vascular plants is known to vary between ecosystems (Eldridge & Greene 1994) and also be species-specific within ecosystems (Escudero,Martinez et al. 2007; Facelli and Springbett 2009).

It is difficult to generalise about the effects of BSCs on seed germination and seedling emergence within a given system, as past research has produced varied results (Prasse and Bornkamm 2000). Crusts can have passive, inhibitory or facilitative effects on seedling emergence (Johansen 1993; Zaady,Gutterman et al. 1997; Facelli and Springbett 2009). For instance, Facelli and Springbett (2009) found that while the presence of BSC significantly reduced seedling emergence for two abundant perennial shrubs (*Atriplex vesicaria* and *Maireana pyramidata*) the latter was far less affected, and this difference could explain its increase under grazing. The annual plant community, which forms a substantial portion of the soil seed bank in arid ecosystems, is the component most likely to be affected by the presence of BSCs and once again the effects can vary between species. Zaady *et al.* (1997) found that germination and seedling emergence of the annual forb *Plantago coronopus* was enhanced by the disturbance and removal of the crust, whereas *Reboudia pinnata* and *Carrichtera annua* were relatively unaffected. The presence of BSC was also found to inhibit the seed germination and

root penetration of two annual grass species, *Bromus tectorum* and *Vulpia microstachys* (Deines,Rosentreter et al. 2007).

The variability of BSC and vascular plant interactions may be attributable to variations in the composition of the crust and the life history stages of both microorganisms and plants. In general, the successional sequence of BSC commences with the establishment of cyanobacteria such as *Microcoleus* spp. followed by algae, which stabilises the soil surface and enables the establishment of lichens and mosses (Belnap and Eldridge 2001). Biological soil crusts are generally categorised into four successional stages: very early, early, mid and late (Belnap and Eldridge 2001). Positive BSC-plant relationships frequently occur during early successional stages of the crust (Eldridge and Greene 1994), and although lichen-dominated BSCs can inhibit seed germination, the degree of impact is also heavily dependent on crust composition (Deines,Rosentreter et al. 2007).

A large proportion of BSC effects on plant community structure is exerted through effects on seedling emergence. Seedling emergence is the culmination of a series of successful events, starting with dispersal of a seed into a 'safe site' where it receives the necessary environmental cues and conditions to overcome dormancy, if required, and to germinate and grow. Biological soil crust and vascular plant interactions often consist of a series of physical, biological and/or chemical processes that vary according to the different stages of seed and seedling development. For instance, cyanobacteria and algae excrete extracellular polysaccharides (EPS) that aggregate soil surface particles and provide prospective safe sites for seeds to accumulate (Eckert,Peterson et al. 1986; Hawkes 2004). However, this tightly-knitted surface may also act as a physical barrier, preventing seeds from entering the soil seed bank and subsequently impeding seedling emergence and root penetration into the soil (Deines,Rosentreter et al. 2007). For example, higher seed densities were found in areas with disturbed crusts or bare surfaces than in intact crusts in the Tengger Desert in China (Li,Jia et al. 2005).

Crusts often modify water availability, temperature and pH of soils, and this greatly affects the conditions required for seeds to overcome dormancy and germinate (Belnap,Prasse et al. 2001). Soil chemical environments are also controlled by the presence of BSCs, which in turn affects the ability of seedlings to establish and survive (Harper and Belnap 2001). Chemical interactions between organisms that form the crust and vascular plants are major processes in arid ecosystems and their effects can vary. Lichens, in particular, are known to produce chemicals that reduce microbial damage to seeds by inhibiting the growth of soil bacteria and fungi; however, these chemicals may also inhibit seed germination and seedling growth in some plants (Hawkes 2004). Conversely, the removal of live cyanobacterial components has been found to inhibit germination and seedling emergence in *Reboudia pinnata* and *Carrichtera annua* (Zaady, Gutterman et al. 1997).

Many studies have investigated the effects of different crust types and crust disturbance on seed germination and seedling emergence in arid ecosystems (Prasse and Bornkamm 2000; Hawkes 2004; Li,Jia et al. 2005; Facelli and Springbett 2009). Some have also combined a range of experiments to investigate various additional mechanisms such as seed accumulation patterns beneath areas with crust (intact and disturbed) and without crust (Prasse and Bornkamm 2000; Li,Jia et al. 2005) and allelopathic effects on seed germination (Hawkes 2004). It is often difficult to isolate interactions from each other, and the strength of the research presented here, is the comprehensive study of multiple BSC-plant interactions. This research combines various experiments to investigate the effect of crust presence and disturbance on seedling emergence and accumulation of propagules within the soil seed bank, as well as allelopathic effects on seed germination.

Understanding BSC and vascular plant interactions is important for the management and restoration of ecosystems where the BSC is lost or disturbed through activities such as grazing and mining. For instance, mine-site restoration depends heavily on the ability of seeds to germinate and seedlings to establish in areas where the BSC has been disturbed, mixed and stockpiled with topsoil seed banks. Biological soil crusts have the capacity to facilitate plant succession to later seres (Bowker 2007) and cyanobacteria provide the cohesive quality of BSCs that enables soil surfaces to withstand erosion (Belnap,Budel et al. 2001). Biological soil crusts are often underexploited as a resource for restoration projects, yet they are critical for re-establishing a functioning ecosystem in the long-term (Bowker 2007; Doudle 2010).

In this study, a series of experiments were conducted to investigate interactions between the BSC and vascular plants in *A. papyrocarpa* open woodland. The goal was to investigate several mechanisms in order to tease apart there effects and importance. The research aimed to answer three key questions: (1) How does late successional stage BSC influence seedling emergence? (2) Does the disturbance of late stage BSC promote seedling emergence? (3) Can early successional stage BSC facilitate seed germination in some species?

5.2 Methods

Study Site

The study site (30°50’17.99”S and 132°12’10.37”E) was located in Yellabinna Regional Reserve, which covers 25,227 km^2 and extends north and north-west of the coastal town of Ceduna in South Australia (Figure 3-2). Mean monthly maximum temperatures recorded between 1922 and 1999 range between 18°C in July and 35°C in January, and mean monthly minimum temperatures range between 4°C in July and 18°C in January (Figure 3-1). Overall rainfall is low and generally consistent during winter months, however, large summer rainfall events that often produce floods, occur during La Niña years (Chesterfield and Parsons 1985; Sinclair 2005; Facelli and Chesson 2008). The

mean annual rainfall calculated from data collected between 1904 and 1999 at Tarcoola is approximately 174 mm (BOM 2012).

Acacia papyrocarpa open woodlands are generally associated with deep calcareous sandy loam soils consisting of a thick layer of brown sandy loam (average 4 m) overlying calcrete (Specht 1972; Doudle 2010). Soil surfaces are fragile and susceptible to wind erosion and disturbance (OES 2006). The BSC occurs mainly in open inter-shrub areas, with patchy and less frequent occurrences beneath canopies of *A. papyrocarpa* (pers. obs. 2008). Interactions between BSCs and vascular plants are most likely to affect the large community of annual and short-lived plants that exist for much of the year as stored seeds within the soil seed bank.

Biological Soil Crust Successional Stages

The BSC associated with the study site has recently been classified by Doudle (2010) into four successional stage types based on Budel, Darienko et al. (2009). Type 1 (light) forms a hard soil surface with slight discolouration. Type 2 (early-mid) forms a thin, light, irregular covering that lacks species diversity. Type 3 (mid-late) is dark, of medium thickness, forms dense and extensive areas and has medium-level species diversity. Type 4 (late) is dark, thick, species diverse and lichen dominated.

Field and glasshouse experiments investigating interactions between BSC and seedling emergence, compared Type 4 BSC against areas of no crust (i.e. loose sand). Likewise, seed extraction experiments compared soil seed banks beneath these two areas. Laboratory experiments investigating chemical effects of BSC on seed germination compared Type 1 BSC, Type 4 BSC with areas of no crust. Although patches of loose sand (2 - 3 cm deep) were generally overlaying physical crusts intergrading into Type 1 early stage BSC, the depth of sand was considered sufficient to trap seeds and form a soil seed bank different to areas with BSC. These patches were the closest representative areas of no crust.

Seedling Emergence in Patches with Different Crust Cover

A field study was conducted between February 2009 and February 2010 to determine if there were any differences between the emergence of seedlings in patches covered with Type 4 late stage crust and areas with no crust.

Quadrats

At the study site, forty quadrats (50 cm x 50 cm) were established in open inter-shrub patches within a 50 m^2 area. Half of them were in areas originally with Type 4 late stage BSC (i.e. crust) and half in areas of loose sand (i.e. no crust). Individual cages were placed on top of each plot to protect the surface from trampling. Crust and no crust quadrats were paired and half of each quadrat (i.e. 25 cm x 50 cm) was scratched using a block of wood with 50 nails protruding approximately 5 mm. This method lightly

scratched the surface to simulate disturbance without turning over the soil (Figure 5-1). This provided a factorial design with split plots for disturbance. Seedling abundances were recorded towards the end of winter (July and August) when seedling emergence commences, in late spring (November) after peak seedling emergence and again in February of the following year.


(a)


(b)

Figure 5-1. Light scratching of one half of each quadrat with nails < 5mm long (a) and placement of the cage to protect the quadrat (b).

Intact Cores

To separate environmental variables and maximise seedling emergence, the experiment was replicated in a glasshouse using intact soil cores collected in February 2009 (Figure 5-2). Intact cores were collected with sections of PVC pipe (90 mm diameter x 5 cm deep) pushed into the ground and retrieved so that the soil structure, and more importantly, the crust or surface remained undisturbed (Facelli and Springbett 2009). Cores were collected from areas with Type 4 late stage BSC and loose sand to replicate the field study. After placing the cores in the glasshouse, half of the cores of each type had their surfaces lightly scratched using five nails (5 mm long) to simulate disturbance (Figure 5-3 b). The cores were watered by automatic overhead sprayers twice a day for ten minutes to maintain a constant level of moisture. Cores were moved within the glasshouse once a week to avoid position effects. Seedling emergence was recorded weekly, and the experiment ceased after four weeks as moss had started to grow on the surfaces of some cores.


(a)


(b)

Figure 5-2 Extracting soil cores. Petri dish lid protects the crust surface (a) and placing the base onto the soil core (b).

Seed Extraction

Seeds were extracted from soil samples collected in patches with different lichen cover to determine whether differences in the soil seed bank can contribute to differences in seedling emergence. Fifteen soil samples were collected in February 2010 from an area adjacent to the field experiment. Each sample consisted of 10 small cores 5 cm x 5 cm and 3 cm deep. These were collected from areas with Type 4 late stage BSC (i.e. crust) and loose sand (i.e. no crust). Samples were transported to a drying room (22 ± 2°C) where they were stored for approximately nine months prior to seed extraction.

Samples were sieved (2800 µm and 1400 µm) to break up aggregates and thoroughly mixed. A 200 g sub-sample was then placed into a two-litre plastic container with one litre of tap water. A modified Malone (1967) floatation method was used: air pressurised through an aquarium air stone aerated samples and floated organic matter, including propagules, to the surface. The organic matter was then scooped from the solution and air dried. A dissecting microscope was used to inspect samples for the presence of propagules. Each propagule was photographed and measured (i.e. maximum lateral and longitudinal dimensions) and divided into viable and non-viable categories based on whether they were intact (viable), shrivelled or damaged (non-viable).

Seedling Emergence from Intact Cores with or without Crust

The PVC cores used in the previous experiment were used to determine precise emergence rates of seeds of known viability and germination capacity beneath crusts. Seeds were inserted into freshly collected intact cores, following the procedure established by Facelli and Springbett (2009).

Five species were selected to represent different plant families that were locally abundant and that naturally occurred in inter-shrub areas with BSC (Table 5-1). Seed availability and an understanding of dormancy-breaking requirements were also considered when choosing species (refer to chapters 6 and 7 of this thesis). Seeds were

collected from > 50 plants per species and kept in paper bags to dry under ambient conditions. Cleaning was required for some collections to remove vegetative material and invertebrates. All seed collections were then transferred to a controlled environment room where they were stored in the dark at 15 ± 2°C with 15% relative humidity.

Dispersal units were left intact for this experiment to reflect their most likely state under natural conditions. Only healthy-looking fruits and seeds were included in the experiments. Four replicates of 25 seeds per species were cut-tested to determine the viability of seed collections. Embryo types, based on categories defined by Martin (1946), were assessed by dissecting approximately six seeds that had imbibed water for several hours. Seeds were cut both longitudinally and transversally, with an inspection of embryo size and shape made under a dissecting microscope (Pound,Facelli et al. 2009).

Table 5-1 Species chosen for insertion into cores and germination experiments, with details of pulse treatments used to overcome physiological dormancy and maximise germination.

Species and Family	Date Collected and Storage Time	Dispersal Unit Tested	Seed Embryo	Viability (%)	Pre-Treatments
Enneapogon cylindricus N.T. Burb. Gramineae	Feb 2009 14 months	Caryopsis	Lateral	98	GA_3 250mg/L (0.7mM) pH 3.0 for 24 hours
Eriochiton sclerolaenoides F. Muell. Chenopodiaceae	Oct 2008 19 months	Fruit	Peripheral	80	None
Lepidium phlebopetalum F. Muell. Cruciferae	Sept 2007 32 months	Seed	Bent	99	GA_3 500 mg/L (1.4mM) pH 2.9 for 24 hours
Maireana trichoptera J. M. Black Chenopodiaceae	Oct 2008 19 months	Fruit	Peripheral	96	GA_3 250mg/L (0.7mM) pH 3.0 for 24 hours
Rhodanthe floribunda (DC) Paul G. Wilson Compositae	Oct 2007 31 months	Achene	Spathulate	99	GA_3 500 mg/L (1.4mM) pH 2.9 for 24 hours

Fresh cores were collected using identical methods for retrieving intact soil cores described previously, and these freshly collected cores were then transported to a drying room (22 ± 2°C) where they were stored for approximately three months. Cores were moistened prior to inserting the seeds. Four replicate cores were used for each

species, with 25 seeds arranged in five rows < 10 mm below the soil surface of each individual core. This was done carefully by inserting them into pre-drilled holes in the sides of the cores to avoid cracking the surface (Figure 5-3 a). Emergence from Type 4 late stage BSC (i.e. crust) and loose sand (i.e. no crust) were compared in this experiment. Half of the cores of each type had their surface scratched and the other half had their surface left intact (Figure 5-3 b). Each combination of factors was replicated four times. Cores were placed into the glasshouse and watered by automatic overhead sprayers twice a day for ten minutes to maintain a constant level of moisture (Figure 5-3 c). Seedling emergence was recorded once a week. The experiment ceased after four weeks as moss had started to grow on the surfaces of some cores.

(a) (b) (c)

Figure 5-3 Inserting seeds into soil cores (a); scratching the surface (b); and the placement of replicates into the glasshouse (c).

Seed Germination - Effects of Crust Leachates

To determine whether BSC has the potential to inhibit germination through chemical leachates, we applied under laboratory conditions crust leachates to seeds of the five species used in the previous experiment. Crust samples were collected in February 2010 from a 50 m^2 area adjacent to the field experiment site. A handheld garden spade was used to carefully scrape the soil surface < 5 mm deep. Three soil surface types were collected: Type 1 early stage BSC, Type 4 late stage BSC and loose sand (Figure 5-4 a, b & c). Four 500 g bags were collected, with each bag constituting one replicate. Samples were transported to a drying room (22 ± 2°C) where they were stored for approximately three months prior to the commencement of experiments.

(a) (b) (c)

Figure 5-4 Three different soil surfaces were used to study effects of BSC leachates on seed germination: type 1 early stage BSC (a), type 4 late stage BSC (b) and loose sand (c).

Samples were passed through 3350 µm, 2360 µm and 1700 µm sieves in order to break down aggregates and mix samples thoroughly. Approximately 25 g of crust sample was used to fill the base of each 70 mm glass Petri dish, which was then levelled off and covered with a single Whatman™ filter paper. A treatment of sterilised crust was added to evaluate the effect of living lichens on germination. This treatment involved microwaving samples for ten seconds to eradicate BSC components.

Seeds of selected species were then carefully placed on top of the filter paper and irrigated with sterilised reversed osmosis (SRO) water. Four replicates of 25 seeds were used in each treatment (Table 5-2). Clear plastic was wrapped around each stack of four replicate Petri dishes to minimise moisture loss. Germination was recorded weekly for six weeks and defined as radical emergence ≥ half the length of the dispersal unit. At the time of scoring, each Petri dish was repositioned within its stack as well as within the incubator, to avoid potential position effects.

Table 5-2 Summary of the seven treatments used to investigate the effects of BSC leachates on seed germination.

Treatments	Soil Surface	Sterilisation Microwave Oven	Incubator Conditions and Irrigation
1	Filter paper (control)	None	Constant 15°C 12 hour light period Weekly irrigation 1ml SRO water
2	Type 4 late stage BSC	None	
3		10 seconds	
4	Type 1 early stage BSC	None	
5		10 seconds	
6	Loose sand (no crust)	None	
7		10 seconds	

Statistical analysis

Seedling Emergence – Quadrats and Intact Cores

Non-parametric permutational multivariate analysis of variance test - PerMANOVA (Anderson 2007) was used to analyse data, with alpha 0.05 used to determine significance (PerMANOVA version 1.6). Data were transformed to fourth root and analyses based on Euclidean distances. Pair-wise *a-posteriori* comparisons were undertaken to detect differences between treatments. Data was converted to per m^2 for ease of comparison.

Seed Extraction

Viable and non-viable propagules were distributed equally across three size classes based on maximum longitudinal dimensions: small (0 - 0.7 mm); medium (0.7 - 2.0 mm);

and large (2.0 - 6.1 mm). Analyses were conducted separately on viable and non-viable propagule data, as well as combined viable and non-viable data. PerMANOVA was used to determine differences between size classes and sample areas as above.

Seeding Emergence (Intact Cores) and Germination (Allelopathic Effects)

Mixed between-within subjects analysis of variance (i.e. Split-plot ANOVA) was used to analyse data for each species at 7 and 28 days (cores) and 7 and 42 days (allelopathic effects). Alpha 0.05 was used to determine significance (PASW version 18 2010, formerly SPSS). A positive interaction between time and treatment indicated that the significance of some treatments changed over time. Data from different times were then analysed separately using PerMANOVA (Anderson 2001). Final data were analysed when no positive interaction was found. Data were transformed to fourth root and analyses based on Euclidean distances. Pair-wise *a-posteriori* comparisons were undertaken to detect differences between treatments.

5.3 Results

Seedling Emergence in Patches with Different Crust Cover

Quadrats

Seedlings were classified into nine taxa (Figure 5-5). It was difficult to distinguish between *Cephalipterum drummondii* and *Rhodanthe floribunda* seedlings and consequently these two species were combined. Similarly, some seedlings could not be identified to genera or species levels within the chenopod (Chenopodiaceae) and grasses (Gramineae) groups, and in *Crassula* and *Zygophyllum* genera. The chenopod group includes *Enchylaena tomentosa*, *Eriochiton sclerolaenoides*, *Maireana* spp. and *Sclerolaena* spp.. *Austrostipa nitida* was the most common grass species identified at the study site.

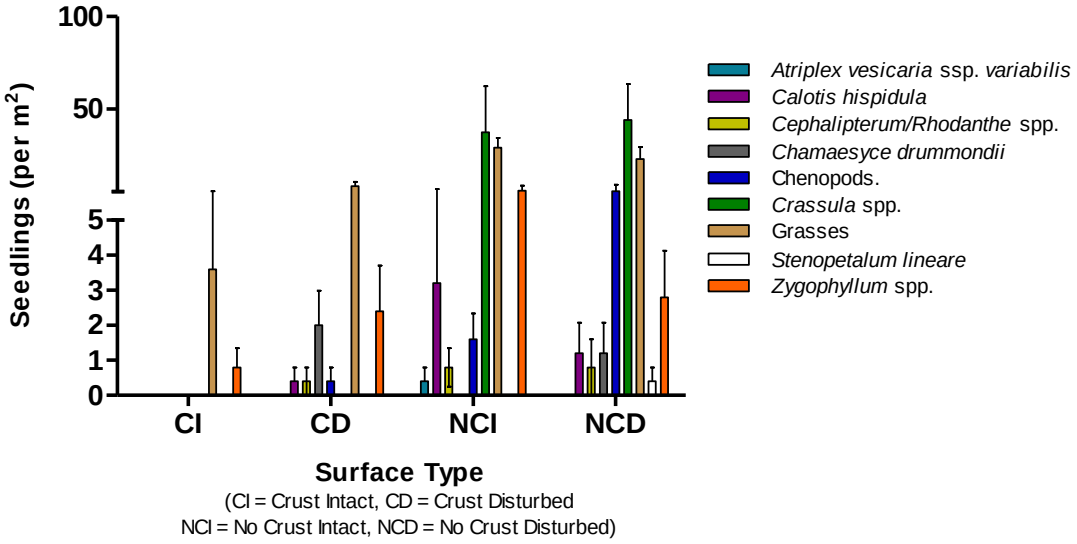

Figure 5-5 Mean number of seedlings per taxon across four different treatments (crust intact and disturbed and no crust intact and disturbed).

Interactions between crust presence and crust disturbance were observed in both the mean number of seedlings recorded (P = 0.0001, df = 3, SS = 16.1495) and the mean number of taxa (P = 0.0001, df = 3, SS = 7.1985) (Figure 5-6). Seedlings recorded in CI quadrats were significantly lower than all other treatments: CD (P = 0.0111, t = 2.8696), NCI (P = 0.0001, t = 6.2194) and NCD (P = 0.0002, t = 5.2948). This pattern was similar for number of taxa, which was lower in CI quadrats than all other treatments: CD (P = 0.0138, t = 2.8100), NCI (P = 0.0001, t = 5.8581) and NCD (P = 0.0003, t = 4.7900). The mean numbers of seedlings were also lower in CD quadrats than NCI (P = 0.0009, t = 3.3799) and NCD (P = 0.0090, t = 2.7610). In contrast, mean number of taxa in CD quadrats was only lower than NCI (P = 0.0141, t = 2.5788). There were no differences in mean seedling numbers (P = 0.8013, t = 0.2513) and taxa (P = 0.5022, t = 0.6859) between NCI and NCD treatments. A comparison of emergence patterns and survival is represented over time (Figure 5-7).

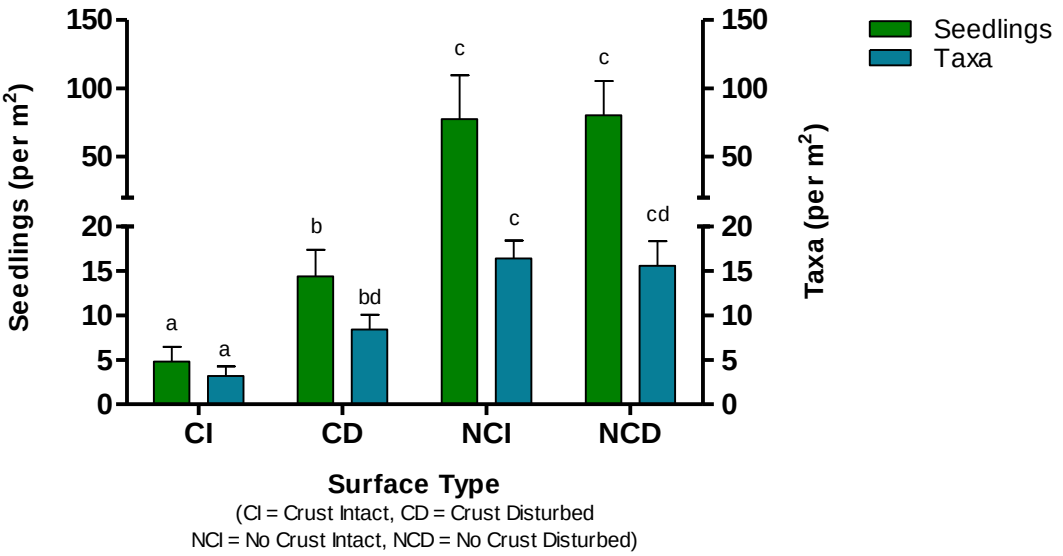

Figure 5-6 Mean numbers of seedlings and taxa recorded in quadrats. Letters denote significant differences between treatments ($P < 0.05$), with seedlings and taxa compared separately.

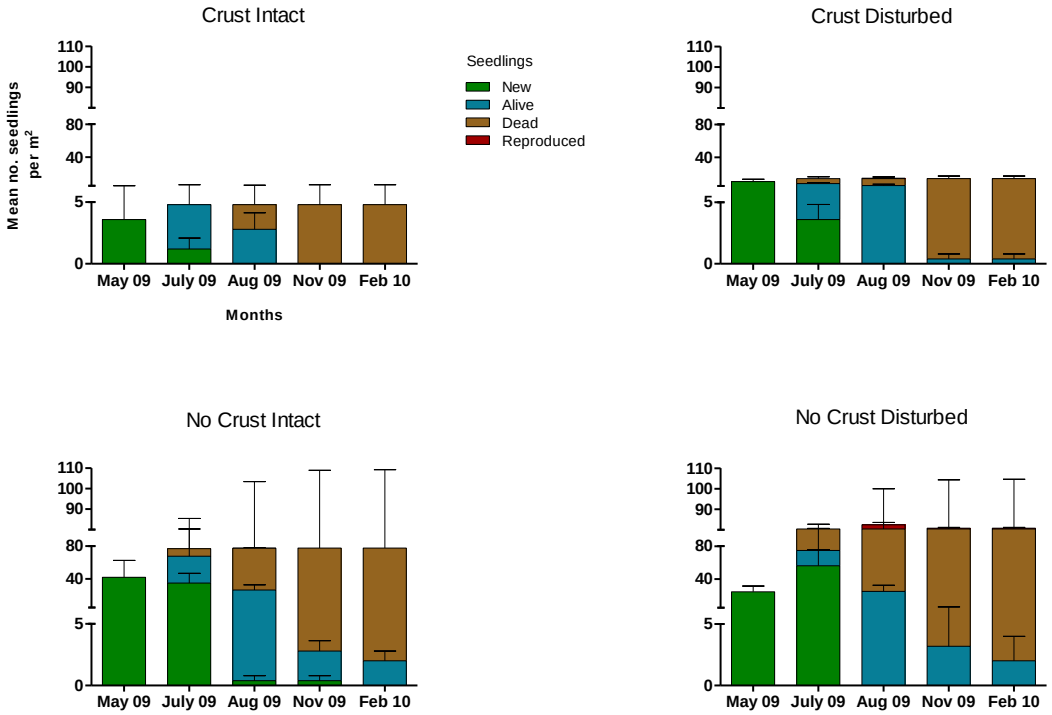

Figure 5-7 Patterns of emergence and survival recorded from each surface type over nine months.

Intact Cores

Seedlings were classified into five groups as defined in the previous field experiment (Figure 5-8).

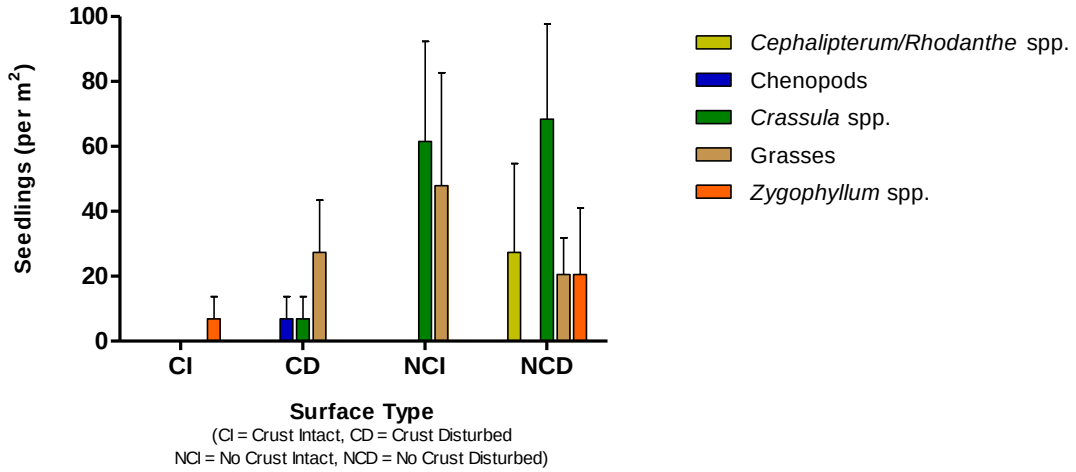

Figure 5-8 Mean number of seedlings per taxon across four different treatments (crust intact and disturbed and no crust intact and disturbed).

Interactions between crust presence and crust disturbance were observed in both the mean number of seedlings recorded (P = 0.0407, df = 3, SS = 1.9290) and the mean number of taxa (P = 0.0406, df = 3, SS = 1.5905) (Figure 5-9). Mean number of seedlings (P = 0.0095, t = 3.0717) and taxa (P = 0.0109, t = 3.0877) were significantly lower in CI core samples than NCD cores.

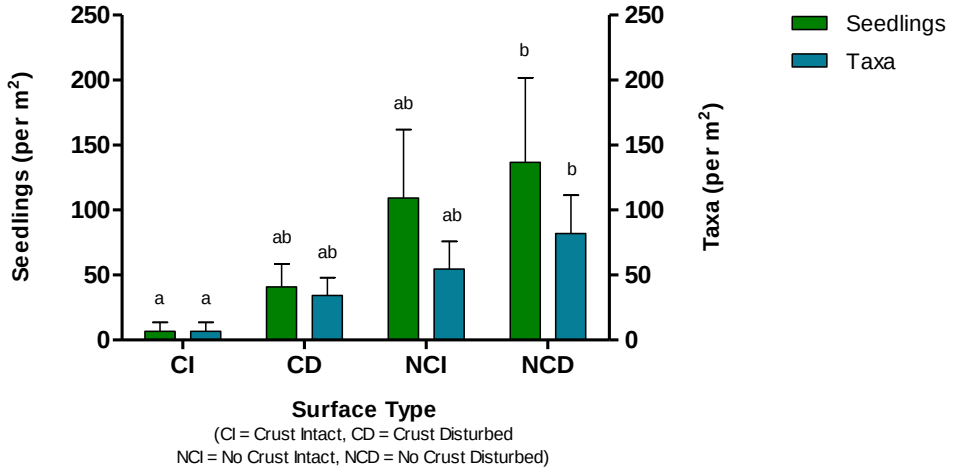

Figure 5-9 Mean number of seedlings and taxa recorded in soil cores. Letters denote significant differences between treatments (P < 0.05), with seedlings and taxa compared separately.

Seed Extraction

More large viable propagules were extracted from soil samples collected in areas with no crust than soil samples collected beneath crust (P = 0.0352, df = 1, SS = 1.3883). The presence of medium-sized propagules was higher in no crust soil samples for both non-viable (P = 0.0085, df = 1, SS = 2.3914) and combined viable and non-viable (P = 0.0253, df = 1, SS = 1.9337) treatments.

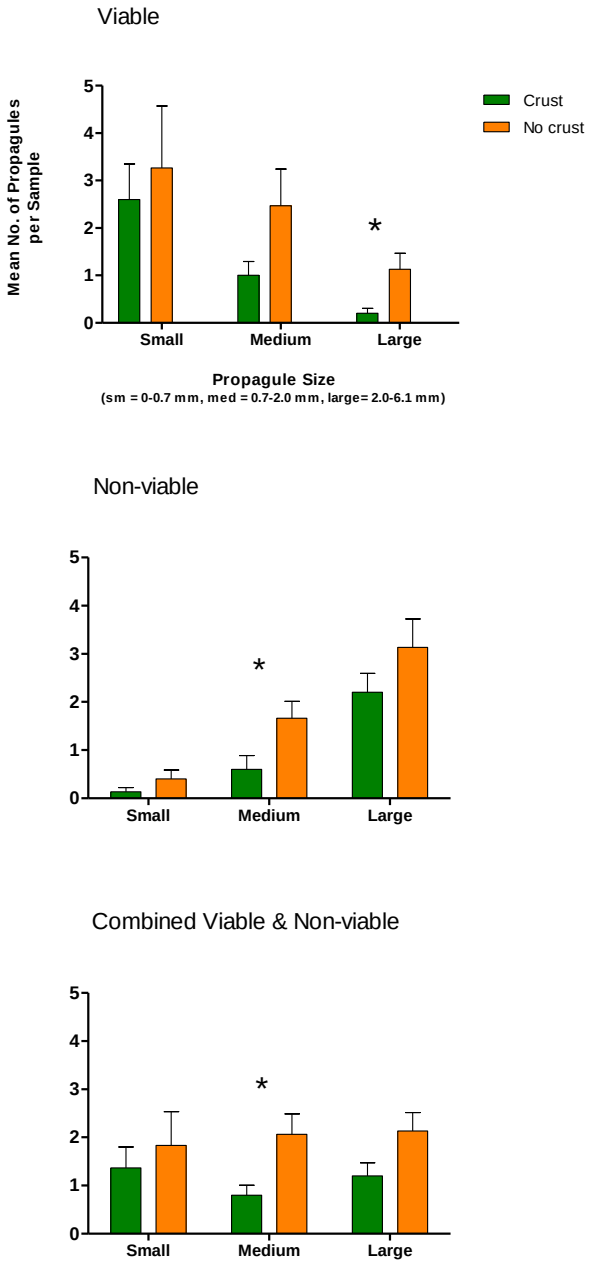

Figure 5-10 Seed extraction results showing different sized propagules accumulate differently between areas with crust and without crust. Asterisks indicate significant differences between treatments ($P < 0.05$).

Seedling Emergence from Intact Cores with or without Crust

The presence of crust and its condition (i.e. disturbed or not disturbed) had different effects on the five species examined (Figure 5-11). *Enneapogon cylindricus* (P = 0.0109, df = 3, SS = 1.8423) achieved only 11% seedling emergence in CI cores compared with 58% in CD cores (P = 0.0285, t = 5.8816). For *E. sclerolaenoides* (P = 0.0025, df = 3, SS = 2.8768), final seedling emergence was significantly lower in CI cores (12%) than all other treatments: CD (62%, P = 0.0285, t = 5.3891), NCI (59%, P = 0.0265, t = 5.1820) and NCD (58%, P = 0.0268, t = 5.6648). Seedling emergence in *Lepidium phlebopetalum* (P = 0.0118, df = 3, SS = 1.4396) was significantly lower in CI cores (23%) than CD cores (67%, P = 0.0285, t = 3.1748), although emergence had not levelled after 28 days in all four treatments. No significant differences were observed between treatments for *M. trichoptera* (P = 0.2454, df = 3, SS = 0.5700). For *R. floribunda* (P = 0.0016, df = 3, SS = 14.7239), no seedlings were recorded in CI cores and this was significantly different to all other treatments: CD (31%, P = 0.0285, t = 11.2596), NCI (19%, P = 0.0265, t = 14.8829 and NCD (34%, P = 0.0268, t = 7.6512).

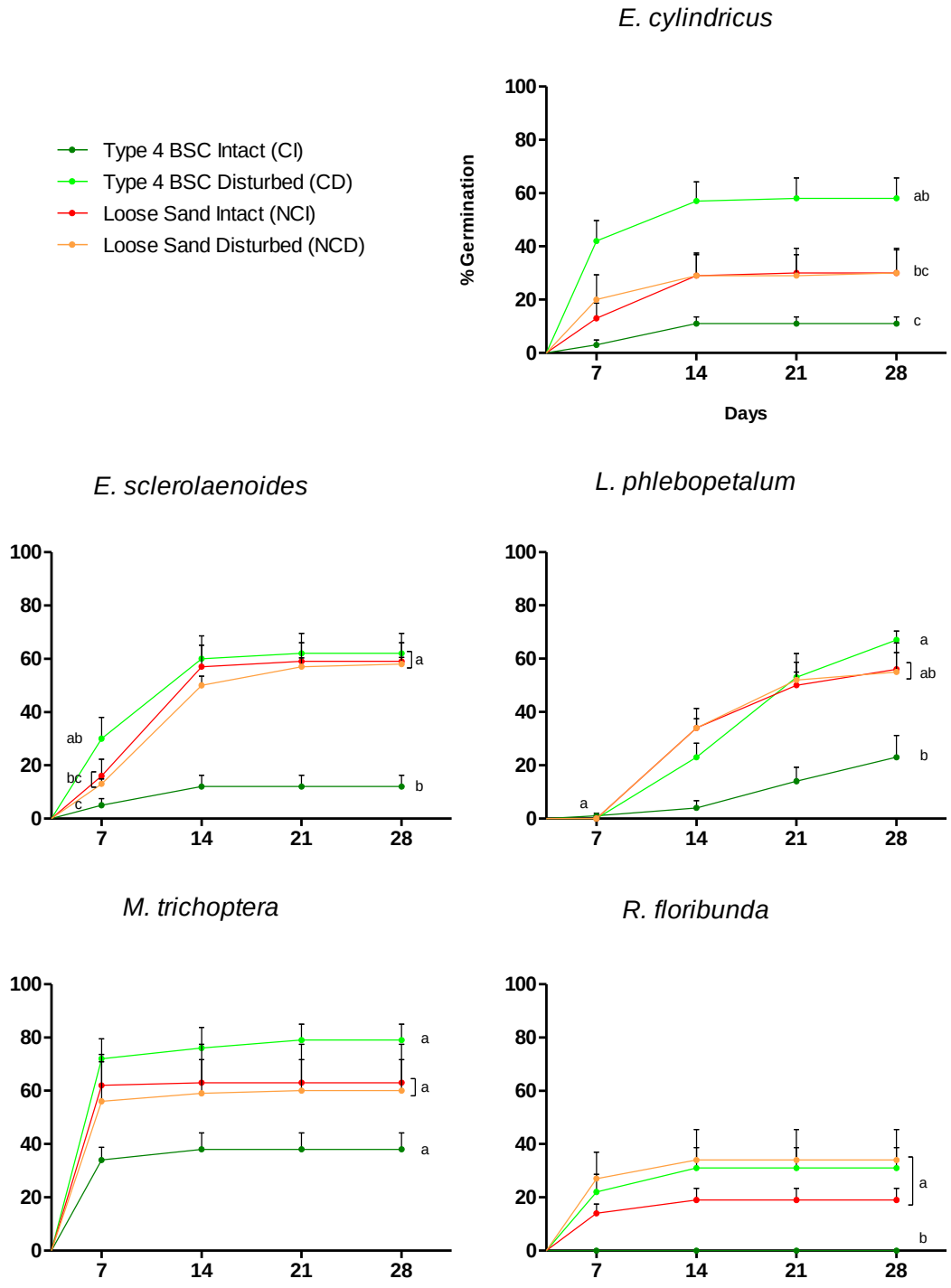

Figure 5-11 Seedling emergence of five different plant species (*E. cylindricus*, *E. sclerolaenoides*, *L. phlebopetalum*, *M. trichoptera* and *R. floribunda*) inserted into soil cores with intact or disturbed Type 4 late stage BSC (crust) and loose sand (no crust). Letters denote significant differences between treatments ($P < 0.05$)

Seed Germination - Effects of Crust Leachates

Crust leachates had different effects on the germination of the five species studied (Figure 5-12). No differences were observed in germination responses between treatments for *E. sclerolaenoides* (P = 0.4554, df = 6, SS = 0.0898) and *M. trichoptera* (P = 0.2911, df = 6, SS=0.0274). For *E. cylindricus* (P = 0.0320, df = 6, SS = 0.0884), there was no difference between the control (84%) and all other treatments (P values > 0.05); however, mean germination in Type 1 BSC - sterilised treatment was higher (94%) than both Type 4 BSC (84%, P = 0.0270, t = 4.9288) and Type 4 BSC – sterilised (74%, P = 0.0287, t = 2.6505). Strong differences between treatments were recorded in the germination responses of *L. phlebopetalum* and *R. floribunda*. For *L. phlebopetalum* (P = 0.0001, df = 6, SS = 47.8546), seed germination after seven days was significantly lower in the control (0%), Type 4 BSC (2%) and Type 4 BSC - sterilised (4%) than all other treatments (74%-90%) (P values < 0.05). After 42 days, however, final results across all treatments were statistically the same (90% - 97%) (P values > 0.05). For *R. floribunda* (P = 0.0001, df = 6, SS = 3.3566), germination was lower in Type 4 BSC (23%) and Type 4 BSC – sterilised (29%) treatments than the control (93%), Type 1 BSC (82%), Type 1 BSC - sterilised (89%), loose sand (55%) and loose sand – sterilised (P values < 0.05).

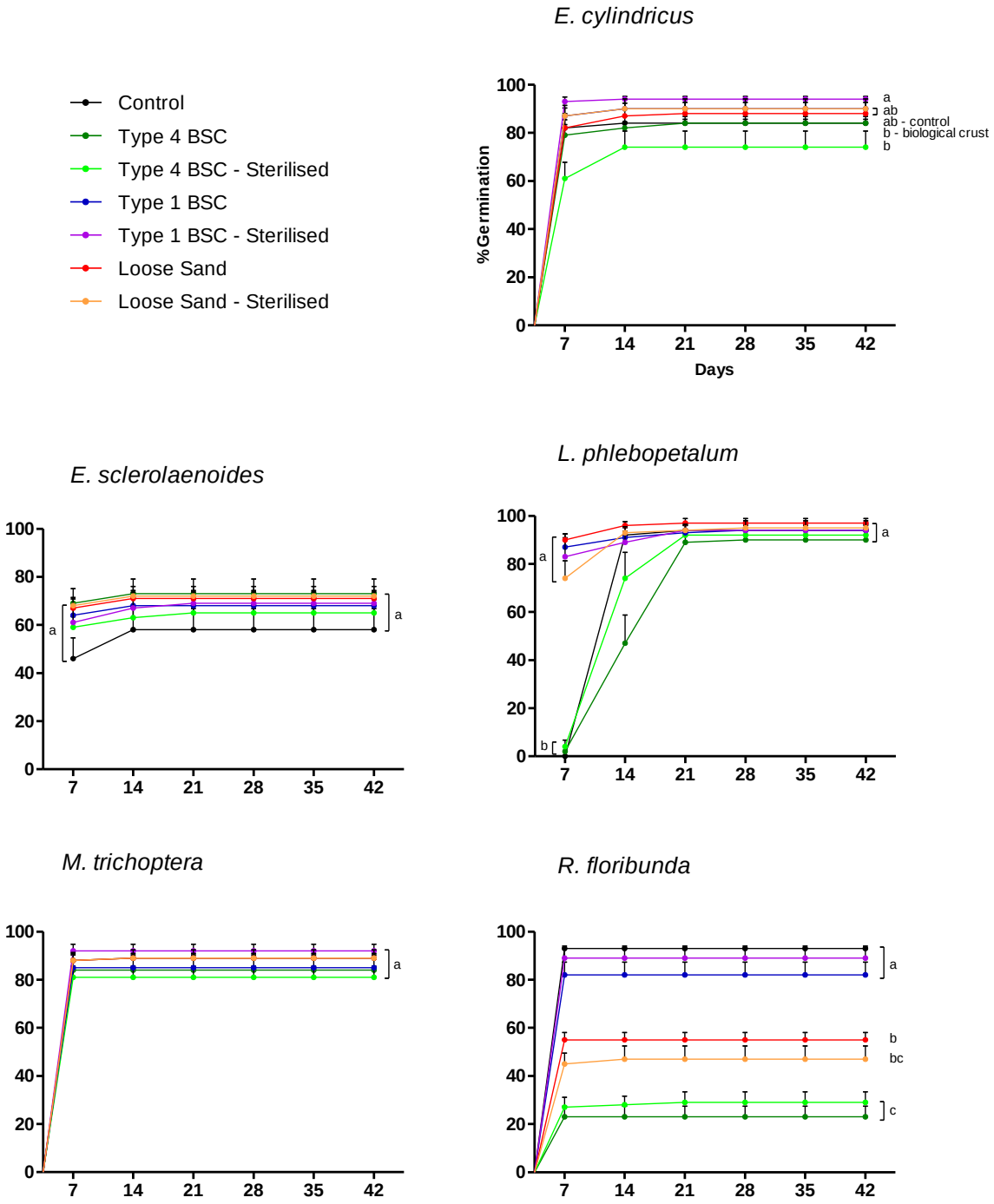

Figure 5-12 Seed germination results of five plant species (*E. cylindricus*, *E. sclerolaenoides*, *L. phlebopetalum*, *M. trichoptera* and *R. floribunda*) after the application of crust leachates treatments. Letters denote significant differences between treatments ($P < 0.05$)

5.4 Discussion

This study identified several mechanisms through which BSCs can affect the plant community in *A. papyrocarpa* open woodlands. Overall, the presence of late successional stage BSC inhibited seedling emergence in this ecosystem. Since the strength of the effects seems to be species specific and the distribution of BSC is patchy, its presence contributes to the spatial heterogeneity of annual plant communities in arid lands, which is a factor known to increase species diversity (Chesson,Gebauer et al. 2004).

As found in previous studies, seedling numbers and seedling diversity were far greater in loose sand treatments than Type 4 late stage BSC (Prasse and Bornkamm 2000; Escudero,Martinez et al. 2007; Facelli and Springbett 2009). Disturbing late stage BSC, without removing it completely, increased seedling emergence and seedling diversity in field quadrats. These results suggest that one of the principal causes of seedling inhibition was physical in nature, since this manipulation would not have removed the chemical effects of BSC. Overall, the core experiment produced similar patterns to the field experiment and disturbing areas without crust (i.e. loose sand) did not affect seedling numbers or diversity in either experiment.

In the field, patterns of low seedling survival were similar across all treatments. Although BSC may suppress recruitment in favourable years, it is the variation in levels of precipitation that has the over-riding impact on recruitment success (Hawkes and Menges 2003). This is important as it suggests that BSCs can contribute to covariance between environmental conditions and densities, which is an ingredient of the storage effect (Facelli,Chesson et al. 2005).

The results from seed extraction show the capacity of BSC to influence the composition of the soil seed bank. The presence of medium-sized (non-viable and combined data) and large-sized (viable) propagules was reduced in the soil seed bank beneath BSC. Boeken and Shachak (1994) also observed that larger propagules were more abundant in pits and loose soil mounds than in the undisturbed crust matrix in a semiarid shrubland in the Negev. Other studies comparing the germinable soil seed bank between areas with crust (intact and disturbed) and without crust, found that intact crust reduces the capacity of propagules to enter the soil seed bank (Prasse and Bornkamm 2000; Li,Jia et al. 2005). Li, Jia et al. (2005) found seed size to be a determinant factor in patterns of seed accumulation, with higher germination results from smaller-sized *Eragrostis poaeoides* seeds than larger-seeded *Bassia dasyphylla* in soils with crust.

Plants have developed a range of strategies to ensure their inclusion in the soil seed bank. These include seed shape and a range of dispersal mechanisms, such as trypanocarpy, to assist penetration through the soil surface. Many seeds and

propagules are tapered and cylindrical, with some having appendages that twist themselves into the soil by hygroscopic movement i.e. *Erodium* spp. and *Austrostipa* spp. (Schoning,Espadaler et al. 2004). The hard impenetrable nature of BSCs, particularly late successional stages, can form a physical barrier to propagules (Deines,Rosentreter et al. 2007). This suggests that the soil seed banks beneath BSCs may be older than in areas without BSCs. Seeds would more easily accumulate beneath loose sand and consequently soil seed banks in these areas would be continually replenished, which has important implications for the distribution of annual plant populations.

Variations in seed predation may also contribute to the differences observed between the two types of seed banks. Indeed, Hawkes and Menges (2003) have shown that seed predation is often increased within the lichen matrix. Nevertheless, results from our preliminary investigation of soil seed banks beneath late stage BSC and loose sand has shown strong compositional differences. Further study is needed to determine the effects of other successional stage crusts on patterns of propagule distribution and accumulation, as seedling densities vary beneath different successional stage crusts due to varying entrapment abilities of crusts (Su,Li et al. 2007).

Evidence of BSC physically inhibiting seedling emergence was also observed at a species level, when seeds of five species were inserted into soil cores. Seedling emergence was lower in CI cores than CD cores (i.e. *E. cylindricus* and *L. phlebopetalum*) and all other core types (i.e. *E. sclerolaenoides* and *R. floribunda*). When considered with results from seed germination experiments, it becomes evident that the influence of BSC on seedling emergence is both species-specific and often results from the combination of more than one type of process.

Results from experiments showed that BSC had no allelopathic effects on the seed germination of two chenopod species, *E. sclerolaenoides* and *M. trichoptera*. For these two species, the effect of late stage BSC on seedling emergence is therefore assumed to be physical inhibition of seedling emergence and potential exclusion from the soil seed bank due to large propagule size. It is possible that all of the five species examined in our experiments would be restricted from entering the soil seed bank by late stage BSC, due to their large propagule size (> 7 mm long).

The remaining three species showed evidence that BSC leachates affect seed germination. Overall seed germination of *E. cylindricus* was very high (> 70%) across all treatments, including the control, however, germination was significantly higher when seeds were placed on Type 1-sterilised BSC than on Type 4 BSC and Type 4-sterilised BSC. These results are difficult to explain, however, they do suggest that this species may have a degree of susceptibility to chemicals and extracellular polysaccharides (EPS) released by lichens, cyanobacteria and algae, which are known to inhibit seed germination in some plants (Hawkes 2004). Early-colonising lichen species often

disperse photobiont-containing tissue in order to avoid the need to find photobionts on site (Belnap,Prasse et al. 2001). Biochemical thermolabile compounds should have been destroyed in the sterilising process and their production by micro-organisms stopped. As a consequence the effects of microbes on seed germination should have been prevented. Some chemicals or elements, however, may have been released during the sterilising process and this may have affected germination of *E. cylindricus* seeds in the two sterilised treatments.

Germination of *L. phlebopetalum* seeds was accelerated in all Type 1 early stage BSC and loose sand treatments, with germination in the control and Type 4 late stage BSC treatments delayed between 7 and 14 days. This result provides additional evidence that early-successional stage BSC facilitates seed germination in some species (Eldridge and Greene 1994). Type 1 BSC had a neutral effect on *R. floribunda* seed germination, with results highest in Type 1 BSC treatments along with the control. Clear delineations were observed between results from loose sand and Type 4 BSC treatments, with both Type 4 BSC treatments showing strong negative effects on the seed germination of *R. floribunda*. Only moderate germination results were recorded in both loose sand treatments, suggesting that there is some component of the sand substrate that inhibits germination in this species, and this may warrant further investigation.

Interactions between BSCs and vascular plants are dynamic and complex. The patchy distribution of BSCs can affect water distribution, seed accumulation within the soil seed bank, conditions for seeds to overcome dormancy and to germinate, as well as seedling emergence. Any increase in vascular plant cover is likely to reduce microbiota cover as a result of increased competition for light and moisture (Eldridge and Greene 1994). Conversely, in arid zones where water availability is low and irregular, BSCs provide an external mechanism through which vascular plants maintain a fraction of seed within the soil seed bank, thus protecting populations against stochastic events such as extended droughts. This also enables fewer, yet stronger plants, to compete with more success for the scarce resources available to them. This series of mechanisms ultimately determines plant communities by influencing species composition, species densities and resource availability. Such heterogeneity in the distribution of species, and hence to species coexistence, enhances diversity within ecosystems.

Conclusions

This research has provided insight into BSC and vascular plant interactions within an intact, spatially and temporally patterned arid ecosystem. Small-scale and well-timed disturbance of BSCs may increase emergence and potentially the establishment of vascular plants (Prasse and Bornkamm 2000). Frequency, intensity and timing is critical however, as increased frequency of disturbance may favour exotic annual plant invasion (Belnap,Prasse et al. 2001). Crusts play a fundamental role in maintaining resilience within arid ecosystems (Lange,Kidron et al. 1992; Eldridge and Leys 2003). They have

the potential to improve rehabilitation outcomes in areas that are disturbed through activities such as mining, through their ability to stabilise soil surfaces as well as capture and retain atmospheric and landscape resources (Doudle 2010).

Further research is needed to determine composition and successional stage effects of BSC on seedling emergence and establishment. Belnap, Prasse et al. (2001) highlight the need to separate crust effects from other influences such as inorganic components of the crust, soil types and climate patterns. Taxonomy of BSCs remains difficult, particularly microbial components and understanding seasonal fluctuations of ephemeral mosses, cyanobacteria and algal populations (Lange,Kidron et al. 1992; Belnap,Budel et al. 2001; George,Roundy et al. 2003). Investigating such finer-scale characteristics of the BSC and their effects on vascular plant germination, seedling emergence and establishment is the next step required in this study.

Chapter 6
Applying Pulse Treatments to Promote Seed Germination in Three Species of *Enneapogon*

E. avenaceus

E. avenaceus caryopsis

E. avenaceus seeds

Applying Pulse Treatments to Promote Seed Germination in Three Species of *Enneapogon*

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Abstract

Grasses play an important ecological role in arid lands by contributing to the annual productivity of these ecosystems. Their re-establishment after disturbance is essential given that they often stabilise soil surfaces and assist to minimise erosion. *Enneapogon* spp. (Gramineae) are important grassy understorey components of *Acacia papyrocarpa* Benth. (Western Myall) open woodland. This paper describes a preliminary investigation into physiological dormancy (PD) in seeds of *E. avenaceus, E. caerulescens* var. *caerulescens* and *E. cylindricus*. A series of pulse treatments using GA_3, KNO_3 and dry heat were applied to seeds, which were then incubated at temperatures and light conditions simulating spring/autumn, summer and winter. Experiments were aimed at promoting germination for inclusion of these species in future seed germination research.

All three species displayed evidence of PD. Most KNO_3 and dry heat pulse treatments failed to promote germination in all species. *Enneapogon avenaceus* and *E. cylindricus* exhibited non-deep PD, as pulse applications of GA_3 overcame dormancy in these species. Both species were capable of germinating under a variety of temperature and light conditions once dormancy was overcome. The use of GA_3 as a possible seed priming agent for these species could be investigated for restoration projects if required. Seed germination in *E. caerulescens* var. *caerulescens* was extremely low, which is indicative of intermediate PD or deep PD.

A more comprehensive study using fresh seeds is needed to fully understand the after-ripening, dormancy and germination requirements in these species. This study forms a basis from which further seed germination research can be undertaken to assist ecological researchers and restoration practitioners.

6.1 Introduction

Grasses contribute to the annual productivity of arid ecosystems and are important components of the ephemeral plant community. Larger perennial grasses provide structure to the ground layer and annual grasses contribute to the diversity of the understorey. Their re-establishment after disturbance is an important ecological process given that grasses often contribute to stabilising soil surfaces and assist to minimise erosion (Huxtable,Koen et al. 2005). Australian arid ecosystems are increasingly being disturbed through activities such as mining. Improving our understanding of the seed biology of grass species is therefore essential for the restoration of arid ecosystems after disturbance. Knowledge of how to pre-treat seeds to bypass dormancy is an initial step towards overcoming the many challenges faced when restoring low rainfall ecosystems, either for the purpose of seed priming or to maximise germination for further experimental research.

This study made the most of an opportunity to collect seeds of three species of *Enneapogon* (Gramineae) simultaneously and from a localised area within an *Acacia papyrocarpa* open woodland. This ecosystem is the dominant vegetation community at a mineral sands mine in Yellabinna Regional Reserve, South Australia. *Enneapogon avenaceus* (Lindl.) C.E.Hubb, *E. caerulescens* (Gaudich.) N.T.Burb. var. *caerulescens and E. cylindricus* N.T.Burb., are tufted grasses with numerous erect or spreading stems, which generally germinate and establish quickly in response to good summer rains. All three species can dominate large and disturbed areas and are consequently recognised as good soil stabilisers (Kutsche and Lay 2003).

The variability and irregularity of rainfall in arid areas of Australia, where so many species of *Enneapogon* occur, may account for their variable life cycles and reproductive strategies. *Enneapogon avenaceus* and *E. caerulescens* var. *caerulescens* are annual or short-lived perennial grasses, and although *E. cylindricus* is perennial, it often behaves like an annual (Burbridge 1941; Kakudidi,Lazarides et al. 1988; Jessop,Dashorst et al. 2006). Both *E. cylindricus* and *E. caerulescens* var. *caerulescens* produce cleistogamous spikelets (Burbridge 1941; Kakudidi,Lazarides et al. 1988), which has an advantage in arid ecosystems in that it requires less plant resources to produce. *Enneapogon* seeds exhibit seed dormancy to enable them to remain inactive until environmental conditions are able to support their germination and growth. Previous germination research identified physiological dormancy (PD) in the seeds of *Enneapogon* spp. (Ooi,Auld et al. 2009).

Ooi, Auld et al. (2009) found that irrigating fresh *E. avenaceus* and *E. cylindricus* seeds without pre-treatment produced little or no germination under a range of incubator conditions: winter 16/6°C, spring/autumn 28/14°C and summer 40/20°C. Low germination rates were recorded for *E. cylindricus* (25%) under spring/autumn conditions and 0% germination was recorded for *E. avenaceus*. Under spring/autumn conditions, germination results were improved for *E. cylindricus* when seeds were dry heat treated for 20 days (41%), 55 days (50%) and 70 days (78%) prior to germination trials. Effects of temperature and age

on seed germination of *E. avenaceus* were investigated by Grice, Bowman et al. (1995). Germination was found to be higher when seeds were six months old when compared with seeds aged 12, 18 and 24 months. Unfortunately no comparison was made with fresh seeds and all germination results were < 20%, which suggests that PD had not been overcome. Results from both studies suggest that an after-ripening period may be required in some species of *Enneapogon* for seeds to be able to overcome dormancy and germinate.

Depending on the plant species, the application of gibberellic acid (GA_3) may circumvent certain types of PD and provides researchers with indications as to the type of PD present. In general, seeds with non-deep PD respond to GA_3. Seeds with intermediate PD may or may not respond and seeds with deep PD do not respond to GA_3 (Baskin and Baskin 1998; Baskin and Baskin 2005). Gibberellic acid does not necessarily break dormancy but instead bypasses the natural blocks to germination, whereby germination may be stimulated even though dormancy characteristics are still retained within the seed (Hilhorst and Karssen 1992; Cohn 1996).

Applications of some nitrates may break seed dormancy in some species. Although the ecological significance of this response is not fully understood, the idea of a gap detection mechanism has been explored (Pons 1989). Nitrate concentrations in soil beneath standing vegetation may be lowered by the absorption of nitrate by the plants, and consequently germination cannot be stimulated. When standing vegetation is disturbed or dies back naturally, then nitrate concentrations in the soil increase due to mineralisation and nitrification, with seed germination stimulated as a result (Pons 1989).

In this research, pulse treatments (acute) were chosen in preference to constant application (chronic) with the intent of maximising germination of these species for their inclusion in future seed germination experiments (refer to Chapter 8 of this thesis). Pulse treatments reduce the risk of compromising treatments through chronic applications of GA_3 (Cohn 1996). As many grasses respond well to dry heat (Baskin and Baskin 1998; Ooi,Auld et al. 2009), our research also included two pulse dry heat treatments. Experiments investigated whether pulse treatments could be used to promote germination in three species of *Enneapogon*.

6.2 Methods

Study Site and Species Description

Seeds were collected from a field study site (30°50’17.99”S and 132°12’10.37”E) located in Yellabinna Regional Reserve. The reserve covers 25,227 km^2 and extends north and north-west of the coastal town of Ceduna in South Australia (Figure 3-2). Mean monthly maximum temperatures recorded between 1922 and 1999 range between 18°C in July and 35°C in January, and mean monthly minimum temperatures range between 4°C in July and 18°C in January (Figure 3-1). Overall rainfall is low and generally consistent during winter months, however, large summer rainfall events that often produce floods, occur during La Niña years

(Chesterfield and Parsons 1985; Sinclair 2005; Facelli and Chesson 2008). The mean annual rainfall calculated from data collected between 1904 and 1999 at Tarcoola is approximately 174 mm (BOM 2012).

Acacia papyrocarpa open woodland has an understorey community of perennial chenopod shrubs and short-lived species. Dominant grass genera include *Austrodanthonia*, *Austrostipa* and *Enneapogon* (Pound,Facelli et al. 2009). Grass species occur within close proximity to each other and often establish themselves in the open inter-shrub areas where the biological soil crust is patchy and contains surface cracks (pers. obs. 2009). These surface cracks are thought to provide 'safe sites' through which seeds can accumulate in the soil seed bank until they receive the right environmental cues for them to germinate and establish (Fowler 1988).

Seed Collection and Preparation

Seed collections were made from > 50 plants per species (Table 6-1). Seed maturity was determined by observing natural dispersal of caryopses from adult plants. Seed collections were kept in paper bags and dried under ambient conditions for approximately three weeks. No cleaning of seed collections was required and they were transferred to a controlled environment room where they were stored in the dark at 15 ± 2°C with 15% relative humidity.

Dispersal units were mostly left intact for this experiment to reflect their likely state in the natural system and to avoid damage to seeds. However, the awns of all *Enneapogon* species were removed with scissors as part of seed preparation to assist moisture uptake. Only healthy-looking fruits and seeds were included in experiments.

Four replicates of 25 seeds per species were cut-tested to determine the viability of seed collections. Seeds containing full and healthy white embryos were classified as viable, whilst seeds containing no embryo or an embryo that was shrunken, shrivelled, brown or dried were classified as non-viable. Embryo types, based on categories defined by Martin (1946), were assessed by dissecting approximately six seeds that had imbibed water for several hours. Seeds were cut both longitudinally and transversally, with an inspection of embryo size and shape made under a dissecting microscope (Pound,Facelli et al. 2009). Average seed weights and lengths were calculated from measuring four replicates of 25 seeds per species. Three measurements are provided for *E. avenaceus*, as each propagule contains three different-sized seeds (small, medium and large) inside individual locules.

Table 6-1 Description of *Enneapogon* spp. seed collections including embryo type, viability of seed collections and seed measurements. Each *E. avenaceus* propagule consists of three different-sized seeds (small, medium and large) and measurements for these are recorded separately.

Species and Storage Time	Seed Embryo	Collection Viability (%)	Average Measurements Per Seed ± Standard Error of Mean		
			Weight (g)*	Length (mm)**	Width (mm)**
E. avenaceus 8 months	Lateral	97	2.84^{-4} (small) $\pm 0.7^{-4}$	1.048 ± 0.0078	0.873 ± 0.0089
			4.39^{-4} (medium) $\pm 0.9^{-4}$	1.219 ± 0.0084	1.051 ± 0.0075
			6.14^{-4} (large) $\pm 0.6^{-4}$	1.422 ± 0.0096	1.077 ± 0.009
E. caerulescens var. caerulescens 8 months	Lateral	95	2.860^{-4} $\pm 0.06^{-4}$	1.121 ± 0.009	0.675 ± 0.004
E. cylindricus 8 months	Lateral	98	3.090^{-4} $\pm 0.09^{-4}$	1.262 ± 0.014	0.669 ± 0.008

*Calculated from the mean of total weights for four replicates of 25 seeds.

**Calculated from mean length and width measurements of four replicates of 25 seeds.

Incubator Conditions and Treatments

Three incubator settings were used to determine which conditions would best promote germination, as little was known about the temperature requirements of *Enneapogon* seeds. Incubator settings were used to simulate average seasonal conditions. Ooi, Auld et al. (2009) found that darkness suppressed *E. cylindricus* germination completely and consequently all incubator conditions included a period of light:

1. **Spring/Autumn:** 10°C (12 hours) / 22°C (12 hours) with 12 hours of light
2. **Summer:** 15°C (10 hours) / 30°C (14 hours) with 14 hours of light
3. **Winter:** 5°C (4 hours) / 15°C (20 hours) with 10 hours of light

The pulse treatments applied to seeds consisted of two different concentrations of GA_3, KNO_3 and dry heat. For GA_3 and KNO_3 pulse treatments, four replicates of 25 seeds were folded into Whatman™ filter paper and placed into 10 ml Eppendorf polypropylene tubes with GA_3 (100 or 250 mg/L) or KNO_3 (100 or 250 mg/L) for a period of 24 hours. Absolute ethanol (1 ml EtOH) was used to aid the dissolution of GA_3 into sterilised reverse osmosis (SRO) water. For dry heat pulse treatments, four replicates of 25 seeds were counted into

plastic weigh pots and placed into an oven pre-heated to either 80°C or 90°C for 15 minutes, with each replicate treated separately (Table 6-2).

Table 6-2 Summary of pulse treatments tested to overcome physiological dormancy in *Enneapogon avenaceus, E. caerulescens* var. *caerulescens* and *E. cylindricus* seeds.

Treatment Solutions and Incubator Conditions			Pulse Treatments
Spring/Autumn	**Summer**	**Winter**	
T1: Control	**T8**: Control	**T15**: Control	None
T2: GA_3 100	**T9**: GA_3 100	**T16**: GA_3 100	100mg/L (0.3mM) GA_3 for 24 hours
T3: GA_3 250	**T10**: GA_3 250	**T17**: GA_3 250	250mg/L (0.7mM) GA_3 for 24 hours
T4: KNO_3 100	**T11**: KNO_3 100	**T18**: KNO_3 100	100mg/L (0.1mM) KNO_3 for 24 hours
T5: KNO_3 250	**T12**: KNO_3 250	**T19**: KNO_3 250	250mg/L (0.25mM) KNO_3 for 24 hours
T6: Heat 80°C	**T13**: Heat 80°C	**T20**: Heat 80°C	Dry heat 80°C for 15 minutes
T7: Heat 90°C	**T14**: Heat 90°C	**T21**: Heat 90°C	Dry heat 90°C for 15 minutes

Seed Germination

Four replicates of 25 seeds were used in each treatment. Seeds were placed into 90 mm glass Petri dishes on top of two Whatman™ filter papers. Filter paper and seeds were initially moistened with approximately 2 ml treatment solution as per Table 6-2 and placed into incubators. Plates were removed from incubators and irrigated weekly using an automated pipette, with 1 ml treatment solution added to each Petri dish and the excess removed by using a transfer pipette. Clear plastic was wrapped around each stack of four replicate Petri dishes to minimise moisture loss. Germination was recorded weekly and defined as radical emergence ≥ half the length of the caryopsis, with germinated seeds removed at the time of recording. To avoid potential position effects, each Petri dish was repositioned within its stack as well as within the incubator at the time of scoring.

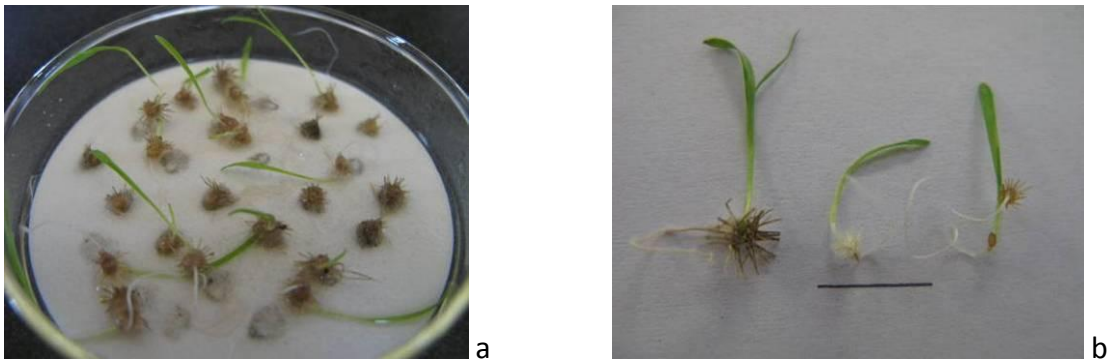
Statistical Analysis

Mixed between-within subjects analysis of variance (i.e. Split-plot ANOVA) was used to analyse data from treatments and controls for each species at 7, 28 and 56 days, with alpha 0.05 used to determine significance (PASW version 18 2010, formerly SPSS). Percentage values were arc-sine transformed prior to analysis to improve normality. A positive interaction between time and treatment indicated that the significance of some treatments changed over time. Data from different times were then analysed separately using non-parametric permutational multivariate analysis of variance tests i.e. PerMANOVA (Anderson 2001). Euclidean distance pair-wise tests were used to differentiate between treatments,

with alpha set at 0.05 (PerMANOVA version 1.6). For each species, statistics are compared within the same time period, across all pulse treatments and three incubator conditions.

6.3 Results

Germination was easily observed in all three *Enneapogon* species (Figure 6-1 a & b). Positive interactions were found between time and treatment in all three species: *E. avenaceus* ($P < 0.05$; F = 7.138; df = 124), *E. caerulescens* var. *caerulescens* ($P = 0.005$; F = 1.853; df = 124) and *E. cylindricus* ($P < 0.05$; F=4.789; df=124), indicating that the significance of some treatments changed over time.



a b

Figure 6-1 *Enneapogon avenaceus* germinants (a) and examples of germinants from each species. From left to right: *E. avenaceus, E. caerulescens* var. *caerulescens* and *E. cylindricus*. Bar = 10mm (b).

E. avenaceus

Final germination results for *E. avenaceus* differed between treatments ($P = 0.0001$, df = 20, SS = 20.4619). Germination was high in all three incubators, indicating that *E. avenaceus* seeds are capable of germinating at varying temperature regimes once dormancy is overcome (Figure 6-2). Final results in the spring/autumn incubator showed that GA_3 pulse treatments (100 mg/L and 250 mg/L) produced consistently higher germination results (68% and 69% respectively) when compared with the control (24%) (P values < 0.05). Results from the GA_3 100 mg/L treatment were significantly higher (59%) than the control (33%) in the summer incubator and GA_3 250 mg/L results were significantly higher (72%) than the control (40%) in the winter incubator (P values < 0.05). Results from controls suggest that a percentage of seeds may not have PD. All KNO_3 and dry heat pulse treatments failed to promote germination in this species.

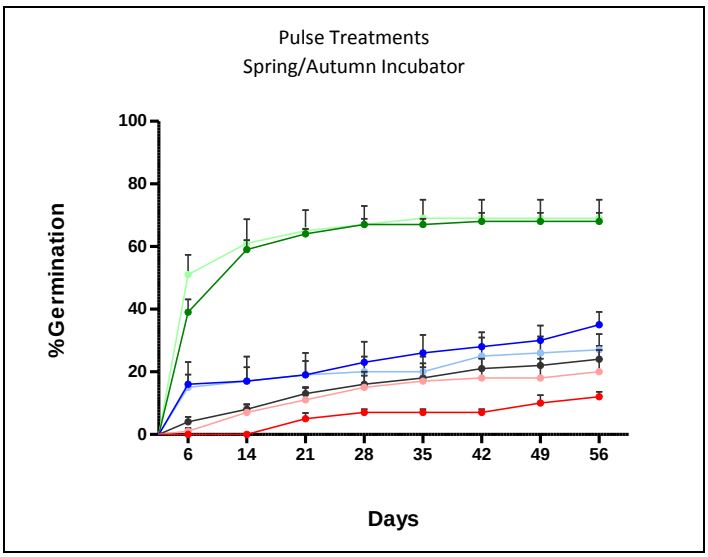
E. caerulescens var. *caerulescens*

Final germination results for *E. caerulescens* var. *caerulescens* differed between treatments ($P = 0.0014$, df = 20, SS = 29.6409). None of the pulse treatments promoted germination in this species (Figure 6-3). In the spring/autumn incubator, results from GA_3 250 mg/L were significantly higher (16%) than results in the control (1%) ($P = 0.0265$, t = 4.3690). In the

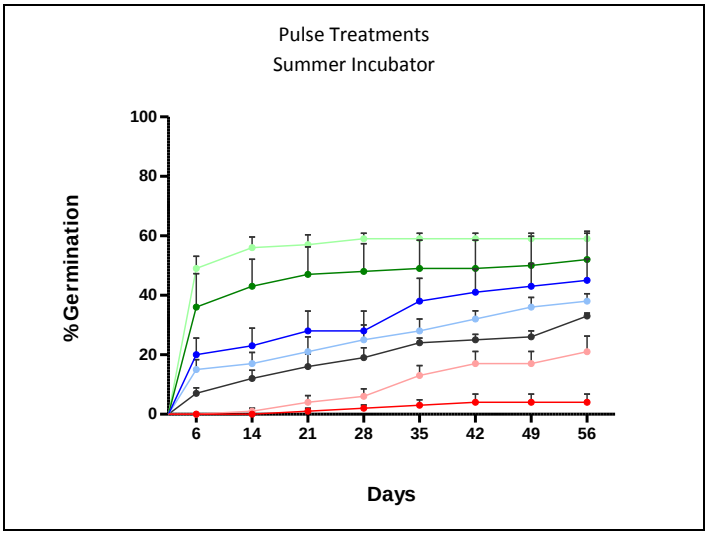
summer incubator, both GA_3 100 mg/L and GA_3 250 mg/L pulse treatments produced significantly higher germination (18% and 24% respectively) when compared with results in the control (1%) (*P* values < 0.05). These results, although significantly higher than controls, left most seed ungerminated, suggesting GA_3 may have produced changes within seeds that were close to a threshold, with some seeds achieving germination whilst others could not.

E. cylindricus

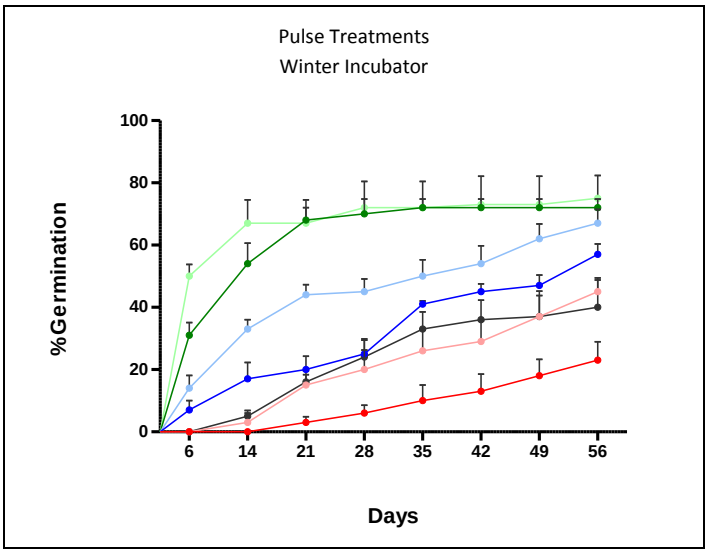
Final germination results for *E. cylindricus* differed between treatments (*P* = 0.0001, df = 20, SS = 46.9774). Germination was consistently higher in all GA_3 100 mg/L and 250 mg/L pulse treatments when compared with controls (*P* values < 0.05) (Figure 6-4). These results indicate that GA_3 was capable of promoting germination in *E. cylindricus* seeds in all incubator conditions. This species shows the capacity to germinate across a range of temperature regimes once dormancy has been overcome. All other KNO_3 and dry heat pulse treatments failed to stimulate germination in this species.



Treatments Spring/Autumn		7 Days	28 Days	56 Days
	T1: Control	EFG	GHIJ	DEF
	T2: GA3 100	A	AB	AB
	T3: GA3 250	AB	A	A
	T4: KNO_3 100	CDE	DFGHIJ	CDE
	T5: KNO_3 250	BCDE	CDEFGHIJ	CD
	T6: Heat 80°C	FG	GHIJK	DEF
	T7: Heat 90°C	G	IK	EF

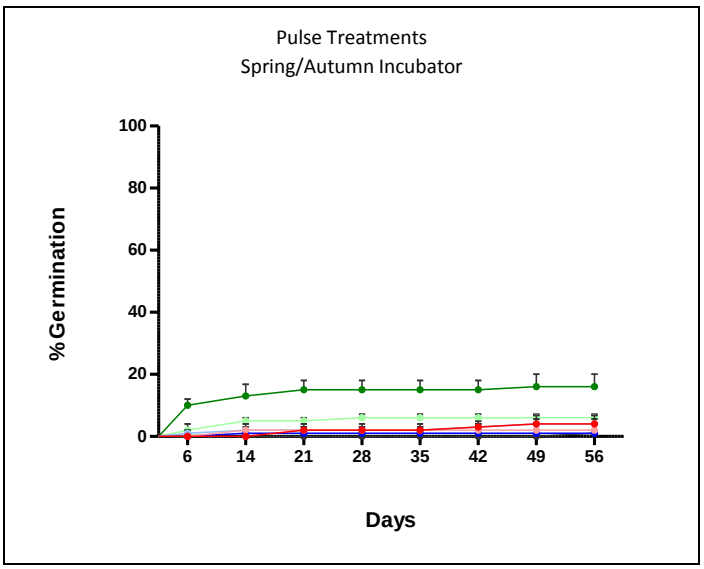


Treatments Summer		7 Days	28 Days	56 Days
	T8: Control	DEF	FGJ	CD
	T9: GA_3 100	A	AB	AB
	T10: GA_3 250	ABC	ABCDF	ABCD
	T11: KNO_3 100	CDE	CDEFG	CD
	T12: KNO_3 250	BCD	CDEFGJ	ABCD
	T13: Heat 80°C	G	IJK	DEF
	T14: Heat 90°C	G	K	F

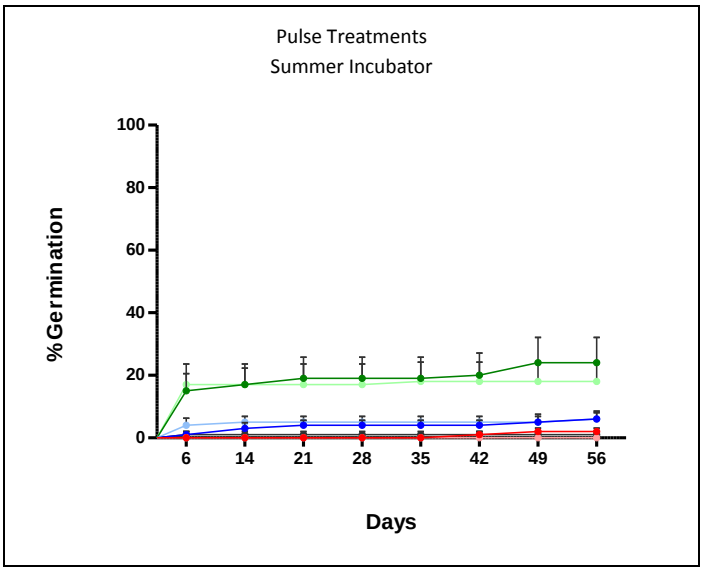


Treatments Winter		7 Days	28 Days	56 Days
	T15: Control	G	CDEFGJ	BCD
	T16: GA_3 100	A	AB	AB
	T17: GA_3 250	ABC	AB	A
	T18: KNO_3 100	CDEF	BCE	AB
	T19: KNO_3 250	DEF	CDEFG	AB
	T20: Heat 80°C	G	EFGHIJ	BC
	T21: Heat 90°C	G	IJK	CDEF

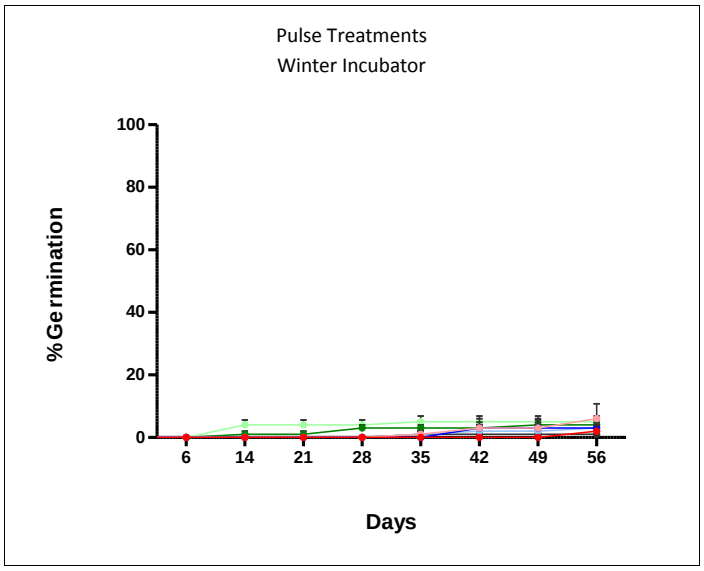
Figure 6-2 *Enneapogon avenaceus* – germination results from pulse treatments. Statistics are compared within the same time period across all pulse treatments and three incubator conditions: spring/autumn, summer and winter. *P* value is significant when < 0.05.



Treatments Spring/Autumn		7 Days	28 Days	56 Days
	T1: Control	C	D	DE
	T2: GA_3 100	ABC	BC	BD
	T3: GA_3 250	A	A	AC
	T4: KNO_3 100	BC	CD	BCDE
	T5: KNO_3 250	C	CD	DE
	T6 Heat 80°C	C	BCD	BDE
	T7: Heat 90°C	C	CD	BDE

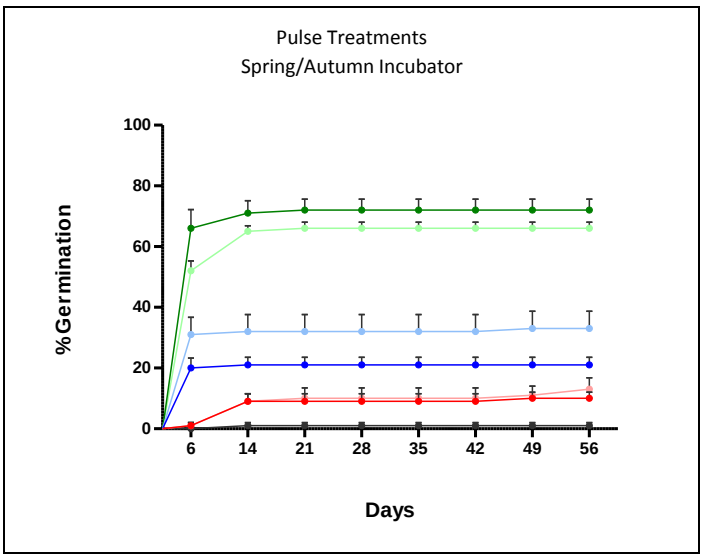


Treatments Summer		7 Days	28 Days	56 Days
	T8: Control	BC	CD	DE
	T9: GA_3 100	A	AB	ABC
	T10: GA_3 250	AB	AB	ABC
	T11: KNO_3 100	ABC	BCD	BDE
	T12: KNO_3 250	BC	BCD	BCDE
	T13: Heat 80°C	C	D	E
	T14: Heat 90°C	C	D	DE

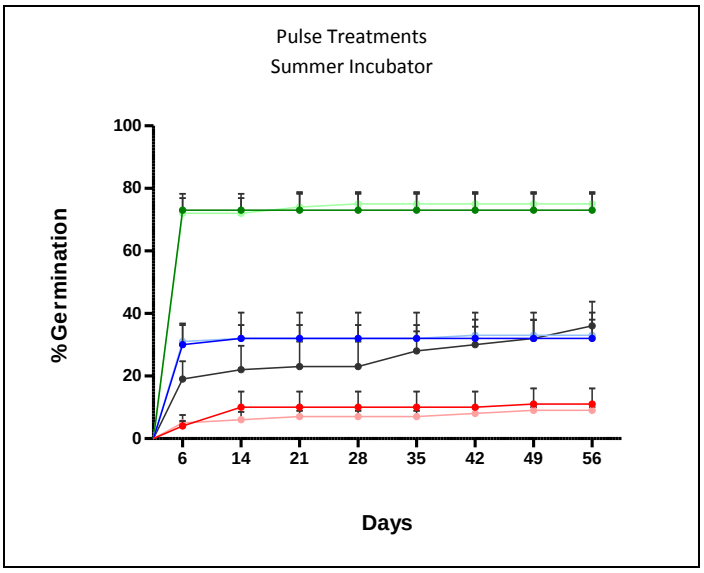


Treatments Winter		7 Days	28 Days	56 Days
	T15: Control	C	D	DE
	T16: GA_3 100	C	BCD	BDE
	T17: GA_3 250	C	CD	BDE
	T18: KNO_3 100	C	D	BCDE
	T19: KNO_3 250	C	D	BDE
	T20: Heat 80°C	C	D	BCDE
	T21: Heat 90°C	C	D	BDE

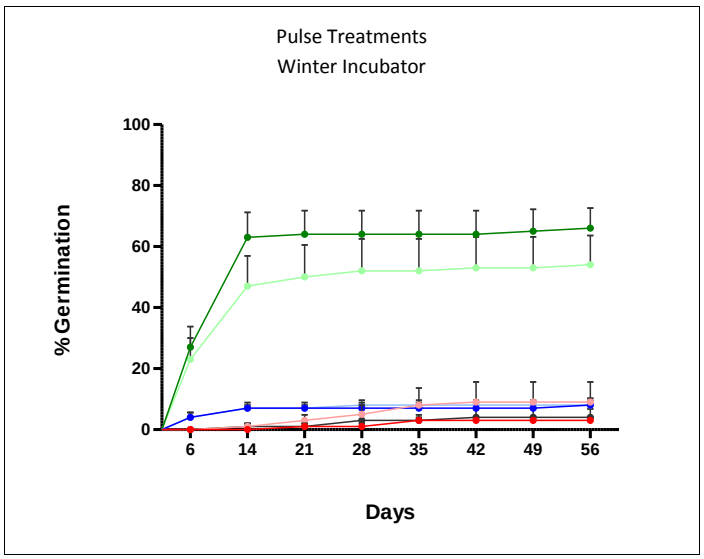
Figure 6-3 *Enneapogon caerulescens* var. *caerulescens* – germination results from pulse treatments. Statistics are compared within the same time period across all pulse treatments and three incubator conditions: spring/autumn, summer and winter. *P* value is significant when < 0.05.



Treatments Spring/Autumn		7 Days	28 Days	56 Days
	T1: Control	I	H	G
	T2: GA3 100	BD	A	A
	T3: GA3 2□0	AB	A	A
	T4: KNO_3 100	CE	BDE	BCD
	T5: KNO_3 250	EF	DE	CD
	T6: Heat 80°C	HI	DEFGH	CDE
	T7: Heat 90°C	HI	EFGH	DE



Treatments Summer		7 Days	28 Days	56 Days
	T8: Control	EFGH	BDEFGH	BCD
	T9: GA_3 100	AB	A	A
	T10: GA_3 250	A	A	A
	T11: KNO_3 100	CE	BD	BCD
	T12: KNO_3 250	DEF	BCDEF	BCDE
	T13: Heat 80°C	FGHI	FGH	E
	T14: Heat 90°C	GHI	DEFGH	CDEFG



Treatments Winter		7 Days	28 Days	56 Days
	T15: Control	I	GH	EFG
	T16: GA_3 100	DE	ABC	AB
	T17: GA_3 250	EFG	AC	A
	T18: KNO_3 100	GHI	FG	EF
	T19: KNO_3 250	GHI	FGH	EFG
	T20: Heat 80°C	I	FGH	DEFG
	T21: Heat 90°C	I	GH	FG

Figure 6-4 *Enneapogon cylindricus* – germination results from pulse treatments. Statistics are compared within the same time period across all pulse treatments and three incubator conditions: spring/autumn, summer and winter. *P* value is significant when < 0.05.

6.4 Discussion

All three *Enneapogon* spp. displayed evidence of PD. Germination responses were different in *E. caerulescens* var. *caerulescens* when compared with *E. avenaceus* and *E. cylindricus*, possibly reflecting certain connections to life history stages of each of the plants, although these were difficult to establish. *Enneapogon avenaceus* and *E. caerulescens* var. *caerulescens* are both annual and short-lived perennial grasses (Burbridge 1941; Kakudidi,Lazarides et al. 1988; Jessop,Dashorst et al. 2006), yet they seem to employ different dormancy and germination strategies. Although *E. cylindricus* is a perennial, its dormancy and germination responses were similar to *E. avenaceus*. It is possible that rainfall variability may account for differences in their life cycles and reproductive strategies.

The germination responses of *E. avenaceus* and *E. cylindricus* to applications of GA_3 were indicative of non-deep PD. It is difficult to determine whether these two species required after-ripening periods, as fresh seeds were unavailable for experiments. Results from Ooi, Auld et al. (2009) suggest that *E. cylindricus* in particular has an after-ripening requirement, whereas dormancy remained unbroken in *E. avenaceus* seeds when after-ripened for 20, 55 and 70 days. Depending on the species, seeds with non-deep PD may after-ripen during dry storage at room temperature (Baskin and Baskin 1998). The seed collections used in our study, were stored in the dark at 15 ± 2°C with 15% relative humidity for 8 months prior to experiments. These conditions may have been sufficient for some degree of dry after-ripening to occur.

Germination was low in seeds of *E. caerulescens* var. *caerulescens*, which is indicative of either intermediate PD or deep PD. Response to cold stratification could be investigated for this species, as seeds with intermediate PD often require a cold stratification treatment (0°C - 10°C and moist) for dormancy to be overcome, whilst seeds with deep PD require extended periods of cold stratification (Baskin and Baskin 1998). Experiments did not include chronic treatments and species may differ markedly in their germination responses between acute and chronic applications of GA_3, sometimes overcoming dormancy in one and not the other (refer to Chapter 7 of this thesis). This makes it difficult to determine the type of PD for *E. caerulescens* var. *caerulescens*.

Enneapogon avenaceus and *E. cylindricus* seeds are capable of germinating at a variety of temperature and light conditions once dormancy has been overcome and water availability is adequate. In contrast, Ooi, Auld et al. (2009) recorded no germination for *E. cylindricus* under winter 16°C / 6°C conditions, with seeds ranging from freshly collected to 70 day-old seeds. The after-ripening process was not sufficient to overcome PD in this species under winter conditions, as it was under autumn/spring and summer conditions. Grice, Bowman et al. (1995) found the optimum germination temperature for seeds of *E. avenaceus* was 20 - 30°C, reflecting spring/autumn and summer conditions. This is not surprising considering that this species generally germinates and establishes in response to high summer rains

(Kutsche and Lay 2003). Our research, however, shows that this species is capable of germinating equally well under cooler conditions.

Pulse applications of GA_3 were capable of overcoming PD in *E. avenaceus* and *E. cylindricus* seeds under all three incubator conditions. The ability to maximise germination rates through the application of GA_3 pulse treatments, enables further research to be undertaken without compromising treatments with chronic applications of GA_3 (Cohn 1996). Consequently, both species could be considered for other future research (Refer to Chapter 8 of this thesis). None of the pulse treatments promoted seed germination in *E. caerulescens* var. *caerulescens* and further research is needed to overcome dormancy in this species.

Dry heat pulse treatments were incapable of promoting germination in seeds of all three species. Future testing of additional dry heat pulse treatments should be considered, experimenting with temperature and duration of exposure. Our results show that the use of nitrates may be inappropriate for overcoming PD in *Enneapogon* species. Soils in arid ecosystems have typically low nutrient levels (Facelli and Temby 2002) and consequently fluctuations in nitrate levels may be minimal and therefore insufficient to stimulate germination. Nitrates have been studied in association with fire responsive species (Keeley and Fotheringham 1998), however fire is not considered to be a major influence on the seed ecology within this ecosystem (Turner,Ostendorf et al. 2008).

Conclusions

This study was designed as a preliminary investigation of PD in three *Enneapogon* species and the results provide some initial insights into how these species respond to the use of pulse-treatments to overcome PD. The use of GA_3 to promote germination in *E. avenaceus* and *E. cylindricus* could be investigated further as a possible seed priming agent for restoration projects. A more comprehensive study using fresh seeds is needed to fully understand after-ripening, dormancy and germination requirements in all three species. A comparison of germination responses of seed aged at 0, 4, 8 and 12 weeks under three incubator conditions (autumn/spring, summer and winter) is recommended. Additional after-ripening times may need to be included based on 12-week germination results. Careful consideration should be given to the way seeds are stored (Ooi,Auld et al. 2009), matching average temperature and humidity conditions to those likely experienced by seeds in the natural soil seed bank.

Chapter 7
Optimising Preparation and Application of Gibberellic Acid for Investigating Physiological Dormancy in Seeds

Atriplex vesicaria ssp. *variabilis* fruit

Austrostipa nitida caryopsis

Maireana radiata fruit

Stenopetalum lineare seed

Optimising Preparation and Application of Gibberellic Acid for Investigating Physiological Dormancy in Seeds

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Abstract

Gibberellic acid (GA_3) is often used in seed ecology research to test seed dormancy status, in particular whether seeds have physiological dormancy (PD). This research investigates methods to improve germination outcomes from the application of GA_3, in particular pulse and chronic applications in conjunction with adjusted pH during solution preparation. This was done by examining germination responses of ten arid zone plant species that commonly occur in *Acacia papyrocarpa* Benth. (Western Myall) open woodland.

A variety of germination responses were observed in experiments, with most species displaying evidence of PD. For three species (*Lepidium phlebopetalum*, *Rhodanthe floribunda* and *Stenopetalum lineare*), germination responses were significantly higher in non-adjusted GA_3 pulse treatments. Both *Rhodanthe floribunda* and *Stenopetalum lineare* were found to be sensitive to chronic exposure to non-adjusted GA_3. As results from screening seed collections for PD may be altered by whether the GA_3 solution is neutral or acidic, consideration should be given to the method of application, chronic versus pulse, prior to adjusting pH values. This research provides technical information pertinent to seed conservation and germination research.

7.1 Introduction

Seeds of many Australian plant species have dormancy mechanisms that prevent them from germinating prematurely after dispersal and following rainfall events (Bell 1999). Physiological dormancy (PD) is the most common form of dormancy (Baskin and Baskin 2004) and requires a chemical change to occur within the seed to enable germination (Fenner and Thompson 2005). Gibberellins or gibberellic acid (usually in the form of GA_3) are often used in seed ecology research to test seed dormancy status, in particular whether seeds have PD and to ascertain the level exhibited i.e. non-deep, intermediate or deep (Baskin and Baskin 2004). In general, seeds with non-deep PD (also referred to as conditionally dormant) respond to GA_3, seeds with intermediate PD may or may not respond and seeds with deep PD do not respond to GA_3 (Baskin and Baskin 1998; Baskin and Baskin 2005).

Seed dormancy is not necessarily broken when chemicals, such as gibberellins, are applied externally to seeds. They may instead bypass the natural blocks to germination whereby germination may be stimulated even though dormancy characteristics are still retained within the seed (Hilhorst and Karssen 1992; Cohn 1996). Gibberellins are often referred to as dormancy-breaking chemicals and referenced material used here may refer to their dormancy-breaking activity.

Identifying PD in seeds is an important first step in seed ecology research, as it can help determine the likelihood that seeds of certain species persist within a soil seed bank. This is important to understand the evolutionary strategies of species in uncertain environments, such as Australian arid zones. This information also assists restoration ecologists to identify species that may need replenishing when restoring disturbed areas and also to single out species that may require or respond to seed priming. Additionally, being able to maximise germination rates through the application of GA_3 pulse treatments, allows seed ecologists to conduct a range of germination experiments without compromising their treatments with chronic applications of GA_3 (Cohn 1996).

Gibberellic acid is a weak acid often applied without buffering or adjusting the pH value (Cohn 2002). Past research has investigated the role of pH in overcoming seed dormancy, with the dormancy-breaking activity of GA_3 known to be pH dependent (Toole and Cathey 1961; Palevitch and Thomas 1976; Cohn,Jones et al. 1989; Cohn 2002). The permeability of seed testas are not always affected by pH values, as Beadle (1952) found in *Atriplex vesicaria* seeds. However, whilst the reduction in cytoplasmic pH has more recently been proposed as an initial step in the dormancy-breaking process, there is no clear consensus as to the function of the pH change (Cohn 1996).

Studies have shown that seeds of some species respond differently to applications of pH neutral gibberellins when compared with acidic gibberellins. Keeley and Fotheringham (1998) found that germination of *Emmenanthe penduliflora* and *Phacelia grandiflora* seeds was only stimulated by acidic GA_3 (pH 3). Similarly, the stimulatory effect of $GA_{4/7}$ on *Apium*

graveolens was enhanced when the solution pH was lowered to 3.5 (Palevitch and Thomas 1976). In contrast, gibberellin solutions, including GA_3, buffered below the pKa value promoted more germination than non-buffered solutions in *Lactuca sativa* and *Lepidium virginicum* seeds (Toole and Cathey 1961). Duration of experiments and hence length of exposure time to gibberellins, were relatively short in all three research studies (i.e. between 7 and 14 days). However, not all species respond to dormancy-breaking chemicals within such a short time-frame. This raises the question of whether long-term exposure to acidic GA_3 affects seed germination response.

In this paper we examine germination responses to the application of pH adjusted and non-adjusted GA_3 in ten arid zone plant species. Past research has investigated PD and seed longevity for all species examined here, excluding *Austrostipa nitida* (Pound,Facelli et al. 2009). This research differs by investigating the method of applying GA_3, in particular the pulse and chronic application of pH neutral and acidic GA_3. Experiments aimed to answer the following questions. (1) Which species have PD and are likely to persist in the soil seed bank? (2) Which species respond to GA_3 pulse treatments? (3) How does neutralising the pH value of GA_3 affect its ability to bypass dormancy in pulse and chronic treatments? Research outcomes were aimed at providing technical information relevant for seed conservation and germination research.

7.2 Methods

Study Site and Species Description

Seeds were collected from a field study site (30°50'17.99"S and 132°12'10.37"E) located in Yellabinna Regional Reserve. The reserve covers 25,227 km^2 and extends north and north-west of the coastal town of Ceduna in South Australia (Figure 3-2). Mean monthly maximum temperatures recorded between 1922 and 1999 range between 18°C in July and 35°C in January, and mean monthly minimum temperatures range between 4°C in July and 18°C in January (Figure 3-1). Overall rainfall is low and generally consistent during winter months, however, large summer rainfall events that often produce floods, occur during La Niña years (Chesterfield and Parsons 1985; Sinclair 2005; Facelli and Chesson 2008). The mean annual rainfall calculated from data collected between 1904 and 1999 at Tarcoola is approximately 174 mm (BOM 2012).

Common understorey plants in *A. papyrocarpa* open woodland were chosen for this study. Species were selected to represent different plant families and life forms that were also locally abundant. *Austrostipa nitida* is a tufted annual or short-lived perennial grass with numerous erect or spreading stems. This grass species can dominate large and disturbed areas and consequently is recognised as a good soil stabilising plant (Kutsche and Lay 2003). *Lepidium phlebopetalum* and *Stenopetalum insulare* are both small erect annuals or short-lived perennial forbs that are very common after good winter-spring rains (Kutsche and Lay 2003), with flowering also recorded throughout the year (Jessop and Toelken 1986).

Rhodanthe floribunda is an erect or spreading forb that is common after heavy cool-season rains and often covers large areas (Kutsche and Lay 2003).

Atriplex vesicaria ssp. *variabilis* is an erect to spreading chenopod shrub that is a dominant species in the shrub layer of *A. papyrocarpa* open woodlands. This species flowers throughout the year in suitable conditions (Jessop and Toelken 1986). *Eriochiton sclerolaenoides* is a small short-lived perennial chenopod shrub that flowers opportunistically throughout all months of the year (Jessop and Toelken 1986). *Maireana erioclada*, *M. radiata* and *M. trichoptera* are erect perennial chenopod shrubs (Kutsche and Lay 2003) that generally flower between June and November (Jessop and Toelken 1986). *Zygophyllum aurantiacum ssp. aurantiacum* is a woody and bushy perennial shrub that flowers during spring and autumn months (Kutsche and Lay 2003). Nomenclature is according to Jessop and Toelken (1986).

Seed Collection and Preparation

Seed collections were made from > 50 plants per species (Table 7-1). Seed maturity was determined by observing the natural dispersal of propagules from adult plants as well as changes to the colour of dispersal units generally from green to brown (Pound,Facelli et al. 2009). Seed collections were kept in paper bags and dried under ambient conditions for approximately three weeks. Cleaning was required for some collections to remove vegetative material and invertebrates. All seed collections were then transferred to a controlled environment room where they were stored in the dark at 15 ± 2°C with 15% relative humidity until experiments commenced.

Dispersal units were left intact for this experiment to reflect their most likely state in the natural system and to avoid damage to seeds. Four replicates of 25 seeds per species were cut-tested to determine the viability of seed collections. Seeds containing full and healthy white embryos were classified as viable, whilst seeds containing no embryo or an embryo that was shrunken, shrivelled, brown or dried were classified as non-viable. Embryo types, based on categories defined by Martin (1946), were also assessed by dissecting approximately six seeds that had imbibed water for several hours. Seeds were cut both longitudinally and transversally, with an inspection of embryo size and shape made under a dissecting microscope (Pound,Facelli et al. 2009). Only healthy-looking fruits and seeds were included in experiments.

Table 7-1 Description of the seed collections used to research methods for optimising the preparation and application of gibberellic acid to overcome physiological dormancy, including seed embryo descriptions and seed viability results.

	Species	Family	Date Collected and Storage Times	Dispersal Unit Tested	Seed Embryo	Viability (%)
1	*Atriplex vesicaria* ssp. *variabilis*	Chenopodiaceae	Oct 2008 14 months	Fruit	Peripheral	72
2	*Austrostipa nitida*	Gramineae	Oct 2009 2 months	Caryopsis	Lateral	100
3	*Eriochiton sclerolaenoides*	Chenopodiaceae	Oct 2008 14 months	Fruit	Peripheral	80
4	*Lepidium phlebopetalum*	Cruciferae	Sept 2007 27 months	Seed	Bent	99
5	*Maireana erioclada*	Chenopodiaceae	Oct 2008 14 months	Fruit	Peripheral	87
6	*Maireana radiata*	Chenopodiaceae	Oct 2008 14 months	Fruit	Peripheral	84
7	*Maireana trichoptera*	Chenopodiaceae	Oct 2008 14 months	Fruit	Peripheral	96
8	*Rhodanthe floribunda*	Compositae	Oct 2007 26 months	Achene	Spathulate	99
9	*Stenopetalum lineare*	Cruciferae	Sept 2007 27 months	Seed	Bent	100
10	*Zygophyllum aurantiacum ssp. aurantiacum*	Zygophyllaceae	Oct 2007 26 months	Seed	Spathulate	95

Incubator Conditions and Treatments

Previous research by Pound et al. (2009) found that nine of the ten selected species (excluding *A. nitida*) were capable of germinating at temperatures ranging between 15°C and 30°C inclusive. This experiment therefore, used one constant incubator setting: 15°C with a 12-hour light period (Table 8-2), as this was one of two incubator conditions (15°C and 30°C) selected for subsequent salinity and water stress experiments (refer to Chapter 8 of this thesis).

For pulse treatments, replicates of 25 seeds were folded into Whatman™ filter paper and placed into 10 ml Eppindorf polypropylene tubes with approximately 10 ml GA_3 (100, 250 or 500 mg/L) for a period of 24 hours. Ethanol (EtOH < 2 ml) was used to aid the dissolution of GA_3 into sterilised reverse-osmosis (SRO) water. Measurements of pH were recorded for each solution of non-pH adjusted GA_3 solution. For pH-adjusted GA_3 solutions the pH level

was increased to 6.5 units using sodium hydroxide (NaOH). Remaining solutions were kept refrigerated in glass Schott™ bottles at 15±2°C and used to irrigate chronic treatments (Table 7-2).

Table 7-2 A summary of pulse and chronic treatments used to research methods for optimising the preparation and application of gibberellic acid to overcome physiological dormancy in seeds.

GA_3 pH Adjusted		**GA_3 pH Non-adjusted**		**Pulse Treatments**	**Weekly Irrigation**	**Incubator Conditions**
T1-T7	**pH**	**T8-T14**	**pH**			
T1: Control SRO water	6.9	**T8**: Control SRO water	6.9	None	SRO water	Constant 15°C with 12-hour light period
T2: Pulse GA_3 100	6.5	**T9**: Pulse GA_3 100	3.3	100mg/L (0.3mM) GA_3 for 24 hours	SRO water	
T3: Pulse GA_3 250	6.5	**T10**: Pulse GA_3 250	3.0	250mg/L (0.7mM) GA_3 for 24 hours	SRO water	
T4: Pulse GA_3 500	6.5	**T11**: Pulse GA_3 500	2.9	500mg/L (1.4mM) GA_3 for 24 hours	SRO water	
T5: Chronic GA_3 100	6.5	**T12**: Chronic GA_3 100	3.3	None	100mg/L (0.3mM) GA_3	
T6: Chronic GA_3 250	6.5	**T13**: Chronic GA_3 250	3.0	None	250mg/L (0.7mM) GA_3	
T7: Chronic GA_3 500	6.5	**T14**: Chronic GA_3 500	2.9	None	500mg/L (1.4mM) GA_3	

Seed Germination

Four replicates of 25 seeds per species were used in each treatment. Seeds were placed into 90 mm glass Petri dishes on top of two Whatman™ filter papers. Plates were irrigated weekly using an automated pipette, with 1 ml solution added to each Petri dish and the excess removed either by tipping it from the plate or using a transfer pipette. Clear plastic was wrapped around each stack of four replicate Petri dishes to minimise moisture loss. Germination was recorded weekly and defined as radicle emergence ≥ half the length of the dispersal unit, with germinated seeds removed at the time of recording. To avoid potential position effects, each Petri dish was repositioned within its stack as well as within the incubator at the time of scoring.

Statistical Analysis

Mixed between-within subjects analysis of variance (i.e. Split-plot ANOVA) was used to analyse data from treatments and controls for each species at 7, 21 and 42 days, with alpha

0.05 used to determine significance (PASW version 18 2010, formerly SPSS). Percentage values were arc-sine transformed prior to analysis to improve normality. A positive interaction between time and treatment indicated that the significance of some treatments changed over time. Data from different times were then analysed separately using non-parametric permutational multivariate analysis of variance tests i.e. PerMANOVA (Anderson 2001). Euclidean distance pair-wise tests were used to differentiate between treatments, with alpha set at 0.05 (PerMANOVA version 1.6). For each species, statistics are compared within the same time period across all pulse and chronic treatments, both pH adjusted and non-adjusted.

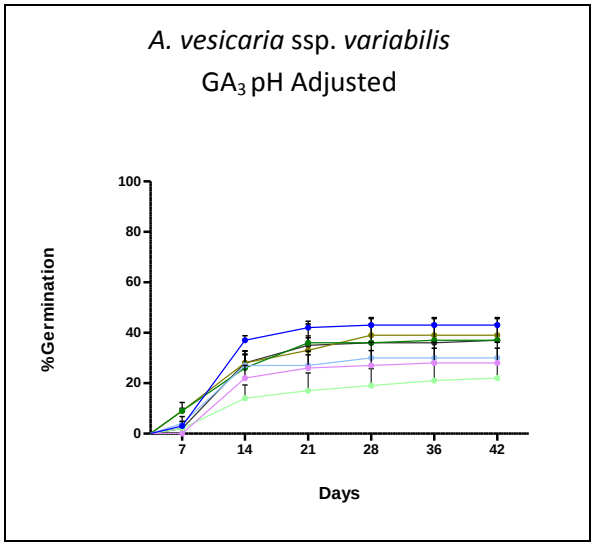
The size of this experiment meant that pH adjusted GA_3 treatments needed to be conducted independently of non-adjusted GA_3 treatments. Although this outcome was not optimal, no delay occurred between the first set of experiments finishing and the second set starting and each set of experiments had their own controls.

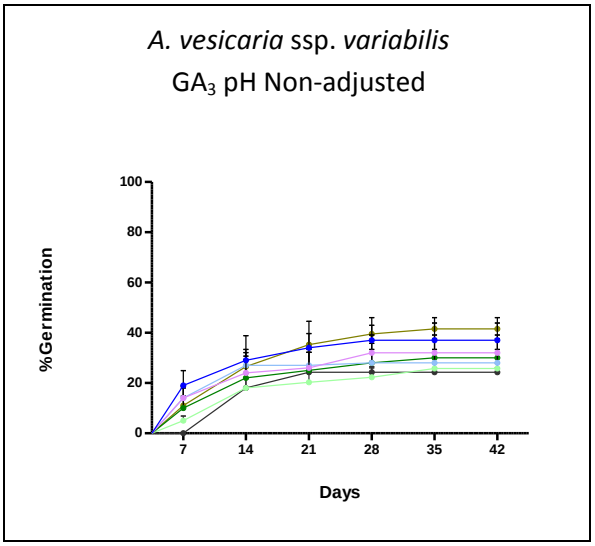
7.3 Results

Fully developed embryos were found in all seeds examined, indicating that neither morphological nor morphophysiological dormancy were present in any of the selected species. In a previous study, results from imbibition tests were used to dismiss the presence of physical dormancy in all species except *A. nitida*, which was not part of their study (Pound,Facelli et al. 2009). The impermeability of *Austrostipa* caryopses (lemma and palea) has been shown to inhibit germination; however, there is no evidence of physical dormancy in seeds of this genus (Gasque and Garcia-Fayos 2003).

Atriplex vesicaria ssp. *variabilis*

A positive interaction was found between time and treatment ($P < 0.05$; F = 1.884; df = 82), indicating that the significance of some treatments changed over time. Differences between treatments were only observed in germination results recorded after 7 days ($P = 0.0020$, df = 13, SS = 22.8968). There were no differences between treatments after 21 days ($P = 0.2175$, df = 13, SS = 2.6428) and 42 days ($P = 0.2775$, df = 13, SS = 2.2418). Pulse and chronic GA_3 treatments, both pH adjusted and non-adjusted, failed to stimulate high levels of germination in *A. vesicaria* ssp. *variabilis* (Figure 7-1).





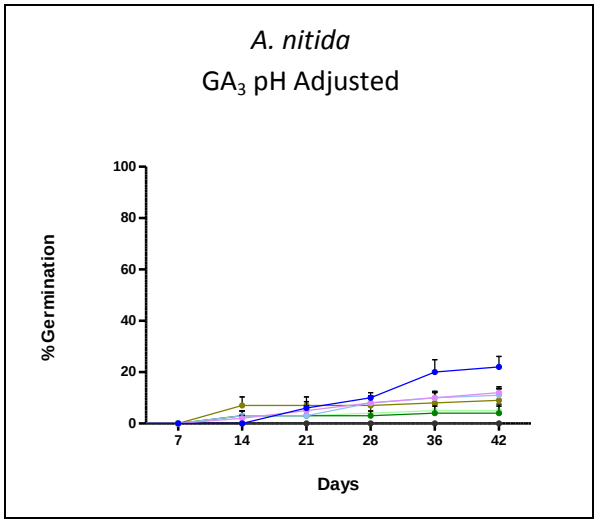
GA_3 Treatments pH Adjusted	7 Days	21 Days	42 Days
Control pH 6.9	bc	a	a
Pulse 100 pH 6.5	ac	a	a
Pulse 250 pH 6.5	abc	a	a
Pulse 500 pH 6.5	a	a	a
Chronic 100 pH 6.5	abc	a	a
Chronic 250 pH 6.5	c	a	a
Chronic 500 pH 6.5	abc	a	a

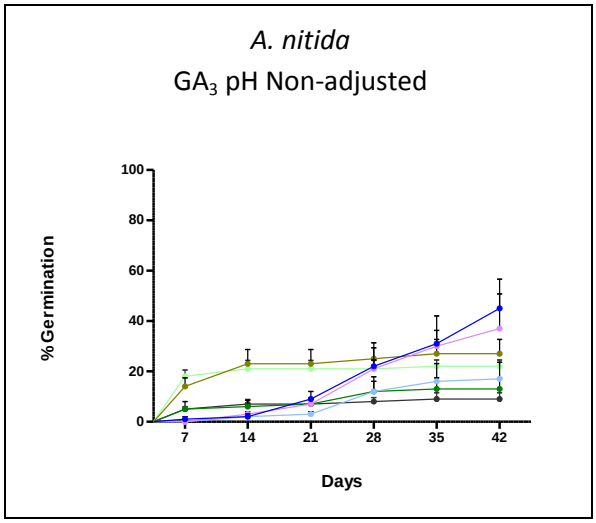
GA_3 Treatments pH Non -adjusted	7 Days	21 Days	42 Days
Control pH 6.9	c	a	a
Pulse 100 pH 3.3	abc	a	a
Pulse 250 pH 3.0	ab	a	a
Pulse 500 pH 2.9	abc	a	a
Chronic 100 pH 6.5	abc	a	a
Chronic 250 pH 6.5	a	a	a
Chronic 500 pH 6.5	ab	a	a

Figure 7-1 *Atriplex vesicaria* ssp. *variabilis*- germination results from GA_3 treatments. Statistics are compared within the same time period across all pulse and chronic treatments, both pH adjusted and non-adjusted. *P* value is significant when < 0.05.

Austrostipa nitida

The significance of some treatments changed over time (P = 0.001; F = 2.542; df = 82). Differences were recorded between treatments at 7 days (P = 0.0001, df = 13, SS = 30.9668), 21 days (P = 0.0106, df = 13, SS = 16.4868) and 42 days (P = 0.0001, df = 13, SS = 24.0837). Low to moderate germination was recorded in *A. nitida* seeds after 42 days (Figure 7-2). Germination results from pulse 250 mg/L GA_3 (pH non-adjusted) and chronic 500 mg/L GA_3 (pH non-adjusted) were significantly higher (27% and 45% respectively) than the control (9%) (P values < 0.05), which is indicative of conditional dormancy. After 42 days, the germination had not levelled-off in two chronic treatments: 250 mg/L and 500 mg/L GA_3 (pH non-adjusted).





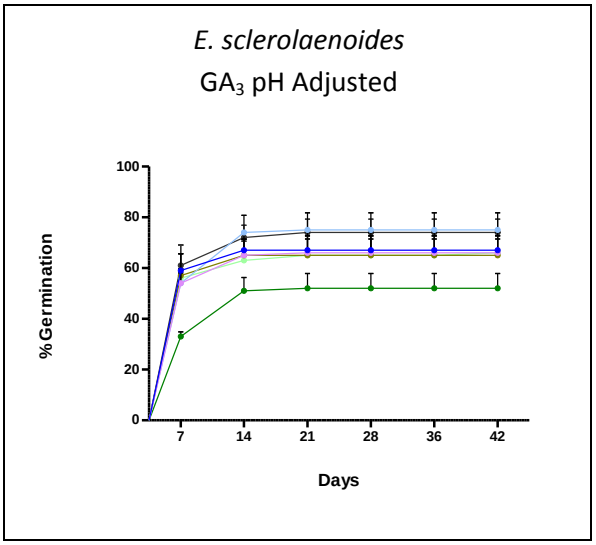
GA_3 Treatments pH Adjusted	7 Days	21 Days	42 Days
Control pH 6.9	d	g	e
Pulse 100 pH 6.5	d	fg	de
Pulse 250 pH 6.5	d	bcdefg	cde
Pulse 500 pH 6.5	d	fg	de
Chronic 100 pH 6.5	d	fg	bd
Chronic 250 pH 6.5	d	efg	bd
Chronic 500 pH 6.5	d	defg	abcd

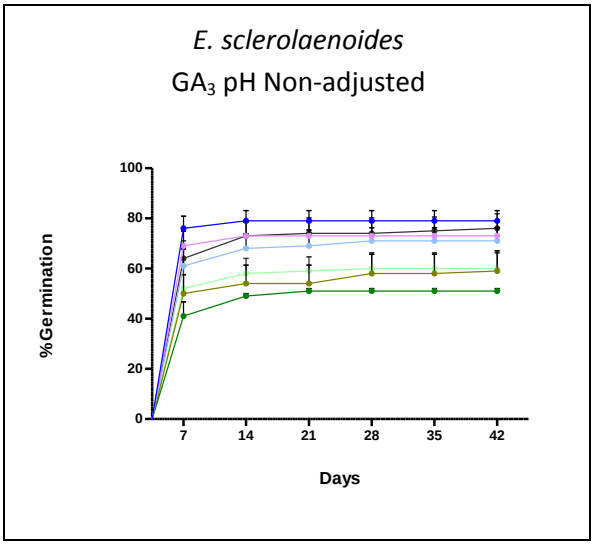
GA_3 Treatments pH Non -adjusted	7 Days	21 Days	42 Days
Control pH 6.9	bc	bdef	bd
Pulse 100 pH 3.3	a	abcd	abc
Pulse 250 pH 3.0	ab	ac	ac
Pulse 500 pH 2.9	abcd	defg	abcde
Chronic 100 pH 6.5	d	fg	abcd
Chronic 250 pH 6.5	d	def	abcd
Chronic 500 pH 6.5	cd	bdefg	ac

Figure 7-2 *Austrostipa nitida* - germination results from GA_3 treatments. Statistics are compared within the same time period across all pulse and chronic treatments, both pH adjusted and non-adjusted. P value is significant when < 0.05.

Eriochiton sclerolaenoides

A positive interaction was found between time and treatment (P = 0.008; F = 2.054; df = 82). Differences were recorded between treatments at 7 days (P = 0.0403, df = 13, SS = 1.0278), 21 days (P = 0.0432, df = 13, SS = 0.5379) and 42 days (P = 0.0486, df = 13, SS = 0.4978). *Eriochiton sclerolaenoides* seeds showed no evidence of PD (Figure 7-3). At day 42, germination was high in all pulse, chronic and control treatments, ranging between 50% and 79%. Germination was significantly lower in non-adjusted pulse 500 mg/L GA_3 (51%) when compared with the control (76%) and chronic 500 mg/L GA_3 (79%) (P values < 0.05), suggesting that the method of application can affect germination response in this species.





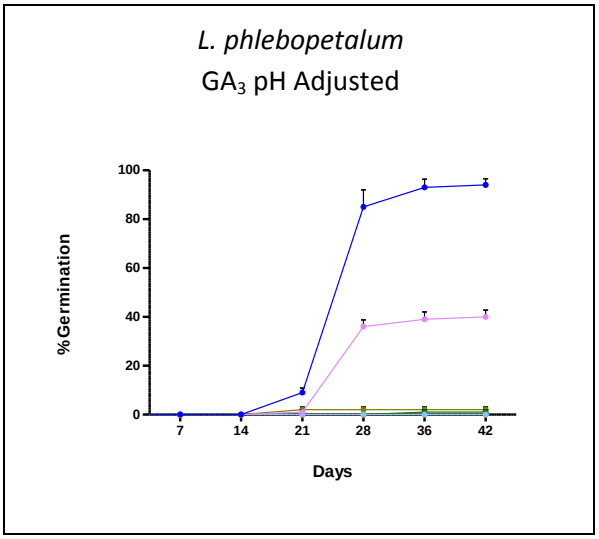
GA_3 Treatments pH Adjusted	7 Days	21 Days	42 Days
Control pH 6.9	abc	ab	ab
Pulse 100 pH 6.5	abcd	abc	abc
Pulse 250 pH 6.5	abcd	abc	abc
Pulse 500 pH 6.5	d	bc	bc
Chronic 100 pH 6.5	abc	ab	ab
Chronic 250 pH 6.5	abc	abc	abc
Chronic 500 pH 6.5	abc	ab	ab

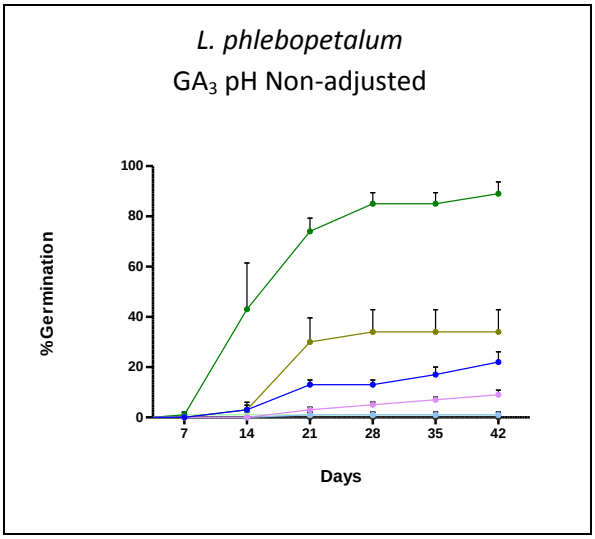
GA_3 Treatments pH Non -adjusted	7 Days	21 Days	42 Days
Control pH 6.9	ab	ab	ab
Pulse 100 pH 3.3	abcd	bc	abc
Pulse 250 pH 3.0	bcd	bc	abc
Pulse 500 pH 2.9	cd	c	c
Chronic 100 pH 6.5	abc	ab	ab
Chronic 250 pH 6.5	ab	ab	ab
Chronic 500 pH 6.5	a	a	a

Figure 7-3 *Eriochiton sclerolaenoides* - germination results from GA_3 treatments. Statistics are compared within the same time period across all pulse and chronic treatments, both pH adjusted and non-adjusted. P value is significant when < 0.05.

Lepidium phlebopetalum

The significance of some treatments changed over time ($P < 0.05$; F = 41.453; df = 82). No differences were recorded between treatments after 7 days (P = 1.0000, df = 13, SS = 0.8571); however, differences were recorded at 21 days (P = 0.0001, df = 13, SS = 50.2374) and 42 days (P = 0.0001, df = 13, SS =78.1013). Final germination of *L. phlebopetalum* seeds was promoted by both chronic 500 mg/L GA_3 (pH adjusted) and pulse 500 mg/L GA_3 (pH non-adjusted) treatments, which achieved significantly higher germination results (94% and 89% respectively) than controls (both 0%) (P values < 0.05) (Figure 7-4). However, germination was delayed by at least 21 days under chronic pH adjusted 500 mg/L GA_3 treatment (P = 0.030, t = 145.46). The equivalent pH non-adjusted result was significantly lower (P = 0.0299, t = 19.7902), suggesting that continual exposure to acidity may be detrimental to germination in this species.





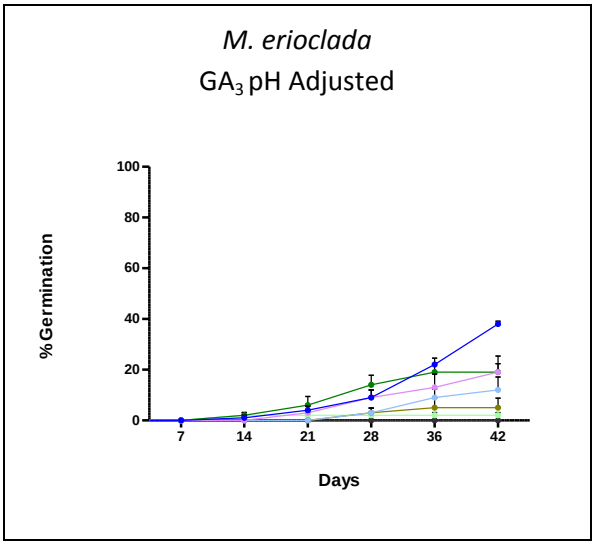
GA_3 Treatments pH Adjusted	7 Days	21 Days	42 Days
Control pH 6.9	a	d	f
Pulse 100 pH 6.5	a	d	f
Pulse 250 pH 6.5	a	cd	ef
Pulse 500 pH 6.5	a	d	ef
Chronic 100 pH 6.5	a	d	f
Chronic 250 pH 6.5	a	cd	b
Chronic 500 pH 6.5	a	bc	a

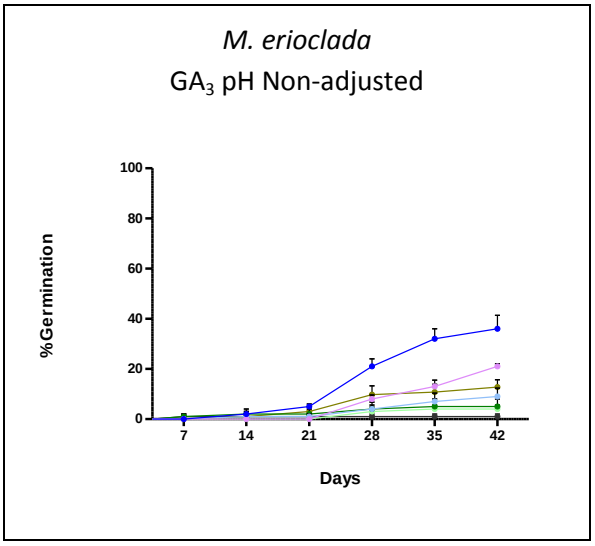
GA_3 Treatments pH Non -adjusted	7 Days	21 Days	42 Days
Control pH 6.9	a	d	f
Pulse 100 pH 3.3	a	cd	ef
Pulse 250 pH 3.0	a	b	bc
Pulse 500 pH 2.9	a	a	a
Chronic 100 pH 6.5	a	cd	ef
Chronic 250 pH 6.5	a	cd	de
Chronic 500 pH 6.5	a	b	cd

Figure 7-4 *Lepidium phlebopetalum* - germination results from GA_3 treatments. Statistics are compared within the same time period across all pulse and chronic treatments, both pH adjusted and non-adjusted. P value is significant when < 0.05.

Maireana erioclada

A positive interaction was found between time and treatment ($P < 0.05$; F = 5.684; df = 82). No differences were recorded between treatments after 7 days ($P = 1.0000$, df = 13, SS = 0.8571); however, differences were recorded at 21 days ($P = 0.0046$, df = 13, SS = 14.2845) and 42 days ($P = 0.0002$, df = 13, SS = 31.9810). In general, low to moderate germination responses were recorded in *M. erioclada* seeds after 42 days (Figure 7-5). Germination results from chronic 500 mg/L GA_3 (pH adjusted) and chronic 500 mg/L GA_3 (pH non-adjusted) were significantly higher (38% and 36% respectively) than controls (0% and 1%) (P values < 0.05), suggesting that seeds were conditionally dormant. Germination had not levelled-off after 42 days in either of these treatments.





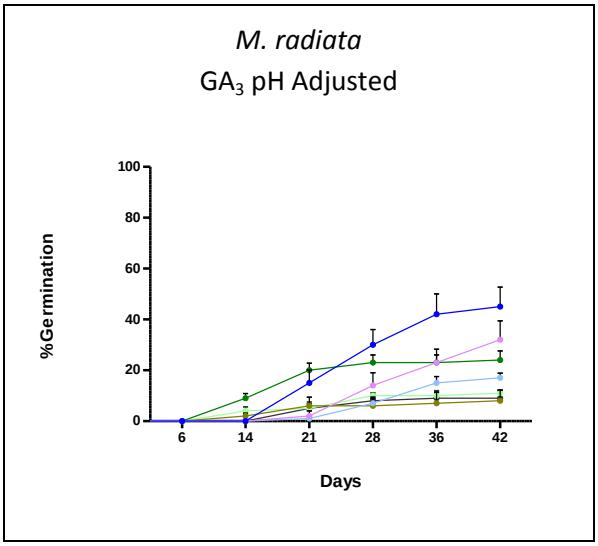
GA_3 Treatments pH Adjusted	7 Days	21 Days	42 Days
Control pH 6.9	a	b	e
Pulse 100 pH 6.5	a	ab	e
Pulse 250 pH 6.5	a	b	cde
Pulse 500 pH 6.5	a	ab	bc
Chronic 100 pH 6.5	a	b	bcde
Chronic 250 pH 6.5	a	ab	bcd
Chronic 500 pH 6.5	a	ab	a

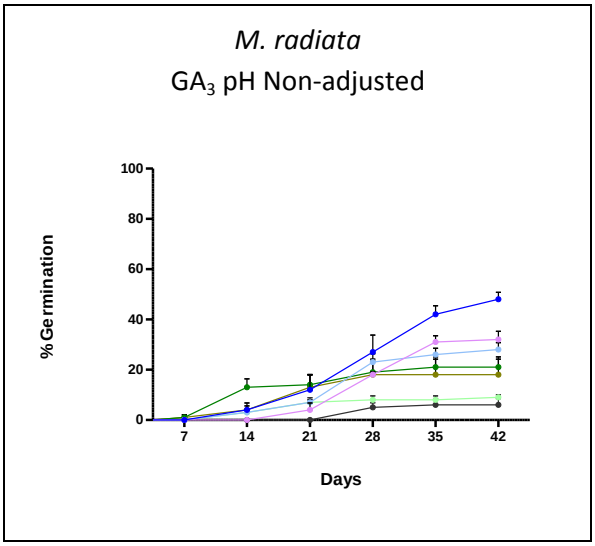
GA_3 Treatments pH Non -adjusted	7 Days	21 Days	42 Days
Control pH 6.9	a	ab	e
Pulse 100 pH 3.3	a	b	de
Pulse 250 pH 3.0	a	ab	bcd
Pulse 500 pH 2.9	a	ab	cde
Chronic 100 pH 6.5	a	ab	cde
Chronic 250 pH 6.5	a	b	b
Chronic 500 pH 6.5	a	a	ab

Figure 7-5 *Maireana erioclada* - germination results from GA_3 treatments. Statistics are compared within the same time period across all pulse and chronic treatments, both pH adjusted and non-adjusted. P value is significant when < 0.05.

Maireana radiata

The significance of some treatments changed over time ($P < 0.05$; F = 7.177; df = 82). There were no differences recorded between treatments after 7 days ($P = 1.0000$, df = 13, SS = 0.8571); however, differences were recorded at 21 days ($P = 0.0017$, df = 13, SS = 20.5747) and 42 days ($P = 0.0025$, df = 13, SS = 10.0263). In general, germination was low to moderate in seeds of *M. radiata* (Figure 7-6). Germination from chronic 500 mg/L GA_3 (pH adjusted and non-adjusted) and chronic 250 mg/L GA_3 (pH non-adjusted) were significantly higher (45%, 48% and 32% respectively) than controls (9% and 6%) (P values < 0.05), indicating that seeds of this species were conditionally dormant. Some chronic treatments in both pH adjusted and non-adjusted treatments had not levelled-off after 42 days.





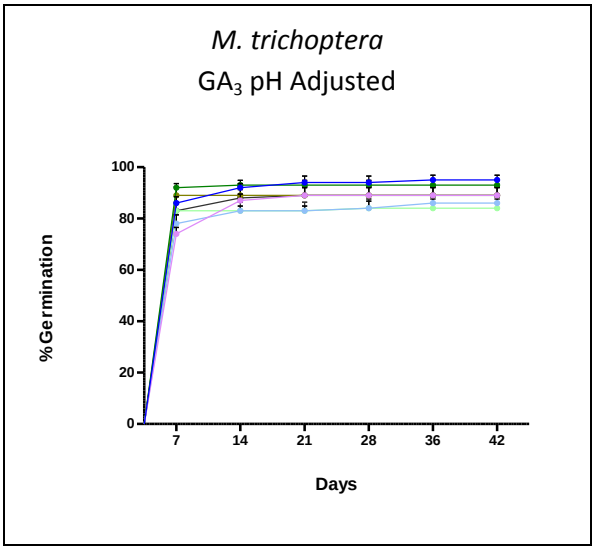
GA_3 Treatments pH Adjusted	7 Days	21 Days	42 Days
Control pH 6.9	a	bde	defghi
Pulse 100 pH 6.5	a	bde	dfgi
Pulse 250 pH 6.5	a	bcde	hi
Pulse 500 pH 6.5	a	ac	bceh
Chronic 100 pH 6.5	a	bde	cdefghi
Chronic 250 pH 6.5	a	bde	abceh
Chronic 500 pH 6.5	a	abc	ab

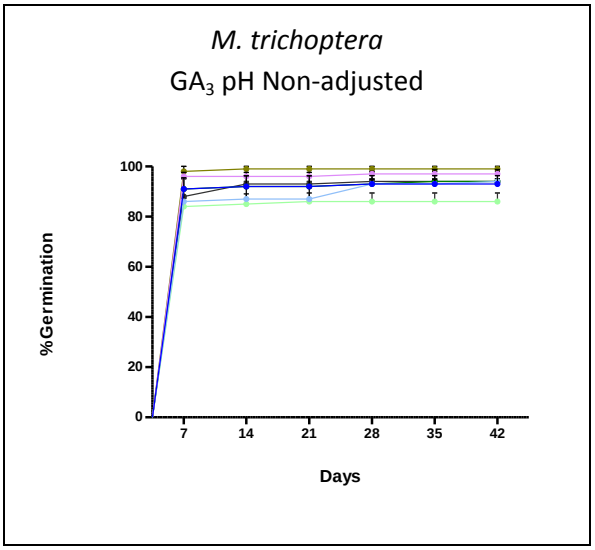
GA_3 Treatments pH Non -adjusted	7 Days	21 Days	42 Days
Control pH 6.9	a	e	i
Pulse 100 pH 3.3	a	bd	fgi
Pulse 250 pH 3.0	a	abcde	bcdefghi
Pulse 500 pH 2.9	a	abc	bcdefghi
Chronic 100 pH 6.5	a	bd	b
Chronic 250 pH 6.5	a	bde	ab
Chronic 500 pH 6.5	a	abcde	a

Figure 7-6 *Maireana radiata* - germination results from GA_3 treatments. Statistics are compared within the same time period across all pulse and chronic treatments, both pH adjusted and non-adjusted. P value is significant when < 0.05.

Maireana trichoptera

A positive interaction was found between time and treatment ($P < 0.05$; F = 2.834; df = 82). Differences were recorded between treatments at 7 days ($P = 0.0003$, df = 13, SS = 0.1764), 21 days ($P = 0.0292$, df = 13, SS = 0.0842) and 42 days ($P = 0.0168$, df = 13, SS = 0.0755). *Maireana trichoptera* showed no evidence of PD (Figure 7-7). At day 42, seeds in all treatments germinated between 84% and 99%.





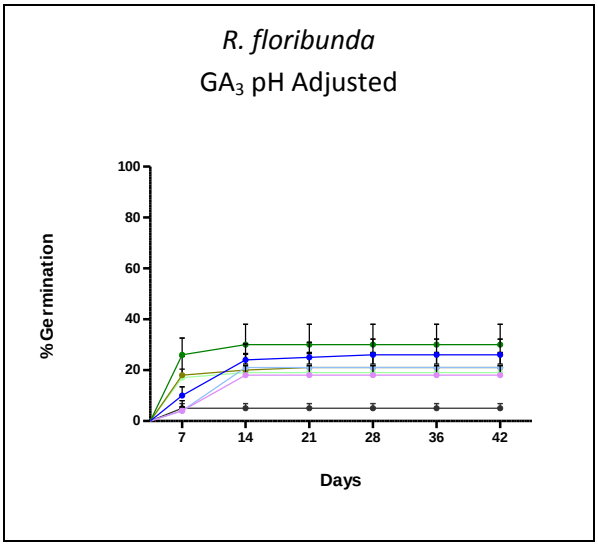
GA_3 Treatments pH Adjusted	7 Days	21 Days	42 Days
Control pH 6.9	cd	abc	ab
Pulse 100 pH 6.5	acd	bc	b
Pulse 250 pH 6.5	abcd	abc	ab
Pulse 500 pH 6.5	abc	abc	ab
Chronic 100 pH 6.5	cd	c	b
Chronic 250 pH 6.5	d	bc	b
Chronic 500 pH 6.5	acd	abc	ab

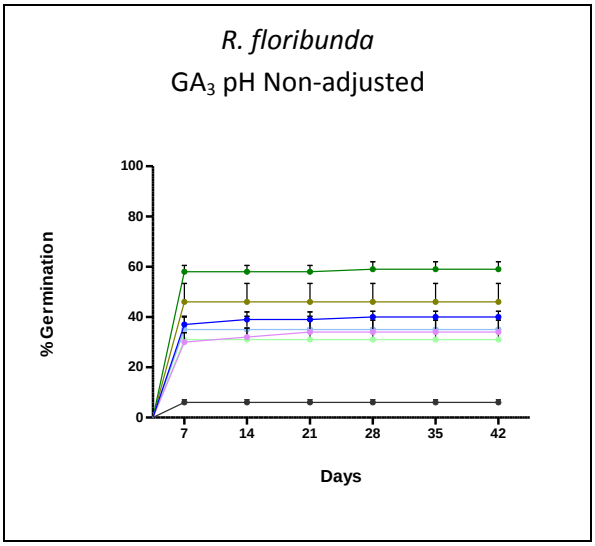
GA_3 Treatments pH Non -adjusted	7 Days	21 Days	42 Days
Control pH 6.9	bc	ab	ab
Pulse 100 pH 3.3	acd	abc	ab
Pulse 250 pH 3.0	a	a	a
Pulse 500 pH 2.9	abcd	abc	ab
Chronic 100 pH 6.5	acd	bc	ab
Chronic 250 pH 6.5	a	ab	a
Chronic 500 pH 6.5	abcd	abc	ab

Figure 7-7 *Maireana trichoptera* - germination results from GA_3 treatments. Statistics are compared within the same time period across all pulse and chronic treatments, both pH adjusted and non-adjusted. *P* value is significant when < 0.05.

Rhodanthe floribunda

A positive interaction was found between time and treatment ($P < 0.05$; F = 3.601; df = 82). Differences were recorded between treatments at 7 days ($P = 0.0001$, df = 13, SS = 21.8229), 21 days ($P = 0.0001$, df = 13, SS = 8.6232) and 42 days ($P = 0.0001$, df = 13, SS = 8.7267). Not adjusting GA_3 treatments improved seed germination results significantly for *R. floribunda* (Figure 7-8). After 42 days, pulse 500 mg/L GA_3 (non-adjusted) stimulated significantly higher germination (59%) than the control (6%) ($P = 0.0299$, t = 14.3635). In contrast, the highest pH adjusted result was in chronic treatment 500 mg/L GA_3, which peaked at only 26% ($P = 0.0307$, t = 2.3879).





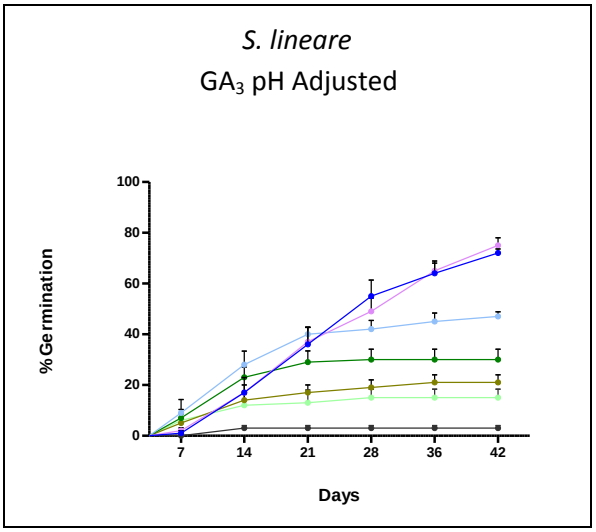
GA_3 Treatments pH Adjusted	7 Days	21 Days	42 Days
Control pH 6.9	d	d	d
Pulse 100 pH 6.5	cd	c	c
Pulse 250 pH 6.5	bcd	bcd	bcd
Pulse 500 pH 6.5	bcd	bcd	bcd
Chronic 100 pH 6.5	d	bcd	cd
Chronic 250 pH 6.5	d	c	c
Chronic 500 pH 6.5	cd	bc	bc

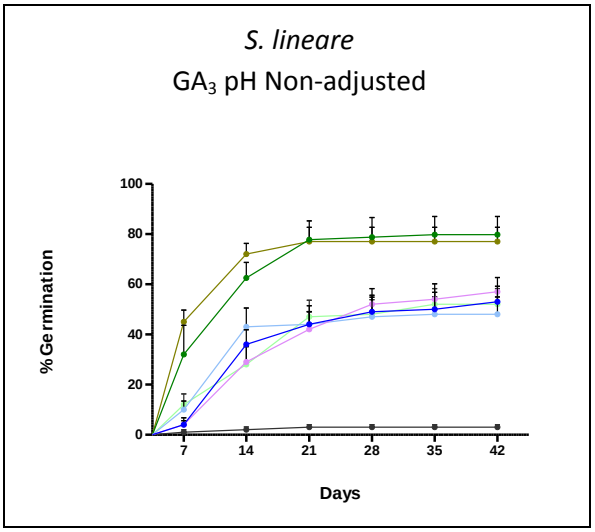
GA_3 Treatments pH Non -adjusted	7 Days	21 Days	42 Days
Control pH 6.9	d	d	d
Pulse 100 pH 3.3	bc	bc	bc
Pulse 250 pH 3.0	ab	abc	abc
Pulse 500 pH 2.9	a	a	a
Chronic 100 pH 6.5	bc	bc	bc
Chronic 250 pH 6.5	bc	bc	bc
Chronic 500 pH 6.5	b	b	b

Figure 7-8 *Rhodanthe floribunda* - germination results from GA_3 treatments. Statistics are compared within the same time period across all pulse and chronic treatments, both pH adjusted and non-adjusted. *P* value is significant when < 0.05.

Stenopetalum lineare

A positive interaction was found between time and treatment ($P < 0.05$; F = 16.789; df = 82). Differences were recorded between treatments at 7 days ($P = 0.0003$, df = 13, SS = 27.7425), 21 days ($P = 0.0001$, df = 13, SS = 19.7794) and 42 days ($P = 0.0001$, df = 13, SS = 21.7559). After 42 days, *S. lineare* germination was ≤ 30% in all pH adjusted GA_3 pulse treatments (Figure 7-9). In contrast, non-adjusted GA_3 pulse treatments 250 mg/L and 500 mg/L stimulated germination well above the control (3%), up to 52% and 79% respectively (*P* values < 0.05). Germination results were also higher than the control in chronic pH adjusted 250 mg/L and 500 mg/L GA_3 treatments, which were 75% and 72% respectively (*P* values < 0.05). Results from equivalent pH non-adjusted chronic treatments were significantly lower (≤ 57%) (*P* values < 0.05), suggesting that continual exposure to acidity may be detrimental to germination in this species.





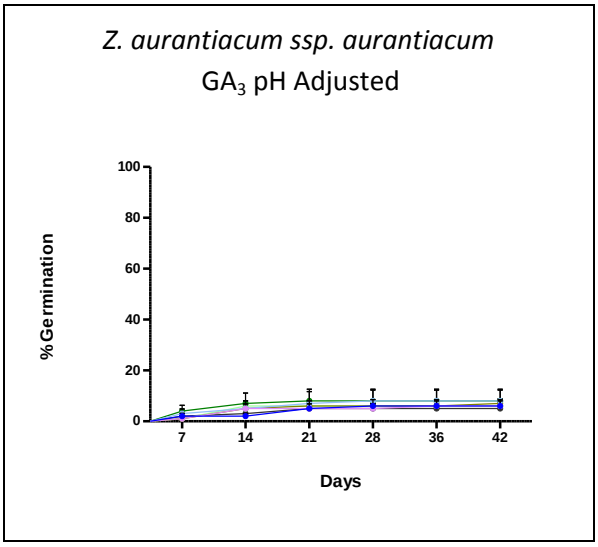
GA_3 Treatments pH Adjusted	7 Days	21 Days	42 Days
Control pH 6.9	d	e	h
Pulse 100 pH 6.5	bcd	de	g
Pulse 250 pH 6.5	bcd	d	fg
Pulse 500 pH 6.5	bcd	cd	efg
Chronic 100 pH 6.5	bcd	c	d
Chronic 250 pH 6.5	cd	c	a
Chronic 500 pH 6.5	cd	cd	a

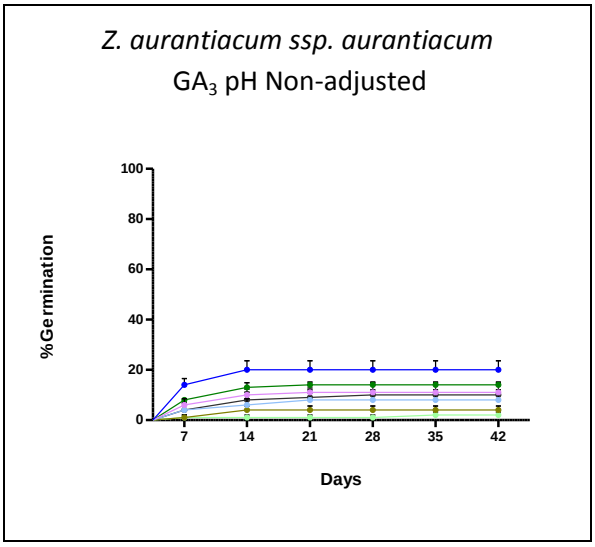
GA_3 Treatments pH Non -adjusted	7 Days	21 Days	42 Days
Control pH 6.9	cd	e	h
Pulse 100 pH 3.3	bc	bc	abcde
Pulse 250 pH 3.0	a	ab	abc
Pulse 500 pH 2.9	ab	a	ac
Chronic 100 pH 6.5	bc	bcd	bdef
Chronic 250 pH 6.5	bcd	c	bcde
Chronic 500 pH 6.5	bcd	c	bcde

Figure 7-9 *Stenopetalum lineare* - germination results from GA_3 treatments. Statistics are compared within the same time period across all pulse and chronic treatments, both pH adjusted and non-adjusted. *P* value is significant when <0.05.

Zygophyllum aurantiacum ssp. aurantiacum

No interaction was found between time and treatment (P = 0.920; F = 0.614; df = 82). Final germination results recorded after 42 days did not differ between treatments (P = 0.2748, df = 13, SS = 7.5121). All *Z. aurantiacum ssp. aurantiacum* seed germination results were very low (≤ 20%) (Figure 7-10), indicating that seeds of this species may have intermediate or deep PD.





GA_3 Treatments pH Adjusted	7 Days	21 Days	42 Days
Control pH 6.9	a	a	a
Pulse 100 pH 6.5	a	a	a
Pulse 250 pH 6.5	a	a	a
Pulse 500 pH 6.5	a	a	a
Chronic 100 pH 6.5	a	a	a
Chronic 250 pH 6.5	a	a	a
Chronic 500 pH 6.5	a	a	a

GA_3 Treatments pH Non -adjusted	7 Days	21 Days	42 Days
Control pH 6.9	a	a	a
Pulse 100 pH 3.3	a	a	a
Pulse 250 pH 3.0	a	a	a
Pulse 500 pH 2.9	a	a	a
Chronic 100 pH 6.5	a	a	a
Chronic 250 pH 6.5	a	a	a
Chronic 500 pH 6.5	a	a	a

Figure 7-10 *Zygophyllum aurantiacum ssp. aurantiacum* - germination results from GA_3 treatments. Statistics are compared within the same time period across all pulse and chronic treatments, both pH adjusted and non-adjusted. P value is significant when < 0.05.

7.4 Discussion

A range of germination responses to pulse and chronic treatments of pH adjusted and pH non-adjusted GA_3 were observed in experiments. *Maireana trichoptera* exhibited no evidence of PD under constant 15°C conditions and is unlikely to persist within the soil seed bank. *Eriochiton sclerolaenoides* also displayed no evidence of PD; however, this finding conflicts with results from Pound, Facelli et al. (2009). In their research, *E. sclerolaenoides* achieved only low to moderate germination results (< 50%) under winter and spring/autumn conditions after chronic application of 100 mg/L pH non-adjusted GA_3. Fresh seeds were use in their experiments, whereas our research used seeds that had been stored for 14 months. Seeds with non-deep PD may after-ripen during dry storage at room temperature (Baskin and Baskin 2005), suggesting that *E. sclerolaenoides* may be conditionally dormant and require an after-ripening period. The rapid decline in seed viability observed by Pound, Facelli et al. (2009) during seed burial experiments, indicates that *E. sclerolaenoides* seeds are unlikely to persist in the soil seed bank.

Five species: *A. vesicaria* ssp. *variabilis*; *A. nitida*; *M. erioclada; M. radiata;* and *Z. aurantiacum ssp. aurantiacum* exhibited evidence of PD which could not be overcome by the range of pulse and chronic GA_3 treatments investigated. Unfortunately it was not possible to discount temperature as a determining factor, as only one incubator condition was tested in the experiments. Germination marks the culmination of both dormancy being broken or bypassed followed by the process of germination (Cohn 1996), with the responsiveness of seeds to GA_3 often determined by factors such as light and temperature (Hilhorst and Karssen 1992). It is therefore difficult to confirm whether incubator conditions were suitable for overcoming dormancy or appropriate for promoting germination in the species chosen, and this warrants further investigation.

The germination response of *A. vesicaria* ssp. *variabilis* was similar to that recorded by Pound, Facelli et al. (2009). The soil seed bank is known to be short-lived for this species, with one study finding only 34% of seeds remained viable after 12 months (Hunt 2001), suggesting that this species is not likely to have deep PD. Interpreting the response of fresh *A. nitida* seeds is complicated by after-ripening requirements, which are commonly associated with this genera (Ralph 1997). Care is needed to avoid mistaking after-ripening with loss of dormancy (Cohn 1996) and consequently it was not possible to determine whether PD is present in this species. Although experiments were terminated prematurely for *A. nitida*, *M. erioclada* and *M. radiata*, Pound, Facelli et al. (2009) concluded that PD were present in all three of these species.

Physiological dormancy was overcome in *L. phlebopetalum*, *R. floribunda* and *S. lineare* seeds by specific pulse and chronic treatments, indicating the presence of non-deep PD or possibly intermediate PD. For all three species, Pound, Facelli et al. (2009) found declines in seed viability over 17 months during seed burial experiments, which suggests that these species are unlikely to be long-lived in the soil seed bank. All three species responded to

pulse treatments, making them suitable for subsequent experimental research (refer to Chapter 8 of this thesis).

The same three species also help to illustrate how neutralising the pH value of GA_3 may affect its ability to bypass PD in pulse and chronic treatments. Only pulse GA_3 treatments that had not been pH adjusted (i.e. acidic) overcame PD in *L. phlebopetalum, R. floribunda* and *S. lineare* seeds. This suggests that neutralising GA_3 in pulse treatments can change seed responses and therefore results of screening seeds for PD. Chronic exposure to pH adjusted 500 mg/L GA_3 delayed seed germination in *L. phlebopetalum* seeds by at least 14 days when compared with the non-adjusted pulse treatment at the same concentration. Conversely, both *L. phlebopetalum* and *S. lineare* displayed sensitivity to chronic exposure to acidic GA_3, with non-adjusted chronic treatments producing statistically lower germination than adjusted chronic treatments. In the case of *L. phlebopetalum*, non-adjusted chronic treatments were inadequate to bypass PD.

Although germination was stimulated in *E. sclerolaenoides* seeds with non-adjusted 500 mg/L GA_3 pulse treatment, germination results were significantly lower than the control and both adjusted and non-adjusted 500 mg/L GA_3 chronic treatments. This suggests that *E. sclerolaenoides* tolerates long-term exposure to acidity but may be affected by the process of leaching. Further support for this interpretation is provided by Pound, Facelli et al. (2009), who found that leaching in water for 72 hours produced low germination rates relative to other treatments of dry heat and dry heat plus leaching.

Conclusions

This research has shown that seeds respond differently to adjusting pH values of GA_3. For some species, the efficacy of using GA_3 pulse treatments was improved with acidic pH, whilst more neutral pH values improved the efficacy of chronic treatments. Seed conservation and seed ecology researchers need to consider the type of application when determining the pH values of dormancy-testing chemicals, as outcomes from screening seed collections for PD may be altered by neutral or acidic solutions. As there is a possibility that key events may have been missed by using one dormancy-testing chemical due to pharmacological complications (Cohn 1996), recommendations for further study include the incorporation of additional incubator conditions for comparison and the investigation of a range of dormancy-testing chemicals.

Chapter 8
Seed Germination of Six Arid Zone Plants under Salinity and Water Stress

Eriochiton sclerolaenoides fruit

Maireana trichoptera fruit

Lepidium phlebopetalum seeds

Rhodanthe floribunda achenes

Seed Germination of Six Arid Zone Plants under Salinity and Water Stress

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Abstract

This research investigated the germination of six arid plant species under salinity and water stress. High salinity levels occur naturally in Australian arid ecosystems. However, the removal of perennial plants through land clearance often results in artificially high salinity levels. Mining activities also alter salinity levels in soils and this has important implications for the restoration of mine-affected areas. Improved understanding of the seed biology of native flora under salinity and water stress is therefore important to inform arid land management and restoration. Different concentrations of sodium chloride (NaCl) and polyethylene glycol (PEG-8000) were used to produce a range of water potentials to assess germination responses. Toxicity and osmotic effects were differentiated through the application of separate NaCl and PEG-8000 treatments.

Plant species varied in their germination responses to salinity and water stress. *Eucalyptus oleosa ssp. ampliata* seeds were the most sensitive to reductions in water potential > 0 MPa. *Lepidium phlebopetalum* and *R. floribunda* seeds were sensitive to reductions in water potentials > -0.25 MPa. Seeds of *E. cylindricus* were moderately tolerant up to -0.5 MPa. *Eriochiton sclerolaenoides* and *M. trichoptera* both showed the capacity to germinate under extremely low water potentials of -2.0 MPa.

Salinity and water stress affected temperature ranges over which *E. cylindricus*, *L. phlebopetalum* and *M. trichoptera* were capable of germinating. *Eriochiton sclerolaenoides* and *M. trichoptera* seeds showed the capacity for selective accumulation of ions to assist in germination. Low water potentials were found to cause delays in the seed germination of two species: *E. sclerolaenoides* and *R. floribunda*. This research shows the potential for salinity and water stress to affect the community structure of species populations, as well as change competitive interactions between seedlings in arid ecosystems.

8.1 Introduction

Salinity can inhibit seed germination, seedling establishment and the long-term survival of plants (Baskin and Baskin 1998). The degree of impact depends strongly on thresholds of individual plants to salinity and water stress, with the germination stage of a plant's life cycle being the most sensitive (Choudhuri 1968). Salinity is a serious problem in Australia, both due to the nature of the environment as to the removal of perennial plants through land clearance and over-grazing. Activities such as mining may also alter soil salinity levels and this has important implications for the restoration of mine-affected areas. Mining has increased in the South Australian arid zone where hyper-saline ground water is the most feasible option for mining operations.

Most arid zone plants have evolved to cope with naturally high salinity gradients. Arid soils are generally characterised by processes of salt accumulation from a combination of factors such as poor surface run-off, evaporation of rain water from depressions or lakes and rising ground water that brings dissolved salts, especially sodium chloride, to the surface (Kovda,Somoilova et al. 1981; Baskin and Baskin 1998). Salinity levels decline with elevation and plants often form concentric bands around areas of high salinity, with less tolerant plants occurring in areas that are less saline (Baskin and Baskin 1998). Variations in soil salinity contribute to spatial and temporal patterning of vegetation in arid ecosystems.

Vegetation patchiness influences soil heterogeneity as nutrients, organic carbon and moisture accumulate in microsites such as those found beneath canopies of trees and shrubs (Sarig,Barness et al. 1994; Facelli and Brock 2000). Research by Facelli and Brock (2000) found that salt accumulation occurred beneath the canopies of *Acacia papyrocarpa* Benth. in grazed chenopod shrublands. Halophytic shrubs such as *Atriplex spp.* and *Maireana spp.* also favour the accumulation of carbonates and other soluble salts in the upper part of the soil profile and are known to transfer chlorides, sulphates and carbonates up to the surface, contributing to the salinisation of top soil (Kovda,Somoilova et al. 1981). Despite having relatively high tolerance levels to salinity, even halophytes are generally salt sensitive in seed germination and seedling emergence stages (Debez,Ben Hamed et al. 2004).

Species vary in their germination response to salinity and water stress, with each species thought to have critical water content requirements for germination (Baskin and Baskin 1998; Fenner and Thompson 2005). The size of a seed may affect its water requirement, with larger seeds having greater absolute requirements (Fenner and Thompson 2005). Large seeds have a relatively smaller surface-to-volume ratio and for seeds up to 1000 mg there is a linear relationship between seed mass and absolute water uptake at full imbibition (Fenner and Thompson 2005).

Salinity may affect seed germination through two processes: 1) salt ions are toxic to the embryo; and 2) salts in solution produce low water potentials that prevent water uptake in seeds (Choudhuri 1968; Romo and Haferkamp 1987; Baskin and Baskin 1998). Species may be affected to varying degrees by one or a combination of both of these stresses. After

testing the effects of different osmotic solutions (i.e. NaCl, Na_2CO_3, Na_2SO_4, PEG and pond water) on the seed germination of five plant species (Compositae, Cruciferae, Gramineae and Typhaceae), Choudhuri (1968) found that NaCl toxicity was the least detrimental. Seed germination of *Cakile maritima* was also found to be inhibited more by osmotic effect than toxicity, with treatment results reversed when seeds were transferred to water (Debez,Ben Hamed et al. 2004).

Water potential can affect the temperature range over which a species is capable of germinating. Under summer temperatures, the germination of *Carrichtera annua* seeds was always very low irrespective of water availability. Under winter and autumn/spring temperatures, the germination of *C. annua* was significantly lower when water potential was reduced to -2.25MPa (Facelli and Chesson 2008). These results refer to autumn and winter retrieval times. Reductions in the temperature range over which a plant may successfully germinate has flow on effects, with reduced opportunities for plants to establish and populations to regenerate in the environment.

Some plant species have evolved strategies to germinate in conditions of relatively high salinity and water stress. Salinity lowers the water potential of the surrounding environment by osmotic effect; however, germination requires a gradient in water potential that is lower in the seed than in the surrounding environment. Plant species are able to achieve this by accumulating ions within the seed (Facelli 2008). Koyro and Eisa (2008) found evidence of ion accumulation in *Chenopodium quinoa* seeds. Germination was accelerated due to low water potentials within the walls of plant ovaries caused by high Na^+ and Cl^- concentrations. *Lepidium perfoliatum* seeds have also demonstrated potential for osmoregulation as well as having ecotypic specialisation, whereby seeds collected from plants in saline habitats were more tolerant than those collected from non-saline habitats (Choudhuri 1968).

In general, very little information is available on the seed biology of species required for the restoration of arid ecosystems in Australia (Commander,Merritt et al. 2009). This research paper focuses on the germination responses to salinity and water stress, of a selection of plant species that occur in *A. papyrocarpa* (Western Myall) open woodland. Previous research has been undertaken in similar grazed open woodlands, including studies of relationships between seed dispersal and seed accumulation, microenvironment properties and seedling establishment (Ireland 1992; Ireland 1997; Meissner and Facelli 1999; Facelli and Brock 2000; Facelli and Temby 2002; Weedon and Facelli 2008). Seed biology and germination research has also been undertaken on a selection of common plant species at the study site (Facelli,Chesson et al. 2005; Pound,Facelli et al. 2009); however, effects of salinity and water stress were not included in either of these studies.

This research undertook a series of laboratory experiments using different concentrations of sodium chloride (NaCl) and Polyethylene glycol (PEG-8000) to produce a range of water potentials to assess germination response of six plant species to salinity and water stress. Experiments aimed to answer the following three questions. (1) What is the germination response of six arid plant species to salinity and water stress? (2) How does salinity and

water stress affect seed germination at different temperatures? (3) Are germination responses the effect of toxicity, osmotic potential or both? Research was aimed at improving our understanding of seed biology in arid zone species with applications for restoration ecology.

8.2 Methods

Study Site

Seeds were collected from a field study site (30°50'17.99"S and 132°12'10.37"E) located in Yellabinna Regional Reserve. The reserve covers 25,227 km^2 and extends north and north-west of the coastal town of Ceduna in South Australia (Figure 3-2). Mean monthly maximum temperatures recorded between 1922 and 1999 range between 18°C in July and 35°C in January, and mean monthly minimum temperatures range between 4°C in July and 18°C in January (Figure 3-1). Overall rainfall is low and generally consistent during winter months, however, large summer rainfall events that often produce floods, occur during La Niña years (Chesterfield and Parsons 1985; Sinclair 2005; Facelli and Chesson 2008). The mean annual rainfall calculated from data collected between 1904 and 1999 at Tarcoola is approximately 174 mm (BOM 2012).

Plant species were selected to represent a range of different plant families and growth forms that were locally abundant. (1) *Enneapogon cylindricus* is a tufted annual or short-lived perennial grass with numerous erect or spreading stems. This species germinates and establishes quickly in response to good summer rains and can dominate large areas, and consequently it is recognised as a good soil stabiliser (Kutsche and Lay 2003). (2) *Eriochiton sclerolaenoides* is a small short-lived perennial chenopod shrub that flowers opportunistically throughout all months of the year (Jessop and Toelken 1986). (3) *Eucalyptus oleosa ssp. ampliata* is a multi-trunked tree referred to as a mallee (Kutsche and Lay 2003) and is generally restricted to sandy rises and some creeklines within *A. papyrocarpa* open woodland (pers. obs. 2008). It is the only species in the study area that stores seeds in hard woody capsules (i.e. serotiny), with seeds of two or three seasons contained within tree canopies (pers. obs.). (4) *Lepidium phlebopetalum* is a small erect perennial forb that is very common after good winter-spring rains (Kutsche and Lay 2003), with flowering also recorded throughout the year (Jessop and Toelken 1986). (5) *Maireana trichoptera* is an erect perennial chenopod shrub (Kutsche and Lay 2003) that flowers between June and November (Jessop and Toelken 1986). (6) *Rhodanthe floribunda* is an erect or spreading forb that is common after heavy cool-season rains and often covers large areas (Kutsche and Lay 2003). Nomenclature is according to Jessop and Toelken (1986).

Seed Collection and Preparation

Seed collections were made from at least 50 plants per species (Table 8-1). Seed collections were kept in paper bags and dried under ambient conditions. Cleaning was required for some collections to remove vegetative material and invertebrates. All seed collections were

then transferred to a controlled environment room where they were stored in the dark at 15 ± 2°C with 15% relative humidity. As collections were made over a 16 month period, storage times prior to experiments ranged between 13 and 29 months.

Dispersal units were left intact for experiments to reflect their most likely state in situ in the natural system and to avoid damage to seeds. Four replicates of 25 seeds per species were cut-tested to determine the viability of seed collections. Seeds containing full and healthy white embryos were classified as viable, whilst seeds containing no embryo or an embryo that was shrunken, shrivelled, brown or dried were classified as non-viable. Embryo types, based on categories defined by Martin (1946), were assessed by dissecting approximately six seeds that had imbibed water for several hours. Seeds were cut both longitudinally and transversally, with an inspection of embryo size and shape made under a dissecting microscope (Pound,Facelli et al. 2009). Only healthy-looking fruits and seeds were included in experiments.

Table 8-1 Description of six plant species included in salinity and water stress experiments, including collection viability results and seed embryo types.

	Species Name	Family	Date Collected	Dispersal Unit	Viability (%)	Seed Embryo
1	*Enneapogon cylindricus* (N.T. Burb.)	Gramineae	Feb 2009	Caryopsis	98	Lateral
2	*Eriochiton sclerolaenoides* (F. Muell.)	Chenopodiaceae	Oct 2008	Fruit	80	Peripheral
3	*Eucalyptus oleosa* (F.Muell. ex Miq.) *ssp. ampliata* (L.A.S.Johnson & K.D.Hill)	Myrtaceae	Oct 2008	Seed	96	Bent
4	*Lepidium phlebopetalum* (F. Muell.)	Cruciferae	Oct 2007	Seed	99	Bent
5	*Maireana trichoptera* (J.M. Black)	Chenopodiaceae	Oct 2008	Fruit	96	Peripheral
6	*Rhodanthe floribunda* (Paul G. Wilson)	Compositae	Oct 2007	Achene	99	Spathulate

Preparation of GA_3 Pulse Treatments

Results from previous germination experiments (refer to Chapter 6 and Chapter 7 of this thesis) provided the most effective pre-treatment to overcome physiological dormancy and to maximise seed germination in each species. *Enneapogon cylindricus* and *M. trichoptera* received pulse treatments with 250mg/L (0.7 mM) GA_3 at pH 3.0 for 24 hours and *L. phlebopetalum* and *R. floribunda* received pulse treatments of 500 mg/L (1.4 mM) GA_3 at pH 2.9 for 24 hours. Approximately 1 ml of ethanol (EtOH) was used to the aid dissolution of GA_3 into sterilised reverse osmosis (SRO) water. Previous germination studies by Pound et al. (2009) found that seeds of *Eucalyptus oleosa ssp. ampliata* were non-dormant and therefore required no pre-treatment.

Preparation of PEG and NaCl

Water potentials were calculated using the following equations:

1. ψ_{NaCl} (MPa) = -0.0045*x* – 0.0218 (R^2 = 0.9981), where x is the concentration of NaCl (mM) (Stevens,Barrett-Lennard et al. 2006).

2. ψ_{PEG} (MPa) = $-1.29x^2T - 140x^2 - 4.0x$, where x is the concentration of PEG by % w/v PEG = $(4 - (5.16\psi T - 560\psi + 16)^{0.5})/(2.58T - 280)$ where *T* is temperature (Michel 1983).

Both NaCl and PEG-8000 were dissolved into SRO water without additives.

Incubator Conditions and Treatments

This experiment used two constant incubator settings, 15°C and 30°C, both with 12-hour light periods (Table 8-2). Pound et al. (2009) showed that many of the species selected for this study were capable of germinating at temperatures ranging between 15°C and 30°C inclusive.

Table 8-2 A summary of water potentials (MPa) and incubator conditions used to examine salinity and water stress on seed germination in six arid plant species.

Treatment	Agent	Water Potentials (MPa)	Incubator Conditions
1	Control	0	15°C constant 12-hour light period
2	Control	0	30°C constant 12-hour light period
3	NaCl	-0.25	15°C constant 12-hour light period
4	NaCl	-0.5	
5	NaCl	-1.0	
6	NaCl	-2.0	
7	NaCl	-0.25	30°C constant 12-hour light period
8	NaCl	-0.5	
9	NaCl	-1.0	
10	NaCl	-2.0	
11	PEG	-0.25	15°C constant 12-hour light period
12	PEG	-0.5	
13	PEG	-1.0	
14	PEG	-2.0	
15	PEG	-0.25	30°C constant 12-hour light period
16	PEG	-0.5	
17	PEG	-1.0	
18	PEG	-2.0	

Seed Germination

Four replicates of 25 seeds were used in each treatment. Seeds were placed into 90 mm glass Petri dishes on top of two Advantec™ glass fibre filters. An automated pipette was used to add each solution, either SRO (control treatments) or SRO supplemented with NaCl or PEG-8000 to achieve water potentials of 0, -2.5, -0.5, -1.0, and -2.0MPa. Excess solution was

removed by either tipping it from the plate or using a transfer pipette. Clear plastic was wrapped around each stack of four replicate Petri dishes to minimise moisture loss. To avoid the concentration build-up of NaCl and PEG-8000 solutions, glass fibre filters were changed each fortnight, with condensation on lids removed with a tissue and fresh solution added. Germination was scored weekly and defined as radicle emergence ≥ half the length of the propagule, with germinated seeds removed at the time of recording. To avoid potential position effects, each Petri dish was repositioned within its stack as well as within the incubator at the time of scoring.

Statistical Analysis

Mixed between-within subjects analysis of variance (i.e. Split-plot ANOVA) was used to analyse data from treatments and controls for each species at 7, 21 and 42 days, with alpha 0.05 used to determine significance (PASW version 18 2010, formerly SPSS). Percentage values were arc-sine transformed prior to analysis to improve normality. Results quoted are from Wilks' lambda distribution scores. A positive interaction between time and treatment indicated that the significance of some treatments changed over time. Data from different times were then analysed separately using non-parametric permutational multivariate analysis of variance tests i.e. PerMANOVA (Anderson 2001). Euclidean distance pair-wise tests were used to differentiate between treatments, with alpha set at 0.05 (PerMANOVA version 1.6). For each species, statistics are compared within the same time period across all NaCl and PEG-8000 treatments and two incubator conditions.

8.3 Results

Enneapogon cylindricus

After 42 days, seed germination in *E. cylindricus* differed between treatments (P = 0.0001, df = 17, SS = 110.9520). Highest germination percentages were recorded in both control treatments at 15°C and 30°C as well as in NaCl and PEG treatments -0.25 MPa and -0.5 MPa at 30°C (P values < 0.05) (Figure 8-1). Germination rates were < 50% in all NaCl and PEG treatments at 15°C. These results indicate that at 30°C seeds of *E. cylindricus* can tolerate moderately low water potentials of -0.5 MPa with germination inhibited at ≥ -1.0 MPa. Seed germination at 15°C is inhibited by relatively small reductions in water potential ≥ -0.25 MPa.

Eriochiton sclerolaenoides

Final germination results differed between treatments (P = 0.0001, df = 17, SS = 75.8622). Only under constant 15°C did *E. sclerolaenoides* achieve its highest germination percentages in the control, NaCl and PEG treatments -0.25 MPa, and NaCl treatments -0.5 MPa and -1.0 MPa (P values < 0.05) (Figure 8-2). Germination rates were < 50% in all NaCl and PEG treatments kept at constant 30°C, including the control (14%). These results show that 30°C inhibits seed germination in *E. sclerolaenoides*. At 15°C, seeds tolerate NaCl to extremely low water potentials of -1.0 MPa (76%) and -2.0 MPa (58%). Seeds were less tolerant of low

water potentials induced by PEG than NaCl, with very low germination recorded at -1.0 MPa (23%) and -2.0 MPa (0%).

Eucalyptus oleosa ssp. *ampliata*

After 42 days, *E. oleosa* ssp. *ampliata* germination results differed between treatments (P = 0.0001, df = 17, SS = 56.3655). Germination peaked at 76% in the control under constant 15°C conditions (Figure 8-3). Germination was inhibited in all other NaCl and PEG treatments at this temperature, with all germination < 11%, indicating that this species is highly sensitive to salinity and water stress. Final germination percentages were < 10% in all NaCl and PEG treatments under constant 30°C conditions, including the control. Higher temperatures (i.e. 30°C) therefore inhibit germination in this species.

Lepidium phlebopetalum

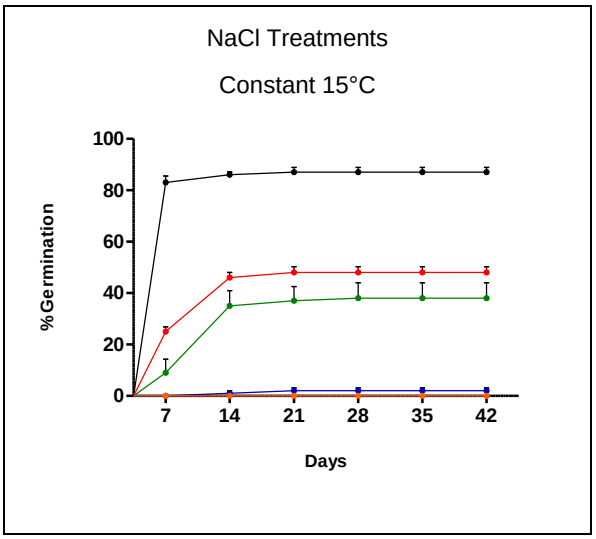
Final germination results differed between treatments (P = 0.0001, df = 17, SS = 99.0243). Peak germination percentages were recorded in both controls and PEG treatment -0.25 MPa at 30°C (P values < 0.05) (Figure 8-4). Germination percentages in all NaCl (30°C) and PEG (15°C) treatments were < 40%. Seeds germinated well at 30°C in PEG treatment -0.25 MPa (66%) but germination was significantly lower in NaCl treatments (< 12%) (P < 0.05). These results suggest that *L. phlebopetalum* seeds are sensitive to toxic ions Na^+ and/or Cl^- and that temperature may affect the degree of toxicity.

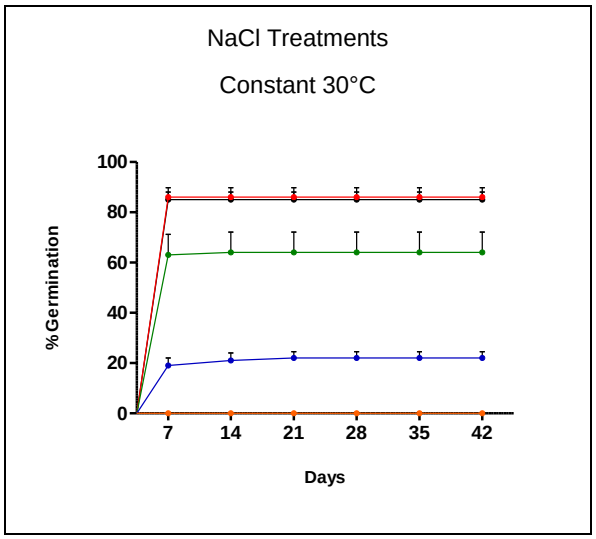
Maireana trichoptera

Final germination results at 42 days showed that germination in *M. trichoptera* differed between treatments (P = 0.0001, df = 17, SS = 70.1541). At constant 15°C, seeds of *M. trichoptera* tolerated NaCl to extremely low water potentials of -1.0 MPa (94%) and -2.0 MPa (70%) as germination was similar to the control (P values > 0.05) (Figure 8-5). Tolerance was reduced at 30°C with germination percentages of 74% at -1.0 MPa and 32% at -2.0 MPa. Seeds were less tolerant of low water potentials induced by PEG than NaCl, with germination lower in PEG treatments -1.0M Pa (15°C: 71% and 30°C: 8%) and -2.0 MPa (15°C: 0% and 30°C: 0%).

Rhodanthe floribunda

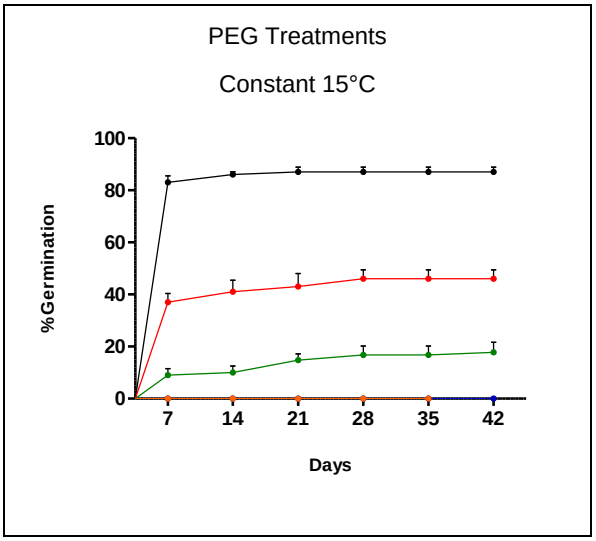
Final germination results for *R. floribunda* varied between treatments (P = 0.0001, df = 17, SS = 94.2723). Germination percentages were highest in both controls and NaCl and PEG treatments -0.25 MPa at 15°C and 30°C (P values < 0.05) (Figure 8-6). Germination was < 20% in all NaCl and PEG treatments -1.0 MPa and -2.0M Pa under both incubator conditions. Seed germination reached approximately 50% in NaCl and PEG treatments -0.5 MPa, excluding PEG treatment at constant 15°C (37%). *Rhodanthe floribunda* seeds showed a sensitivity to reductions in water potentials > -0.25 MPa.

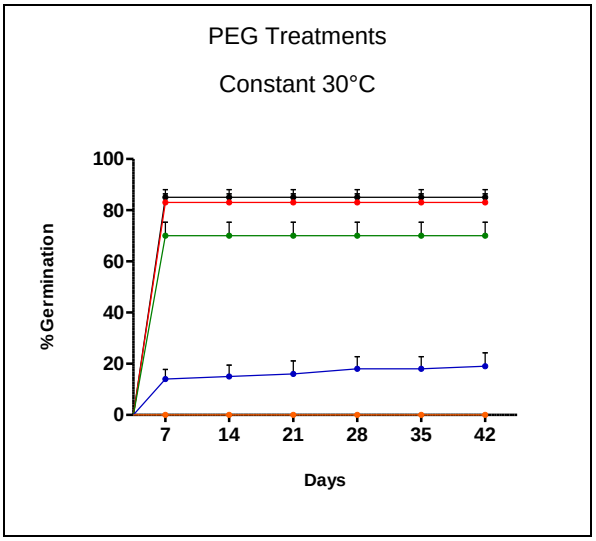




NaCl Treatments Constant 15°C	7 Days	21 Days	42 Days
Control	a	a	a
-0.25 MPa	cd	b	b
-0.5 MPa	def	bc	bc
-1.0 MPa	f	ef	d
-2.0 MPa	f	f	d

NaCl Treatments Constant 30°C	7 Days	21 Days	42 Days
Control	a	a	a
-0.25 MPa	a	a	a
-0.5 MPa	ab	ab	ab
-1.0 MPa	de	cd	c
-2.0 MPa	f	f	d

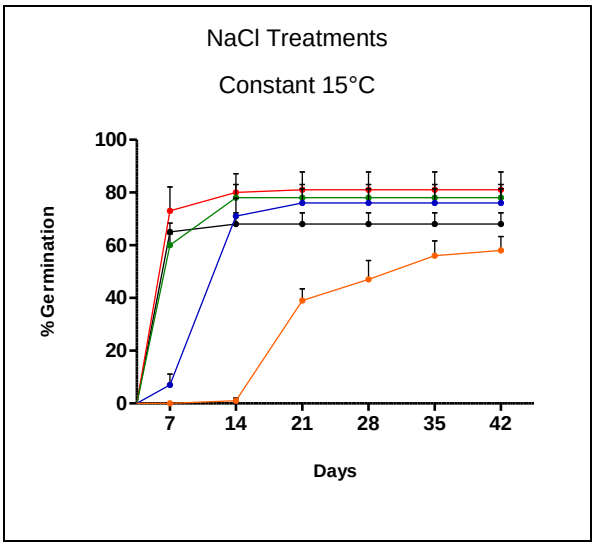


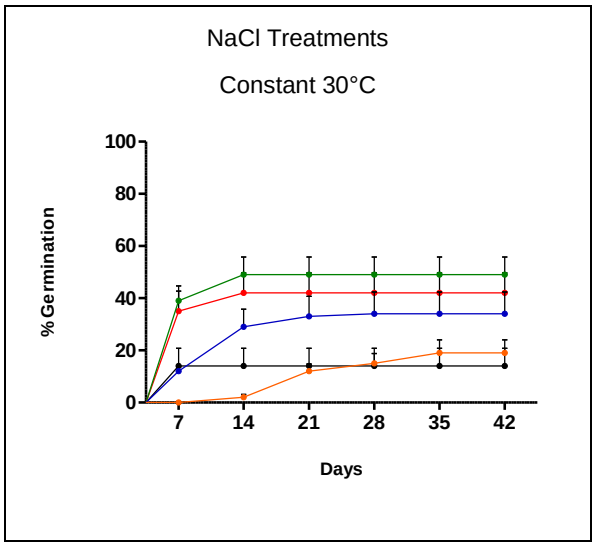


PEG Treatments Constant 15°C	7 Days	21 Day	42 Days
Control	a	a	a
-0.25 MPa	bc	bc	b
-0.5 MPa	e	d	c
-1.0 MPa	f	f	d
-2.0 MPa	f	f	d

PEG Treatments Constant 30°C	7 Days	21 Days	42 Days
Control	a	a	a
-0.25 MPa	a	a	a
-0.5 MPa	a	a	a
-1.0 MPa	de	cde	c
-2.0 MPa	f	f	d

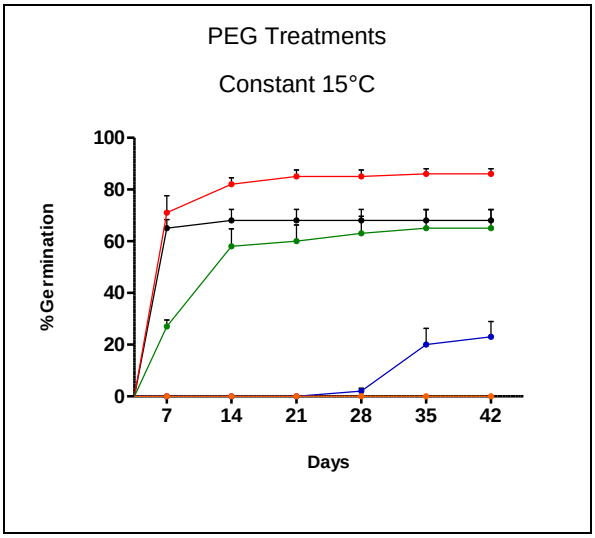
Figure 8-1 *Enneapogon cylindricus* – Germination results from water potential experiments. Statistics are compared within the same time period across all NaCl and PEG treatments and two incubator conditions: constant 15°C and 30°C. *P* value is significant when < 0.05.

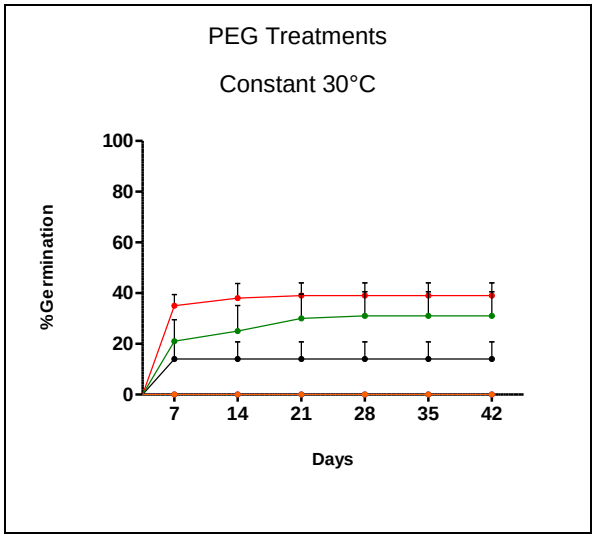




NaCl Treatments Constant 15°C	7 Days	21 Days	42 Days
Control	a	bcd	bc
-0.25 MPa	a	ab	ab
-0.5 MPa	ac	abc	ab
-1.0 MPa	de	abcd	abc
-2.0 MPa	e	ef	bcd

NaCl Treatments Constant 30°C	7 Days	21 Days	42 Days
Control	bde	fgh	ef
-0.25 MPa	bc	def	cde
-0.5 MPa	b	def	cde
-1.0 MPa	d	efg	de
-2.0 MPa	e	g	e

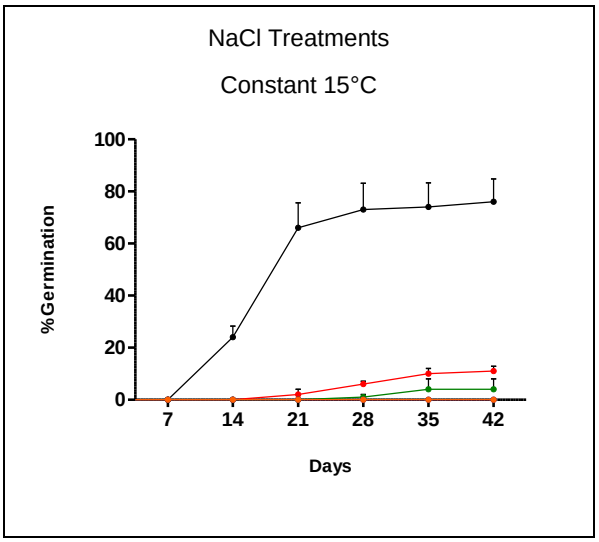


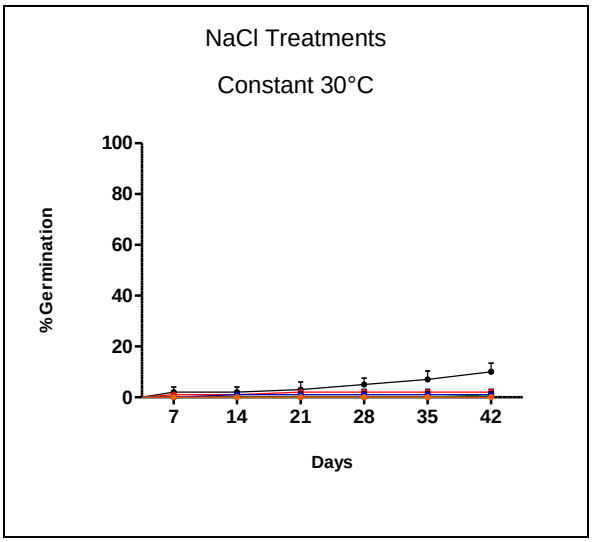


PEG Treatments Constant 15°C	7 Days	21 Day	42 Days
Control	a	bcd	bc
-0.25 MPa	a	a	a
-0.5 MPa	b	bcde	bcd
-1.0 MPa	e	h	e
-2.0 MPa	e	h	f

PEG Treatments Constant 30°C	7 Days	21 Days	42 Days
Control	bde	fgh	ef
-0.25 MPa	b	ef	de
-0.5 MPa	bde	efg	de
-1.0 MPa	e	h	f
-2.0 MPa	e	h	f

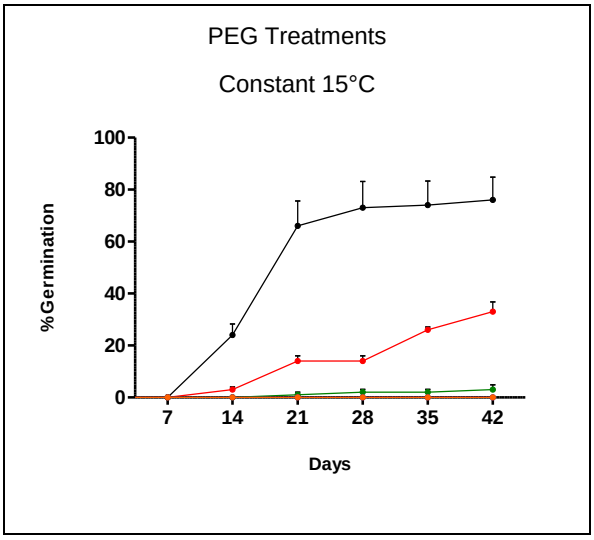
Figure 8-2 *Eriochiton sclerolaenoides* – Germination results from water potential experiments. Statistics are compared across all NaCl and PEG treatments and the two incubator conditions constant 15°C and 30°C within the same time period. *P* value is significant when < 0.05.

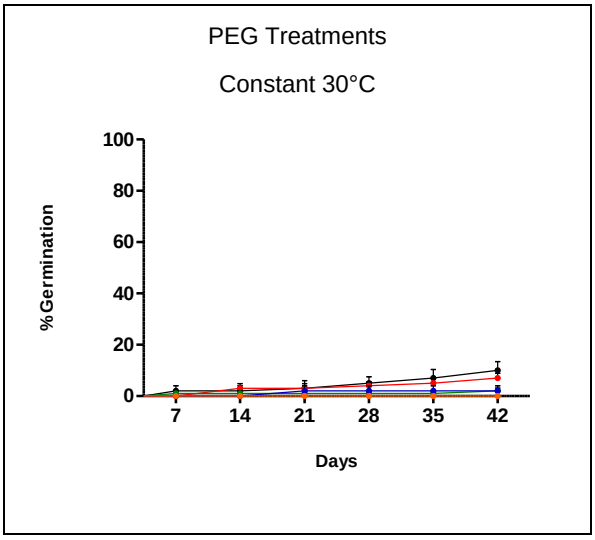




NaCl Treatments Constant 15°C	7 Days	21 Days	42 Days
Control	a	a	a
-0.25 MPa	a	bc	ce
-0.5 MPa	a	c	cdef
-1.0 MPa	a	c	f
-2.0 MPa	a	c	f

NaCl Treatments Constant 30°C	7 Days	21 Days	42 Days
Control	a	bc	cde
-0.25 MPa	a	bc	cde
-0.5 MPa	a	c	df
-1.0 MPa	a	bc	ef
-2.0 MPa	a	c	f

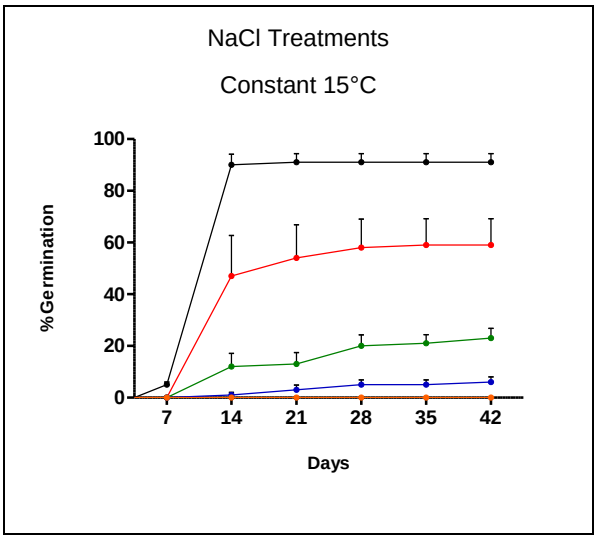


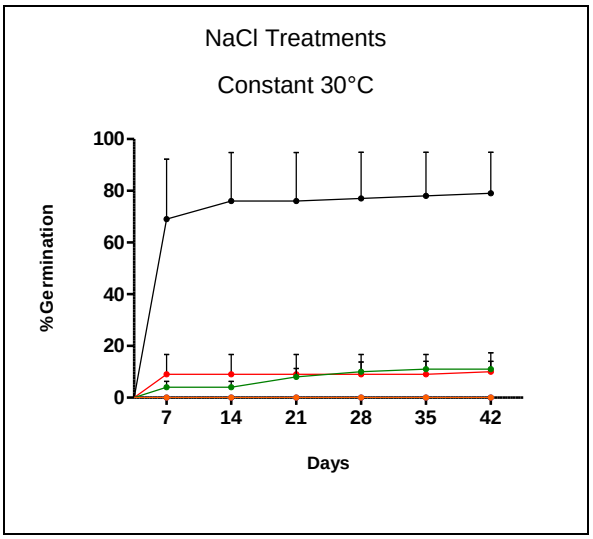


PEG Treatments Constant 15°C	7 Days	21 Day	42 Days
Control	a	a	a
-0.25 MPa	a	b	b
-0.5 MPa	a	c	cdef
-1.0 MPa	a	c	f
-2.0 MPa	a	c	f

PEG Treatments Constant 30°C	7 Days	21 Days	42 Days
Control	a	bc	cde
-0.25 MPa	a	c	df
-0.5 MPa	a	c	df
-1.0 MPa	a	c	df
-2.0 MPa	a	c	f

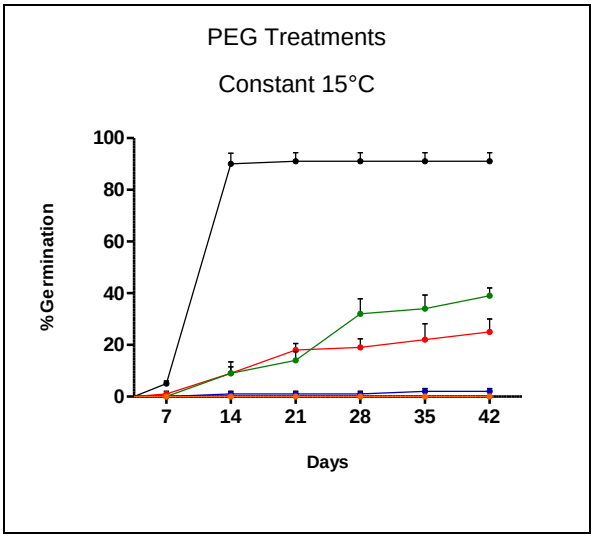
Figure 8-3 *Eucalyptus oleosa ssp. ampliata* – Germination results from water potential experiments. Statistics are compared across all NaCl and PEG treatments and the two incubator conditions constant 15°C and 30°C within the same time period. *P* value is significant when < 0.05.

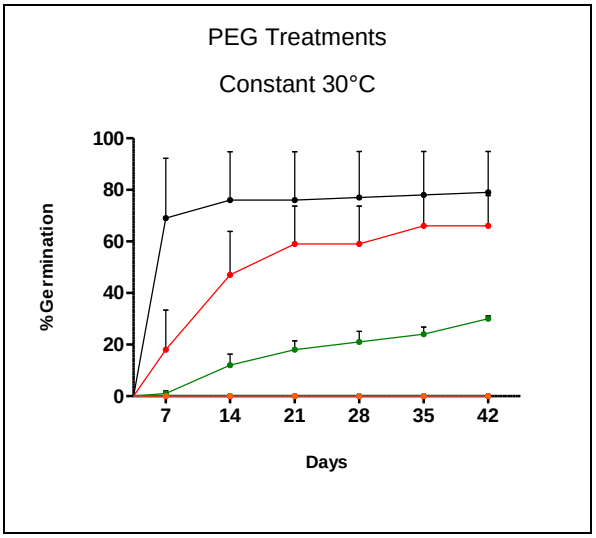




NaCl Treatments Constant 15°C	7 Days	21 Days	42 Days
Control	a	a	a
-0.25 MPa	b	bd	b
-0.5 MPa	b	cefg	de
-1.0 MPa	b	fgh	fgh
-2.0 MPa	b	h	h

NaCl Treatments Constant 30°C	7 Days	21 Days	42 Days
Control	ab	abcd	abc
-0.25 MPa	ab	cdefgh	cdegh
-0.5 MPa	ab	efgh	efg
-1.0 MPa	b	h	h
-2.0 MPa	b	h	h

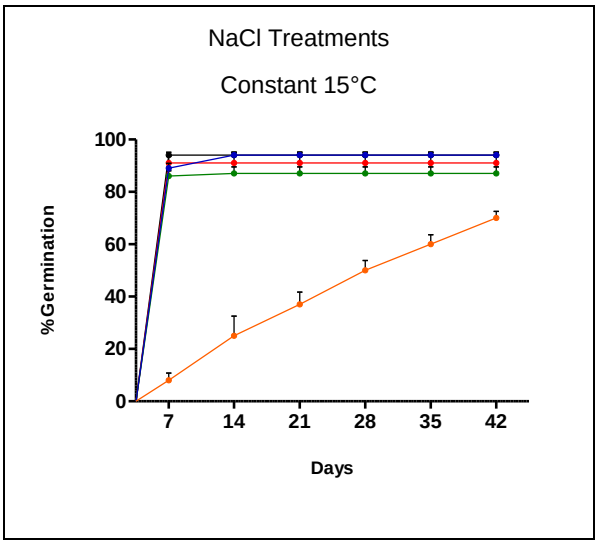


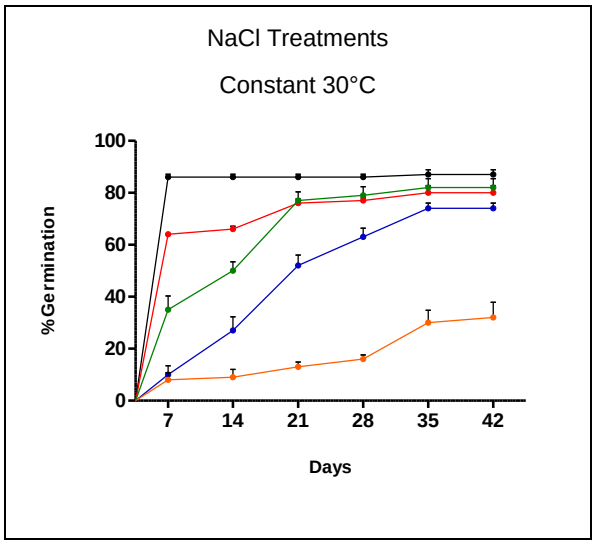


PEG Treatments Constant 15°C	7 Days	21 Day	42 Days
Control	a	a	a
-0.25 MPa	ab	ce	bcd
-0.5 MPa	b	cef	bc
-1.0 MPa	b	gh	gh
-2.0 MPa	b	h	h

PEG Treatments Constant 30°C	7 Days	21 Days	42 Days
Control	ab	abcd	abc
-0.25 MPa	ab	abd	ab
-0.5 MPa	ab	cde	cd
-1.0 MPa	b	h	h
-2.0 MPa	b	h	h

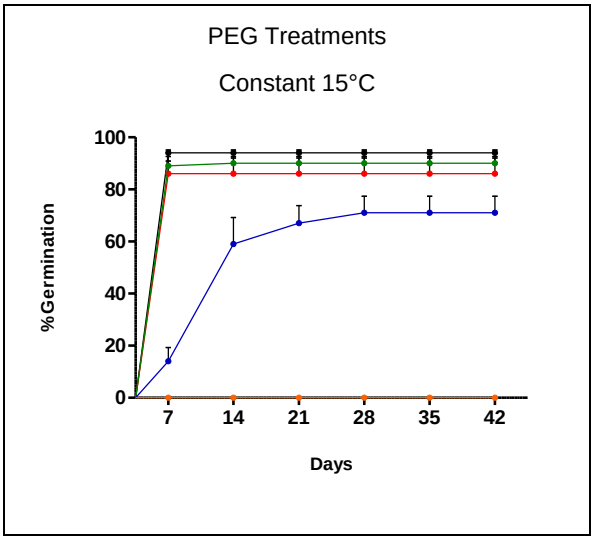
Figure 8-4 *Lepidium phlebopetalum* – Germination results from water potential experiments. Statistics are compared across all NaCl and PEG treatments and the two incubator conditions constant 15°C and 30°C within the same time period. *P* value is significant when < 0.05.

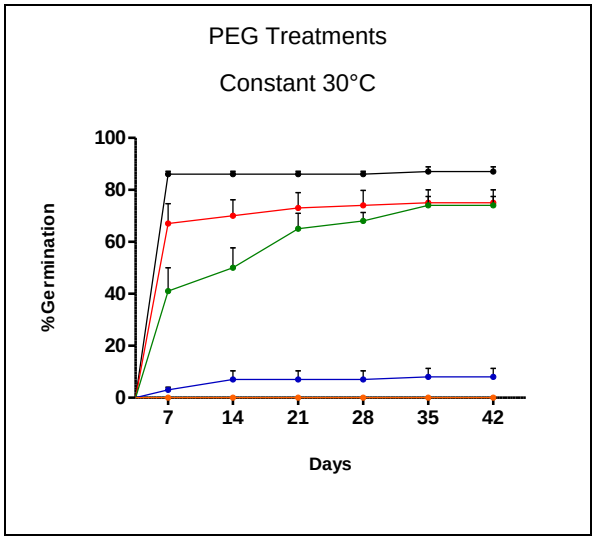




NaCl Treatments Constant 15°C	7 Days	21 Days	42 Days
Control	a	a	a
-0.25 MPa	ab	abd	ab
-0.5 MPa	b	abcd	abc
-1.0 MPa	ab	a	a
-2.0 MPa	ef	g	d

NaCl Treatments Constant 30°C	7 Days	21 Days	42 Days
Control	b	bd	ab
-0.25 MPa	c	ce	bcd
-0.5 MPa	d	bcde	abcd
-1.0 MPa	ef	fg	cd
-2.0 MPa	e	h	e

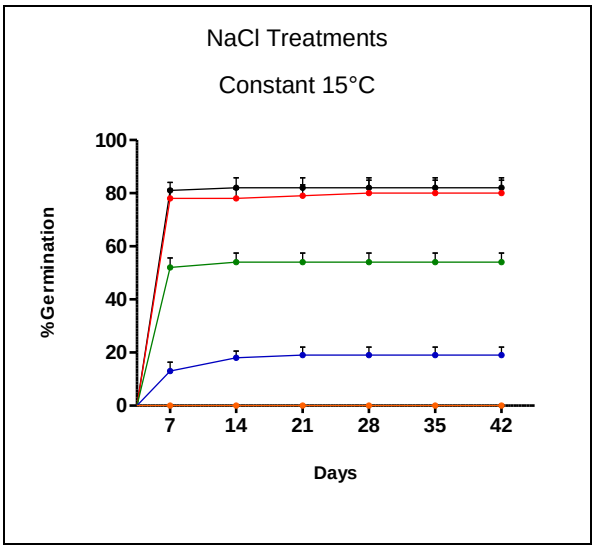


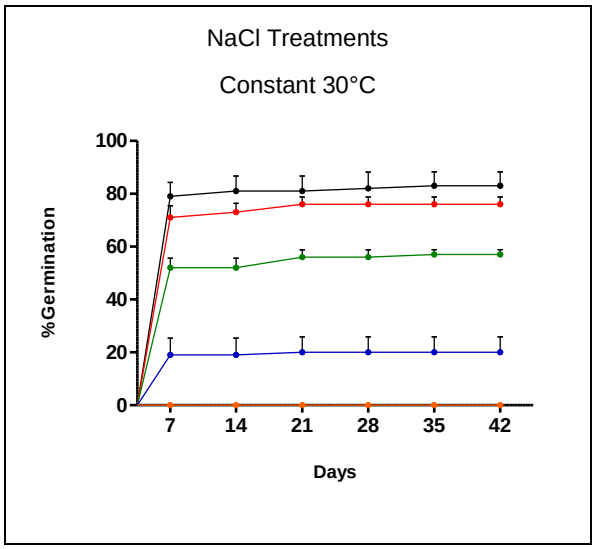


PEG Treatments Constant 15°C	7 Days	21 Day	42 Days
Control	a	a	a
-0.25 MPa	ab	abcde	abcd
-0.5 MPa	ab	abd	ab
-1.0 MPa	de	cef	cd
-2.0 MPa	f	i	f

PEG Treatments Constant 30°C	7 Days	21 Days	42 Days
Control	b	bd	ab
-0.25 MPa	bc	de	bcd
-0.5 MPa	cd	ef	cd
-1.0 MPa	ef	h	f
-2.0 MPa	f	i	f

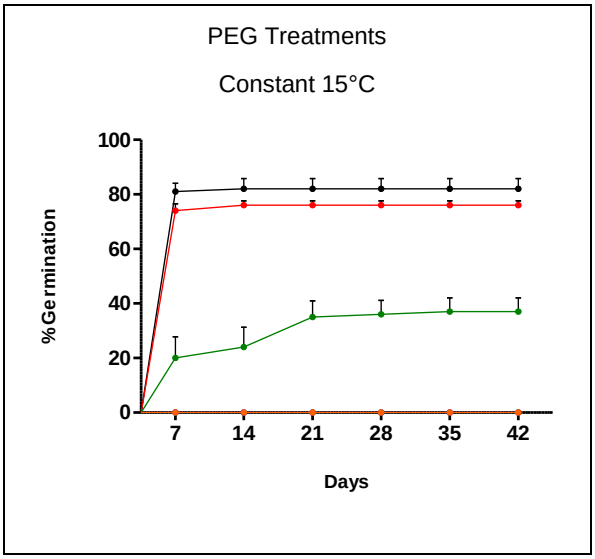
Figure 8-5 *Maireana trichoptera* – Germination results from water potential experiments. Statistics are compared across all NaCl and PEG treatments and the two incubator conditions constant 15°C and 30°C within the same time period. *P* value is significant when < 0.05.

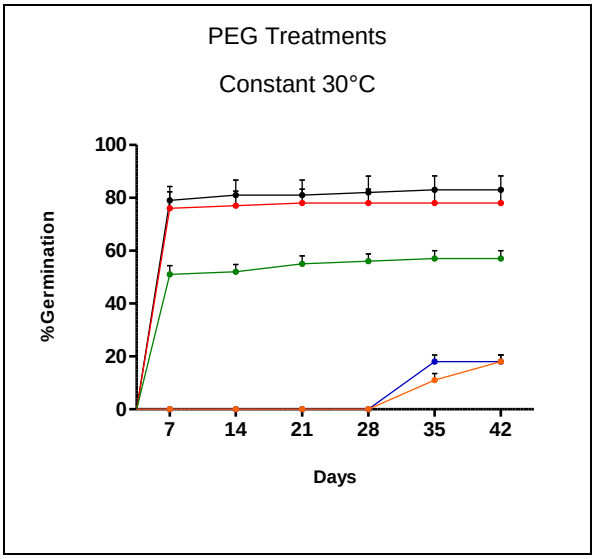




NaCl Treatments Constant 15°C	7 Days	21 Days	42 Days
Control	a	a	a
-0.25 MPa	a	a	a
-0.5 MPa	b	bd	bd
-1.0 MPa	c	e	e
-2.0 MPa	d	f	f

NaCl Treatments Constant 30°C	7 Days	21 Days	42 Days
Control	a	ac	a
-0.25 MPa	ab	a	a
-0.5 MPa	b	bd	b
-1.0 MPa	c	e	e
-2.0 MPa	d	f	f





PEG Treatments Constant 15°C	7 Days	21 Day	42 Days
Control	a	a	a
-0.25 MPa	a	a	a
-0.5 MPa	c	de	de
-1.0 MPa	d	f	f
-2.0 MPa	d	f	f

PEG Treatments Constant 30°C	7 Days	21 Days	42 Days
Control	a	ac	a
-0.25 MPa	ab	ac	ac
-0.5 MPa	b	bc	bc
-1.0 MPa	d	f	e
-2.0 MPa	d	f	e

Figure 8-6 *Rhodanthe floribunda* – Germination results from water potential experiments. Statistics are compared across all NaCl and PEG treatments and the two incubator conditions constant 15°C and 30°C within the same time period. *P* value is significant when < 0.05.

8.4 Discussion

A range of germination responses were recorded from the six plant species examined, each having its own critical water content requirement for germination as previously found in other studies (Baskin and Baskin 1998; Fenner and Thompson 2005). The species studied ranged from sensitive, moderately tolerant to extremely tolerant of salinity and water stress conditions. Importantly, some species were differentially affected by NaCl and PEG, indicating different sensitivity to water availability and toxicity. These factors in turn interacted with temperature to determine germination rates and fractions.

Eucalyptus oleosa ssp. ampliata seeds were the most sensitive to reductions in water potential with very low germination recorded across all NaCl and PEG treatments. *Lepidium phlebopetalum* and *R. floribunda* seeds were very sensitive to reductions in water potentials > -0.25 MPa, whereas seeds of *E. cylindricus* were moderately tolerant up to -0.5 MPa. *Eriochiton sclerolaenoides* and *Maireana trichoptera* both showed the capacity to germinate under very low water potentials of -2.0 MPa.

Salinity and water stress affected the temperature range over which some of the species were capable of germinating. At 30°C seeds of *E. cylindricus* germinated in moderately low water potentials, yet at 15°C seeds were sensitive to even the smallest reductions. Although this grass species generally germinates and establishes after large rainfall events during hot summer months (Kutsche and Lay 2003), it was capable of germinating at the cooler temperature of 15°C once dormancy had been overcome. The results indicate that moderate levels of salinity and water stress will reduce the temperature range over which this species is capable of germinating, restricting germination to higher temperatures.

Salinity and water stress also affected the temperature range over which *M. trichoptera* seeds were capable of germinating. Only at 15°C could seeds germinate >50% under very low water potentials of -1.0 MPa (PEG treatments) and -2.0 MPa (NaCl treatments). Although this species generally germinates during winter, results from controls indicate that germination is also possible at higher temperatures (i.e. 30°C) once dormancy has been overcome. If conditions are suitable, this species has the potential to germinate at other times of the year. Salinity and water stress may impact on this species by restricting the temperature range over which it can germinate to cooler temperatures.

The temperature range over which *L. phlebopetalum* was capable of germinating was affected by salinity. This annual species is very common after consistent winter-spring rains (Kutsche and Lay 2003). At constant 15°C, seeds tolerated a slight reduction in water potential from NaCl whereas germination was very low under constant 30°C. Water stress induced by PEG did not affect the temperature range over which this species could germinate, suggesting that *L. phlebopetalum* seeds may be sensitive to Na^+ and/or Cl^- ions and that the degree of toxicity was increased by the higher temperature. At higher temperatures the seed membrane permeability may be such that it does not exclude ions or the metabolic processes of the seed increase making them more susceptible to ionic toxicity

(Lambers,Chapin et al. 2008). Future research is needed to test whether treatment results reverse once seeds are transferred to water, as per Debez, Ben Hamed *et al.* (2004).

Species differed in their germination response to salinity (NaCl) and water stress (PEG) treatments. For three species, *E. cylindricus*, *E. oleosa ssp. ampliata* and *R. floribunda*, there were no distinguishable patterns in the germination responses between NaCl and PEG treatments. These results suggest that for the range of water potentials tested, NaCl affects the germination of these three species by osmotic effect rather than through toxicity.

Eriochiton sclerolaenoides seeds were capable of germinating at high salinity levels. As with many other species in the Chenopodiaceae family, this species may be utilising Na^+ and/or Cl^- ions to produce gradients in water potential lower in the seed than the surrounding environment. This germination response is typical of seedlings that effect selective accumulation of ions (Facelli 2008). *Maireana trichoptera* also demonstrated high tolerance levels to extremely low water potentials in NaCl treatments, suggesting that selective accumulation also occurs in seeds of this species. Both of these species are halophytic and favour accumulation of carbonates and other soluble salts in the upper part of the soil profile (Kovda,Somoilova et al. 1981).

Salinity and water stress can cause delays in seed germination once seed dormancy has been broken. Although the majority of germination occurred within the first seven days in most treatments, some species experienced lengthy delays in some NaCl and PEG treatments. Most notable was *E. sclerolaenoides*, where seed germination was delayed between 14 and 21 days in NaCl treatments -1.0 MPa and -2.0 MPa at 15°C. The potential impacts of delaying germination in this species are heavily dependent on the timing of germination events and follow-up temperatures and rainfall conditions. Deferred germination could potentially assist survivorship of *E. sclerolaenoides* populations when soil becomes too dry to support seedling establishment. Those seeds with delayed germination would remain alive in the soil seed bank. Conversely, deferred germination may increase the risk of seedling mortality by shifting seedling emergence and establishment events towards unfavourable seasons i.e. towards dry and hot summer months. There can be little doubt that delayed germination caused by salinity and water stress has the potential to affect age and community structure as well as change competitive interactions between seedlings in arid environments.

Rhodanthe floribunda seeds also experienced delayed germination at low water potentials, commencing after 28 days in PEG treatments -1.0 MPa and -2.0 MPa at 30°C. Germination had not levelled after 42 days; however, the germination rate was considered likely to remain low. If this reflects natural system responses, then seedling survival could be at risk from high summer temperatures and unreliable rains. Delays in germination are critical for this species. As an annual it germinates in large quantities when dormancy is broken and suitable conditions occur. If over consecutive seasons germination is delayed and results in low seedling establishment, then the soil seed bank risks becoming depleted over time.

The germination responses of the plant species examined here may be reflecting a connection to some aspect of the life history stage of each of the plants. *Eucalyptus oleosa* ssp. *ampliata* is a long-lived tree that stores seeds from multiple seasons inside capsules within its canopy. Seeds from this species display no dormancy mechanism upon their release from capsules and do not form persistent soil seed banks (Pound,Facelli et al. 2009). The sensitivity shown by *E. oleosa* ssp. *ampliata* seeds to salinity and water stress may reflect the reduced level of contact that seeds have with naturally high salinity levels that occur in the soil seed bank.

Seeds of the two annual forbs, *L. phlebopetalum* and *R. floribunda*, were found to be very sensitive to reductions in water potentials. Both species are common after cool winters and heavy winter/spring rains (Kutsche and Lay 2003). As a consequence, their germination naturally occurs during a time when salinity and water stress would be minimised by cool and wet conditions. This prevents their germination in conditions that may not lead to successful reproduction, thus preserving seeds in the soil seed bank. In contrast, the annual or short-lived grass *E. cylindricus* has a relatively higher tolerance for low water potentials. This species generally germinates in response to good summer rains and therefore at a time when water potentials would be naturally low. It is likely that this species has higher tolerance to water stress and is able to produce seed even when conditions turn unfavourable. Not surprisingly, both halophytic shrubs, *E. sclerolaenoides* and *M. trichoptera* (Chenopodiaceae) showed the greatest capacity of all the plant species examined to germinate under conditions of salinity and water stress.

Implications for Restoration

Any reductions in optimum temperature ranges for seed germination will impact on restoration success, particularly by compounding existing problems of low erratic rainfall and extreme temperatures (Commander,Merritt et al. 2009). *Enneapogon cylindricus* is considered a good soil stabiliser species (Kutsche and Lay 2003) and has demonstrated the capacity to germinate at moderate levels of salinity. Germination will be restricted to warmer months of the year and seedling establishment would be highly dependent on good follow-up rains. In contrast, *Eucalyptus oleosa ssp. ampliata* has no tolerance threshold for salinity and water stress and this species will require targeted measures to ensure its successful inclusion in the restoration programs.

Lepidium phlebopetalum seeds are sensitive to salinity and water stress. The ecotypic response of this species may be worth investigating, by screening seeds collected from less saline areas and comparing them with the germination response of seeds collected from areas higher in salinity (i.e. closer to nearby salt lakes). *Rhodanthe floribunda* is a spreading forb that is common after heavy cool-season rains, often carpeting large areas (Kutsche and Lay 2003). Our study shows that it is a sensitive species and that salinity and water stress may cause depletion of the soil seed bank by delaying germination and increasing the risk of seedling mortality. *Eriochiton sclerolaenoides* and *Maireana trichoptera* are both halophytic plants that tolerate relatively high salinity and low water potentials for germination. These

shrubs have relatively shallow root systems and may be able to establish quickly and reach reproductive maturity in areas of higher salinity.

Further research

Further research should consider screening larger subsets of species to see if trends exist between germination responses to salinity and water stress and specific seed traits, such as seed size or embryo type. Another consideration is the impact of increased salinity on soil microflora, as the seeds of some species are likely to have positive relationships with soil biota such as fungi. This research could also be taken further by investigating different salt ions and their impacts on seed longevity, germination, seedling establishment and seedling survival.

Chapter 9
General Discussion and Recommendations for Further Research

A dingo in the morning light near the study the site, 2008. Photo E Steggles.

9.1 General Discussion

This study investigated the roles of the soil seed bank and the biological soil crust in contributing to spatial and temporal heterogeneity in an *A. papyrocarpa* open woodland. Results add to our understanding of heterogeneity and patch dynamics in arid ecosystems and help to inform the management and restoration of these systems. Understanding the germination responses of key plant species to salinity and water stress is also important to help maximise species return in disturbed sites. This final chapter provides an overall discussion of the research results and a summary of the new information that has been gained during the course of the project. Several of the research outcomes have implications for ecological restoration in arid ecosystems and these are discussed in a separate section.

Heterogeneity and Patch Dynamics

Through documenting key environmental conditions at the study site (Chapter 3), I have demonstrated that *A. papyrocarpa*, *A. vesicaria* ssp. *variabilis* and *M. sedifolia* influence heterogeneity and patch dynamics in *A. papyrocarpa* open woodlands. This is the first study to have characterised heterogeneity concurrently between four patch types: *A. papyrocarpa*, *A. vesicaria* ssp. *variabilis*, *M. sedifolia* and open areas in a system with minimum grazing pressure. Past studies have compared *A. papyrocarpa* with open spaces (Facelli and Brock 2000), *A. vesicaria*, *M. sedifolia* and open spaces (Facelli and Temby 2002), and *M. sedifolia* and open spaces (Weedon and Facelli 2008) and all of these studies were conducted in sites with prolonged histories of sheep grazing. The study site in this research is likely to have a grazing regime similar to that before European settlement. There have also been no previous studies that measured soil nutrient content, soil temperature and soil moisture content simultaneously in these four patch types.

Some of the environmental conditions I measured had similar distribution patterns to those of previous studies undertaken in grazed open woodland. For example, there was a similar distribution pattern of phosphorous in soils beneath *M. sedifolia* and open areas as found by Facelli and Temby (2002). The indication that soils beneath *M. sedifolia* generally have lower moisture contents than other patch types was also observed in a similar grazed system (J. M. Facelli, unpublished data). Overall, the spatial variation in environmental conditions and soil properties observed in the soil seed bank beneath trees, shrubs and open areas, suggest that seeds would receive very different dormancy-breaking cues and germination cues (i.e. soil nutrients, temperature and water availability) in each of the four patch types. This study provides important information on environmental conditions at the study site, which was relevant to my investigation of the spatial and temporal patterns in the germinable soil seed bank (Chapter 4). The results also added valuable information to our general understanding of heterogeneity in arid ecosystems.

A key difference between this study site and a similar grazed system was the relative abundance of perennial species recorded in both seedling monitoring (Chapter 3) and the germinable soil seed bank (Chapter 4). In contrast, most species recorded in the disturbed

system were annual species (Facelli and Temby 2002). There are two potential non-exclusive explanations for this variation between the two systems. Firstly, that grazing can substantially change the spatial distribution of seeds and patterns in the deposition of detritus, and transported particles may be substantially different (Emmerson,Facelli et al. 2010). Secondly, that different life stages of *A. papyrocarpa* trees can contribute to variation in the composition of seedlings. At the study site, *A. papyrocarpa* trees are younger (stage IV) than those in the grazed system (stages IV, V and VI) and lack the procumbent (and sometimes polyprocumbent) canopies often represented there. These variations in canopy structure can influence differently the spatial distribution of seeds and patterns in the deposition of litter. Our research, therefore, adds further weight to the importance of age structure and population dynamics of woody perennial species for ecosystem and community dynamics of arid lands (Facelli and Brock 2000).

Relatively high seedling emergence was recorded from soil samples collected at the end of summer, suggesting that the flora in this system may have a trend towards winter and summer partitioning, more similar to the SW of North America (Baskin,Chesson et al. 1993). This strategy was not previously recorded in this system, with Facelli et al. (2005) barely recording any emergence in samples collected in late spring through to late summer. This implies that summer rains may be more predictable at the study site than in similar systems further east near Whyalla, South Australia. Unfortunately, our investigation of water availability and its effects on the composition of the germinable soil seed bank at different times of the year was limited to only one variable (Chapter 4). In our research, soil samples were submersed in water for 7 days to simulate flooding, which was at the very high end of the water availability spectrum. Water availability has been found to affect seedling emergence in samples from open spaces in a similar grazed system (Facelli,Chesson et al. 2005), and consequently, a more comprehensive study testing a range of different water contents is needed to confirm summer and winter partitioning of species in this system.

In general, higher seedling emergence was recorded beneath *A. vesicaria* ssp. *variabilis* and *M. sedifolia* canopies than open areas, whilst results were generally similar between *A. papyrocarpa* and open areas. The latter may again reflect the age and life stage of the trees occurring at the study site. I attribute such differences to variations in the way seeds accumulate beneath shrub canopies as well as different microhabitat conditions (Chapter 3). *Crassula* spp. seedlings were consistently higher in soils collected beneath *A. vesicaria* ssp. *variabilis* than all other patch types, indicating there are differences in the germinable soil seed bank between the two chenopod shrubs. This last finding, therefore, adds further evidence to research by Facelli and Temby (2002) and Weedon and Facelli (2008), which show that shrubs contribute to heterogeneity in arid systems and that individual shrub species differ in their effects.

Through investigating temporal variability in the germinable soil seed bank, our research showed that canopies of both trees and shrubs can provide suitable conditions for seeds to overcome dormancy in seasons other than autumn. This result suggests that if

environmental conditions are suitable, canopy presence can expand emergence opportunities for some plant species to other times of the year. This finding has potential implications for species coexistence, since it provides an expansion of the temporal niche of some species while also contributing to spatial heterogeneity.

Biological soil crusts were also found to play a critical role in contributing to spatial heterogeneity and patch dynamics at the study site (Chapter 5). My results showed that BSCs not only physically inhibited seedling emergence, but also influenced the accumulation of propagules in open inter-shrub areas. This research should be extended by investigating the effects of different successional stage crusts on seedling emergence and with more detailed soil seed bank composition studies (i.e. seed extraction). My results did show, however, that BSCs play a pivotal role in influencing patterns of soil seed bank distribution and therefore heterogeneity in arid ecosystems.

The interaction between BSC and vascular plants was found to be, to some extent, species-specific and often resulting from more than one process. The successional stage of BSC was shown to be critical in determining whether their influence on seed germination was facilitative or inhibitive. My results show that BSCs ultimately determine plant communities in open inter-shrub areas by influencing species composition, species densities and resource availability. Such information adds to the general understanding of BSCs and their contribution to heterogeneity in the distribution of species, and hence to species coexistence and enhanced diversity within ecosystems.

Seed Dormancy and Germination

My preliminary investigation of physiological dormancy (PD) in three *Enneapogon* species (Chapter 6) provides some initial insights into how these species respond to the use of pulse-treatments to overcome PD. I found that all three *Enneapogon* spp. displayed evidence of PD. The germination response of *E. avenaceus* and *E. cylindricus* to applications of GA_3 were indicative of non-deep PD, whilst *E. caerulescens* var. *caerulescens* showed evidence of having either intermediate or deep PD. The primary purpose of this research was to investigate methods to maximise germination for their subsequent inclusion in salinity and water stress experiments. A more comprehensive study using fresh seeds is needed to fully investigate after-ripening, dormancy and germination requirements in all three species.

To my knowledge, no previous studies have investigated methods for improving germination outcomes from the application of GA_3, in particular pulse and chronic applications in conjunction with adjusted pH during solution preparation (Chapter 7). As results from testing seed dormancy and germination can be altered by whether the GA_3 solution is neutral or acidic, my research provides valuable technical information for seed conservation and germination research. The results show that seeds of various species respond differently to adjusting pH values of GA_3. For some species, the germination response to GA_3 pulse treatments was improved with acidic pH, whilst more neutral pH values improved the efficacy of chronic treatments. I recommend that seed conservation and ecology

researchers consider the type of application when determining the pH values of dormancy-testing chemicals, as results from screening seed collections for PD may be altered by neutral or acidic solutions.

In Chapter 8, I investigated germination responses in six arid plant species from different families and life history stages, under salinity and water stress conditions. The plant species varied in their germination responses, ranging from highly sensitive (*Eucalyptus oleosa ssp. ampliata*) to sensitive (*Lepidium phlebopetalum* and *Rhodanthe floribunda*), and moderately tolerant (*Enneapogon cylindricus)* to highly tolerant *(Eriochiton sclerolaenoides* and *Maireana trichoptera).* Salinity and water stress was also shown to affect the temperature ranges over which some species were capable of germinating (*E. cylindricus*, *L. phlebopetalum* and *M. trichoptera*). Two chenopod species, *E. sclerolaenoides* and *M. trichoptera*, showed the capacity for selective accumulation of ions to assist germination. The implications of my results are that salinity and water stress can affect community structure of species populations, as well as change competitive interactions between seedlings in arid ecosystems. These findings have increased our understanding of the seed biology of native flora under salinity and water stress, which is important to inform arid land management and restoration.

9.2 Implications for Restoration Ecology

Understanding differences in microenvironmental conditions and their influence on plant communities is important to inform and direct management and restoration in arid ecosystems. It is often difficult to obtain pre-disturbance data that is site-specific, particularly at the microenvironmental scale. In Chapter 3 of this thesis, I provide important information on environmental conditions between four different patch types, which can be used to inform post-mine restoration at the Jacinth-Ambrosia mineral sands mine located adjacent to the study site. Such information emphasises the importance of patch dynamics, particularly created by tree and shrub canopies, for providing sites where seeds can accumulate and receive the necessary conditions to overcome dormancy and germinate, and for seedlings to survive and grow. Restoration efforts should include the recreation of some of this spatial heterogeneity to reproduce functional aspects of the system.

Soil seed banks vary both spatially and temporally across a landscape and this is critical information to assist the management and restoration of arid ecosystems. Mine-site restoration in particular, relies on the ability of seeds to germinate and seedlings to establish from re-spread topsoil (Doudle 2010). In Chapter 4 of this thesis, I identified a range of common species that are likely to emerge from respread topsoil at Jacinth-Ambrosia. My results also show that patch type influences seed accumulation and seedling emergence, further demonstrating the role of trees and shrubs in creating heterogeneity in plant diversity. Understanding spatial and temporal heterogeneity in the soil seed bank and the requirements of individual species to overcome dormancy, germinate and emerge, may help to maximise species return.

Biological soil crusts are often underexploited as a resource for restoration projects, yet they are critical for re-establishing a functioning ecosystem in the long-term (Bowker 2007; Doudle 2010). Crusts have the capacity to facilitate plant succession to later seres (Bowker 2007) and cyanobacteria provide the cohesive quality of BSCs that enables soil surfaces to withstand erosion (Belnap,Budel et al. 2001). My research in Chapter 5 shows that in the short-term, early successional stage crust has the capacity to accelerate seed germination in some species. In the long-term, late successional stage BSCs influence the distribution of the soil seed bank in open inter-shrub areas and can physically inhibit seedling emergence, ensuring fewer, yet stronger plants, compete with more success for the scarce resources available to them. Since the strengths of the effects seem to be species specific and the distribution of BSC is patchy, its presence contributes to the spatial heterogeneity of annual plant communities in arid lands, which is a factor known to increase species diversity. This has potentially important implications for the success of restoration projects in the long-term.

My research showed that small-scale and well-timed disturbance of BSCs may increase emergence and potentially the establishment of vascular plants. However, the frequency, intensity and timing is critical, as increased frequency of disturbance may favour exotic annual plant invasion (Belnap,Prasse et al. 2001). This is certainly an area that requires further research, as controlling introduced weed species is a major issue in restoration projects.

Understanding seed biology is vital to ensure the establishment of seedlings in restoration projects (Commander,Merritt et al. 2009). In particular, knowledge of dormancy and germination requirements is essential to maximise the return of recalcitrant species to restored areas. Grasses play an important ecological role in arid lands by contributing to the annual productivity of these ecosystems, and their re-establishment after disturbance is essential given that they often stabilise soil surfaces and assist to minimise erosion. My preliminary investigations of physiological dormancy (PD) in three species of *Enneapogon* (Chapter 6) suggest that *E. caerulescens* var. *caerulescens* may be a recalcitrant species that may require targeted measures to return it to disturbed areas. The use of GA_3 could be investigated further as a possible seed priming agent for *E. avenaceus and E. cylindricus*, should these species prove difficult to re-establish in disturbed areas. In Chapter 7, I provided information to optimise the preparation and application of GA_3, which has implications for seed priming, to assist the return of recalcitrant species and to maximise species return in respread topsoil.

Increased soil salinity can affect seed germination, seedling establishment and the long-term survival of plants. Therefore, improving our understanding of individual species responses to increased salinity levels and water stress, particularly at the seed germination stage, is critical for restoration success. In Chapter 8, I found that salinity can affect the temperature range over which seeds are able to germinate, which has implications for restoration

programs, as any reduction in temperature range for seed germination will compound existing problems of low erratic rainfall and extreme temperatures.

My research identified some species that tolerate high levels of salinity and water stress, in particular *Eriochiton sclerolaenoides* and *Maireana trichoptera*. These shrubs have relatively shallow root systems and will likely be able to establish and reach reproductive maturity in areas of high salinity. *Enneapogon cylindricus,* a good soil stabiliser species (Kutsche and Lay 2003), has also demonstrated the capacity to germinate at moderate levels of salinity and may therefore be successful in restoration programs. In contrast, *Eucalyptus oleosa ssp. ampliata* has no tolerance threshold for salinity and water stress and this species will require targeted measures to ensure its successful inclusion in the restoration of this ecosystem. *Lepidium phlebopetalum* and *Rhodanthe floribunda* seeds are sensitive to salinity and water stress, however, the ecotypic response of these species may be worth investigating, by comparing germination responses of seeds collected from areas higher in salinity (i.e. closer to nearby salt lakes) with less saline areas.

9.3 Further Research

In summary, while this project produced several very important insights into the functioning of the system, it has also raised several questions. The main recommendations for further research are listed below:

Soil Seed Bank Research

1. Investigate winter and summer partitioning of plant species by testing a range of water availability treatments on soil collected from different patch types (*A. papyrocarpa*, *A. vesicaria* ssp. *variabilis*, *M. sedifolia* and open areas) and collected at different time periods (summer, autumn, winter and spring).
2. Examine differences in seed accumulation beneath the canopy of *A. papyrocarpa* i.e. between the canopy edge and the trunk.
3. Examine the role of other large shrubs and tree species that may contribute to spatial patterns in the soil seed bank, in particular *Alectryon oleifolius* (Bullock bush).
4. Study the effects of stock piling top soil on the seed viability of selected species.

Biological Soil Crust and Plant Interactions

1. Explore the effects of different successional stage BSCs on seed accumulation and seedling emergence.
2. Screen more plant species for BSC leachate effects on seed germination under a range of different temperature and moisture conditions (i.e. simulating winter, autumn/spring and summer conditions).
3. Examine salinity and water stress effects on crust-plant interactions including seed germination and seedling emergence.
4. Investigate the effects of shade on BSC-plant interactions.
5. Study interactions between BSC and common weed species at the study site (i.e. **Carrichtera annua* and **Brassica tournefortii*).

Seed Germination – Enneapogon spp.

1. Conduct more comprehensive germination experiments to fully investigate after-ripening, dormancy and germination requirements of all three *Enneapogon* spp.
2. Investigate the use of GA_3 as a possible seed priming agent for *E. avenaceus* and *E. cylindricus*.

Optimising Pre-treatment to Overcome Physiological Dormancy

1. Incorporate a range of dormancy-testing chemicals and additional incubation conditions to continue investigations into optimising pre-treatments for overcoming PD.

Seed Germination under Salinity and Water Stress

1. Screen a larger subset of species to see if any trends exist between germination response to salinity and water stress and specific seed traits, such as seed size or embryo type.
2. Investigate salinity effects on soil microflora, as the seeds of some species are likely to have positive relationships with soil fungi and increased salinity may impact on this.
3. Include different salt ions and investigate their impacts on seed longevity, germination, seedling establishment and seedling survival.
4. Investigate ecotypic variation in germination responses in key plant species, including *L. phlebopetalum* and *R. floribunda*.

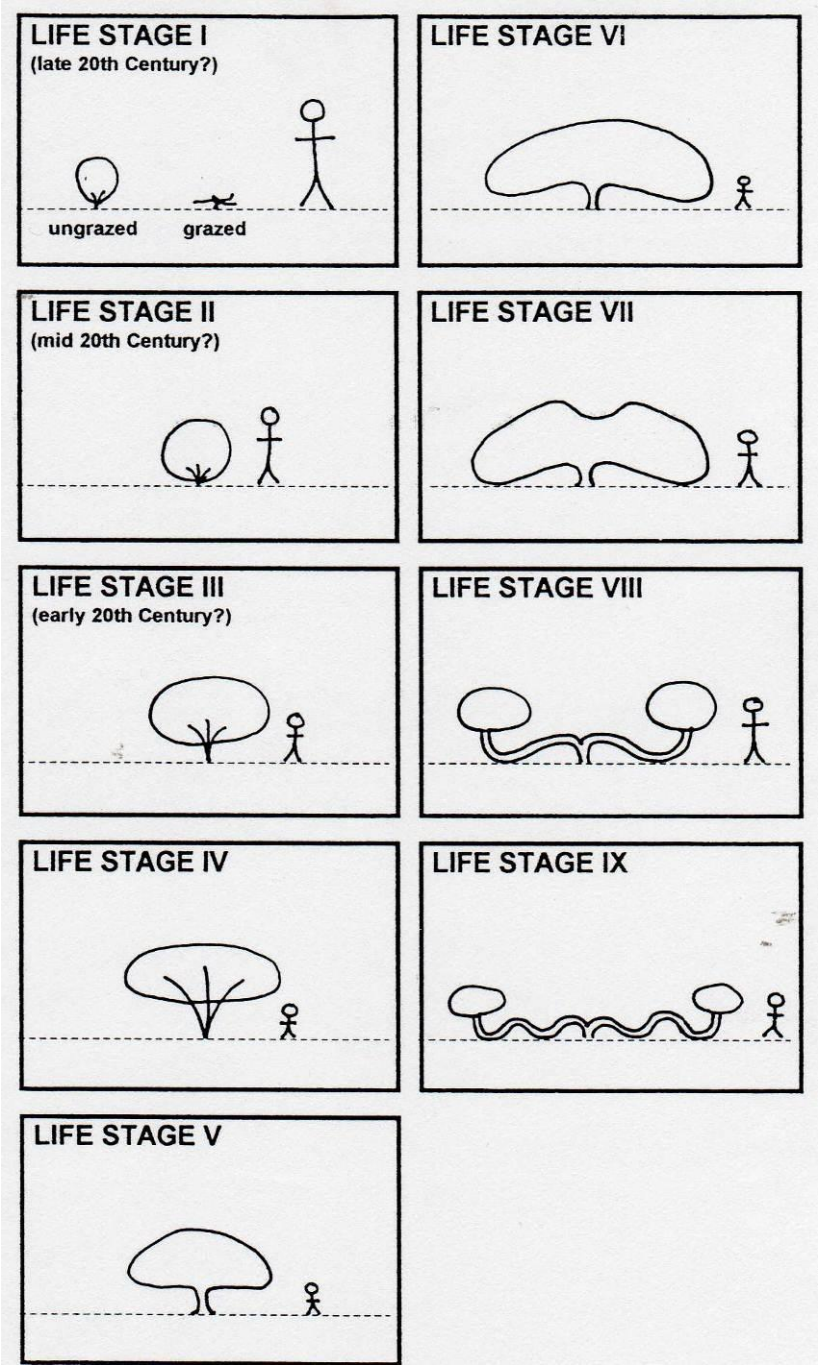
9.4 Conclusions

This research project was a rare opportunity to investigate key natural processes within a remote and intact arid ecosystem. The results have added to our understanding of ecological processes within *A. papyrocarpa* open woodland. Overall, this thesis highlights the importance of trees and shrubs in creating spatial patterns that contribute to plant diversity. It also demonstrates the role of BSCs in influencing patterns of seed accumulation and seedling emergence. Important information was also gained from germination experiments, including optimising pre-treatment methods for overcoming physiological dormancy applicable to a range of plant species. The germination response of a selection of plant species to salinity and water stress conditions also provides important information that is pertinent to restoration at the study site. Several aspects of my research also have relevance for informing the management and restoration of other arid ecosystems.

Many of the study components need further research particularly within the context of increasing human activities in arid ecosystems. These include further investigations into the ways in which seeds disperse and accumulate in a landscape, seed biology, seedling ecology and BSC-plant interactions in rehabilitated areas.

Appendix 1
Life Stage System for
Acacia papyrocarpa

Source: (Ireland 1997)



Appendix 2

Plant Species Recorded at the Study Site

Genus/Species	**Common Name**	**Family**
Acacia oswaldii	Umbrella Wattle	Leguminosae
Acacia papyrocarpa	Western Myall	Leguminosae
Alectryon oleifolius	Bullock Bush	Sapindaceae
Amyema quandang	Grey Mistletoe	Loranthaceae
Arabidella glaucescens	Cress	Cruciferae
Atriplex vesicaria ssp. *variabilis*	Bladder Saltbush	Chenopodiaceae
Austrodanthonia caespitosa	Wallaby Grass	Gramineae
Austrostipa eremophila	Rusty Spear-grass	Gramineae
Austrostipa nitida	Balcarra Spear-grass	Gramineae
Austrostipa platychaeta	Flat-awn Spear-grass	Gramineae
Brachyscome ciliaris var. *ciliaris*	Variable Daisy	Compositae
Calandrinia eremaea	Dryland Purselane	Portulaceae
Calotis hispidula	Hairy Burr-daisy	Compositae
Casuarina pauper	Black Oak	Casuarinaceae
Cephalipterum drummondii	Pompom Head	Compositae
Chamaesyce drummondii	Caustic Weed	Euphorbiaceae
Chenopodium curvispicatum	Cottony Goosefoot	Chenopodiaceae
Crassula colligata ssp. *lamprosperma*	Australian Stonecrop	Crassulaceae
Crassula colorata var. *colorata*	Dense Crassula	Crassulaceae
Cratystylis conocephala	Bluebush Daisy	Compositae
Dodonaea viscosa ssp. *angustissima*	Narrow-leaf Hop-bush	Sapindaceae
Enchylaena tomentosa var. *tomentosa*	Ruby Saltbush	Chenopodiaceae
Enneapogon avenaceus	Common Bottle-washers	Gramineae
Enneapogon caerulescens var. *caerulescens*	Blue Bottle-washers	Gramineae
Enneapogon cylindricus	Jointed Bottle-washers	Gramineae
Eremophila alternifolia	Narrow-leaf Emubush	Myoporaceae
Eremophila latrobei ssp. *glabra*	Crimson Emubush	Myoporaceae
Eremophila scoparia	Broom Emubush	Myoporaceae
Eriochiton sclerolaenoides	Wooly-fruit Bluebush	Chenopodiaceae
Erodium carolinianum	Clammy Heron's-bill	Geraniaceae
Erodium cygnorum	Blue Heron's-bill	Geraniaceae
Eucalyptus oleosa ssp. *ampliata*	Red Mallee	Myrtaceae
Euphorbia tannensis ssp. *eremophila*	Desert Spurge	Euphorbiaceae
Frankenia serpyllifolia	Thyme Sea-heath	Frankeniaceae
Goodenia pinnatifida	Cut-leaf Goodenia	Goodeniaceae
Lepidium phlebopetalum	Veined Peppercress	Cruciferae
Lotus cruentus	Red-flower Lotus	Leguminosae
Lycium australe	Australian Boxthorn	Solanaceae
Maireana erioclada	Rosy Bluebush	Chenopodiaceae
Maireana georgei	Satiny Bluebush	Chenopodiaceae
Maireana integra	Entire-wing Bluebush	Chenopodiaceae

Genus/Species	Common Name	Family
Maireana radiata	Radiate Bluebush	Chenopodiaceae
Maireana sedifolia	Pearl Bluebush	Chenopodiaceae
Maireana trichoptera	Hairy-fruit Bluebush	Chenopodiaceae
Maireana villosa	Silky Bluebush	Chenopodiaceae
Minuria cunninghamii	Bush Minuria	Compositae
Myoporum platycarpum ssp. *platycarpum*	False Sandalwood	Myoporaceae
Pelargonium australe	Austral Stork's-bill	Geraniaceae
Phlegmatospermum cochlearinum	Oval Podded Cress	Brassicaceae
Plantago drummondii	Dark Plantain	Plantaginaceae
Ptilotus obovatus var. *obovatus*	Silver Mulla Mulla	Amaranthaceae
Pycnosorus pleiocephala	Soft Billybuttons	Compositae
Rhagodia parabolica	Mealy Saltbush	Chenopodiaceae
Rhagodia spinescens	Spiny Saltbush	Chenopodiaceae
Rhagodia ulicina	Intricate Saltbush	Chenopodiaceae
Rhodanthe floribunda	White Everlasting	Compositae
Rhodanthe haigii	Haig's Everlasting	Compositae
Rhodanthe stuartiana	Stuart's Everlasting	Compositae
Salsola australis	Buckbush	Chenopodiaceae
Santalum acuminatum	Quandong	Santalaceae
Santalum spicatum	Sandalwood	Santalaceae
Scaevola spinescens	Spiny Fan flower	Goodeniaceae
**Schismus barbartus*	Arabian Grass	Gramineae
Sclerolaena diacantha	Grey Bindyi	Chenopodiaceae
Sclerolaena obliquicuspis	Oblique-spined Bindyi	Chenopodiaceae
Sclerolaena patenticuspis	Spear-fruit Bindyi	Chenopodiaceae
Sclerolaena uniflora	Small-spine Bindyi	Chenopodiaceae
Senecio glossanthus	Annual Groundsell	Compositae
Senna artemisioides ssp. *coriacea*	Broad-leaf Desert Senna	Leguminosae
Senna artemisioides ssp. *petiolaris*	Flat-stalk Senna	Leguminosae
Senna cardiosperma ssp. *gawlerensis*	Gawler Ranges Senna	Leguminosae
Sida petrophila	Rock Sida	Malvaceae
Sida spodochroma	Sida	Malvaceae
**Sonchus oleraceus*	Common Sow-thistle	Compositae
Stenopetalum lineare	Narrow Thread-petal	Cruciferae
Tetragonia eremaea	Native Spinach	Aizoaceae
Typha domingensis	Narrow-leaf Bulrush	Typhaceae
Vittadinia eremaea	Desert New Holland Daisy	Compositae
Zygophyllum apiculatum	Pointed Twinleaf	Zygophyllaceae
Zygophyllum aurantiacum ssp. *aurantiacum*	Shrubby Twinleaf	Zygophyllaceae
Zygophyllum eremaeum	Pale-flower Twinleaf	Zygophyllaceae
Zygophyllum ovatum	Dwarf Twinleaf	Zygophyllaceae

Appendix 3
Species Identified in the Biological Soil Crust in Areas Adjacent to the Study Site

Source: (Doudle 2010)

Cyanobacteria (11)	**Algae (2)**	**Lichens (17)**	**Mosses (3)**
Chroococcus sp.; *Gloeocapsopsis* sp.; *Microcoleus vaginatus* Vauch; *Microcoleus* cf. *lacustris*; *Microcoleus* cf. *paludosus*; *Nostoc commune* Vauch; *Nostoc* cf. *flagelliforme*; *Porphyrosiphon* cf. *fuscus*; *Scytonema* sp.; *Stigonema* sp.; *Schizothrix* cf. *antarctica*	2 unidentified	*Buellia* sp.; *Chondropsis semiviridis* F. Muell. ex Nyl Nyl.; *Collema* sp.; *Diplochistes* spp. (2); *Endocarpon* spp. (2); *Eramastrella crystallifera* Taylor G. Schneider; *Fulgensia* sp.; *Heppia* sp.; *Lecidea* sp.; *Peccania* sp.; *Peltula patellata* Büdel; *Placidium* sp.; *Psora crenata* Taylor Reinke; *Psora decipiens* Hedwig Hoffm.; cf. *Xanthoparmelia* sp.	*Goniometrium enerve* Hook. Et Wils.; 2x Unidentified

Appendix 4
A Comparison of Methods to Assess the Soil Seed Bank

Reference	Location	Enumeration method	Author(s) comments
(Malone 1967)	Agricultural land, USA	Seed extraction by floatation with magnesium sulphate	This method is improved (100% efficiency) with the dispersion of soil in an aqueous solution of sodium hexa-metaphosphate and sodium bicarbonate.
(Thorsen and Crabtree 1977)	Nine weed species added to soil, USA	Seed extraction after washing	Washer constructed from readily available materials and was found to function efficiently.
(Fay and Olson 1978)	Agricultural land, USA	Washing soil in nylon bags followed by manual counting	Rapid: 'total manipulation time was approximately 10 minutes'. Nb. not appropriate for very small seeds.
(Standifer 1980)	Agricultural land, USA	Seed extraction after sieving and a laboratory seed separator; seedling emergence used to test viability	Seed extraction technique needs improvement, is difficult and tedious.
(Poiani and Johnson 1988)	Prairie wetlands, USA	Seedling emergence and seed extraction after sieving	Both methods produced similar estimates of seed bank composition. Seedling emergence was more reliable and efficient.
(Gross and Renner 1989)	Agricultural land, USA	Elutriation (washing) followed by manual counting	Washing was reliable and did not affect seed viability of three weed species. Separating, classifying and counting seeds was time consuming.
(Gross 1990)	Agricultural land, USA	Seedling emergence, seedling emergence following cold stratification, elutriation (washing) and manual counting, and floatation on salt density gradient	Floatation abandoned due to inaccuracy and inefficiency. More species detected with cold-stratification. Seed-density estimates higher in washed samples, although included non-viable seeds. Germination methods provide more complete listing of species.
(Brown 1992)	Forest seed bank, Canada	Seed extraction following floatation method (Malone 1967) and seedling emergence	Both methods produced similar estimates of seed bank composition. Careful consideration needs to be given to the objectives of the study before choosing a method.
(Buhler and Maxwell 1993)	Agricultural land, USA	Seed extraction by floating using K_2CO_3-centrifugation and image-analysis	High recovery of seeds; K_2CO_3 affected seed viability; image capture compared favourably with manual seed counting and has potential for improvement.
(Cardina and Sparrow 1996)	Agricultural land, USA	Seedling emergence and seed extraction following sieving	No method gave consistently highest correlation between core and field emergence densities.
ter Heerdt, Verweij *et al.* 1996)	Marsh land, the Netherlands	Seedling emergence after removing the soil by sieving (seed concentration)	Sieving increases the number of seedlings that emerge when compared to unconcentrated samples. Germination rates were 81-100% of viable seeds. Greatly reduces glasshouse space required.
(Luschei,Buhler et al. 1998)	Agricultural land, USA	Seed extraction by floating using K_2CO_3-centrifugation	This method can reduce seed viability.

Reference	Location	Enumeration method	Author(s) comments
(Traba,Levassor et al. 1998)	Mediterranean *dehesa*, Spain	Seedling emergence after removing soil by sieving (i.e. seed concentration)	Concentrating samples can lead to under-estimations of soil bank composition due to losses of small seeds.
(Ferrandis,Herranz et al. 1999)	Cistaceae family, Mediterranean ecosystem, Spain	Seedling emergence and seed extraction	Combination of both techniques recommended when a physically-dormant hard-seed component is expected in the soil seed bank.
(ter Heerdt,Schutter et al. 1999)	Dune slacks, the Netherlands	Seedling emergence method and the effects of water supply	There is a danger of using a 'standard' watering regime for seedling emergence method. Species requirements vary.
(Ishikawa-Goto and Tsuyuzaki 2004)	Mount Usu, Japan	Seed extraction by floating using K_2CO_3-centrifugation and seedling emergence	Floatation detected more species and seeds, however many did not germinate. Use of a single method should be avoided in estimating seed bank size.

Appendix 5
Plant Species Recorded in the Soil Seed Bank Study

Species Identity Confirmed		Family	Description	Total Frequency of Occurrence		
				No. Replicates	No. Specimens	Total Percentage
1	*Atriplex vesicaria* ssp. *variabilis*	Chenopodiaceae	Perennial shrub	17	18	0.20
2	*Austrostipa nitida*	Gramineae	Annual grass	198	302	3.27
3	*Brachyscome ciliaris* var. *ciliaris*	Compositae	Annual composite	29	43	0.47
4	*Calandrinia eremaea*	Portulacaceae	Annual forb	85	194	2.10
5	*Calotis hispidula*	Compositae	Annual composite	35	72	0.78
6	*Casuarina pauper*	Casuarinaceae	Perennial tree	3	3	0.03
7	*Cephalipterum drummondii*	Compositae	Annual composite	28	53	0.57
8	*Chamaesyce drummondii*	Euphorbiaceae	Annual forb	78	115	1.25
9	*Chenopodium curvispicatum*	Chenopodiaceae	Perennial shrub	14	16	0.17
10	*Crassula colligata* ssp. *lamprosperma*	Crassulaceae	Annual herb	659	7869	85.31
11	*Crassula colorata* var. *colorata*	Crassulaceae	Annual herb			
12	*Enchylaena tomentosa* var. *tomentosa*	Chenopodiaceae	Perennial shrub	1	1	0.01
13	*Eriochiton sclerolaenoides*	Chenopodiaceae	Short-lived perennial shrub	11	20	0.22
14	*Eucalyptus* sp.	Myrtaceae	Perennial tree	2	2	0.02
15	*Euphorbia tannensis* ssp. *eremophila*	Euphorbiaceae	Short-lived perennial shrub	4	4	0.04
16	*Frankenia serpyllifolia*	Frankeniaceae	Perennial shrub	7	11	0.12
17	*Lepidium phlebopetalum*	Cruciferae	Annual forb	8	11	0.12
18	*Lotus cruentus*	Leguminosae	Annual forb	3	3	0.03
19	*Maireana radiata*	Chenopodiaceae	Short-lived perennial shrub	2	4	0.04
20	*Pelargonium australe*	Geraniaceae	Annual forb	3	3	0.03
21	*Rhodanthe floribunda*	Compositae	Annual composite	20	48	0.52
22	*Salsola australis*	Chenopodiaceae	Short-lived perennial shrub	25	29	0.31
23	**Schismus barbartus*	Gramineae	Annual exotic grass	9	11	0.12
24	*Sclerolaena obliquicuspis*	Chenopodiaceae	Short-lived perennial shrub	30	51	0.55
25	*Senecio glossanthus*	Compositae	Annual composite	1	1	0.01
26	*Sida spodochroma*	Malvaceae	Perennial shrub	1	1	0.01
27	**Sonchus oleraceus*	Compositae	Annual exotic forb	1	1	0.01
28	*Stenopetalum lineare*	Cruciferae	Annual forb	26	31	0.34
29	*Tetragonia eremaea*	Aizoaceae	Annual forb	8	13	0.14
30	*Typha domingensis*	Typhaceae	Perennial rush	8	8	0.09
31	*Vittadinia eremaea*	Compositae	Annual composite	6	18	0.2
32	*Zygophyllum ovatum*	Zygophyllaceae	Short-lived perennial shrub	185	268	2.91

Appendix 6
Soil Seed Bank Results:
Indicator Species Analysis (Area)

Species or Taxa*	Label	Maximum Group	Collection Date	IV	Mean	S. Dev	*P***
Atriplex vesicaria ssp. *variabilis*	Av	*A. papyrocarpa*	Winter2	33.3	31.2	3.90	*0.5236*
		A. vesicaria	Autumn1	30.8	30.8	0.31	*0.0389*
		A. vesicaria	Spring1	40.0	35.0	2.89	*0.0796*
		A. vesicaria	Autumn2	28.6	30.3	3.10	*1.0000*
		A. vesicaria	Spring2	38.9	32.1	3.94	*0.0777*
		A. vesicaria	Summer2	35.7	30.3	3.05	*0.2357*
		M. sedifolia	Summer1	30.8	30.8	0.31	*1.0000*
Brachyscome ciliaris var. *ciliaris*	Bc	*A. vesicaria*	Autumn1	47.6	42.7	4.08	*0.2380*
		A. vesicaria	Winter1	42.9	34.3	4.76	*0.1009*
		A. vesicaria	Spring1	35.7	35.7	0.36	*0.3176*
		A. vesicaria	Autumn2	37.0	35.7	5.32	*0.4307*
		A. vesicaria	Winter2	42.1	33.7	4.36	*0.0311*
		A. vesicaria	Summer2	30.8	30.8	0.31	*0.5450*
Calandrinia eremaea	Ce	*A. papyrocarpa*	Autumn1	40.0	36.1	5.34	*0.2772*
		A. papyrocarpa	Winter1	42.1	32.2	3.92	*0.0409*
		A. papyrocarpa	Spring1	43.9	40.0	6.71	*0.3090*
		A. papyrocarpa	Summer1	64.3	43.5	8.75	*0.0215*
		A. papyrocarpa	Spring2	55.6	41.5	8.07	*0.0834*
		A. papyrocarpa	**Summer2**	**59.3**	**37.6**	**6.07**	***0.0029***
		M. sedifolia	Autumn2	34.4	38.3	6.44	*0.6463*
		M. sedifolia	Winter2	30.0	32.3	3.79	*0.5918*
Calotis hispidula[1]	Ch[1]	*A. vesicaria*	Winter1	40.0	36.5	5.42	*0.3621*
		A. vesicaria	Spring1	36.8	33.7	4.33	*0.3891*
		A. vesicaria	Winter2	47.6	36.7	5.84	*0.0834*
		M. sedifolia	Autumn1	33.3	31.2	3.86	*0.3541*
		M. sedifolia	Autumn2	51.4	45.5	8.65	*0.3001*
		M. sedifolia	Spring2	40.0	35.3	5.23	*0.1029*
		M. sedifolia	Summer2	37.5	43.7	6.69	*0.9584*
Calotis hispidula and *Vittadinia eremaea* seedlings combined[1]	Ch/Ve[1]	*A. vesicaria*	Winter1	30.8	30.8	0.3	*0.1870*
		A. vesicaria	Summer1	38.9	34.7	5.0	*0.3081*
		A. vesicaria	Autumn2	37.9	35.5	5.2	*0.3672*
		A. vesicaria	Spring2	44.4	33.8	4.3	*0.0426*
		M. sedifolia	Autumn1	50.0	36.9	6.0	*0.0595*
		M. sedifolia	Spring1	35.7	35.7	0.4	*1.0000*
		M. sedifolia	Winter2	31.8	35.8	5.2	*0.8376*
		M. sedifolia	Summer2	53.6	43.7	6.3	*0.0495*
Cephalipterum drummondii[2]	Cd[2]	***A. vesicaria***	**Autumn2**	**60.9**	**38.8**	**6.59**	***0.0028***
		A. vesicaria	Summer2	35.9	37.9	6.39	*0.5579*
		M. sedifolia	Autumn1	40.0	35.0	4.85	*0.1111*
		Open	Winter1	35.7	30.3	3.09	*0.2461*
Cephalipterum drummondii and *Rhodanthe floribunda* seedlings combined[2]	Cd/Rf[2]	*A. vesicaria*	Winter1	35.7	30.3	3.1	*0.0283*
		A. vesicaria	Spring1	41.2	38.1	3.5	*0.2206*
		A. vesicaria	Summer1	34.9	35.4	5.1	*0.5108*
		A. vesicaria	Autumn2	45.9	37.5	6.2	*0.1128*
		A. vesicaria	Winter2	46.2	33.4	4.3	*0.0162*
		A. vesicaria	Spring2	31.8	33.4	4.3	*0.8062*
		M. sedifolia	Summer2	38.9	32.2	3.9	*0.1363*
		Open	Autumn1	31.6	33.8	4.3	*0.5428*

Species or Taxa*	Label	Maximum Group	Collection Date	IV	Mean	S. Dev	P**
Chenopodium curvispicatum	Cc	*A. papyrocarpa*	Winter1	*30.8*	30.8	0.31	*1.0000*
		A. papyrocarpa	Summer1	*33.3*	31.2	3.91	*0.4408*
		A. papyrocarpa	Autumn2	*33.3*	31.1	3.85	*0.3136*
		A. papyrocarpa	Spring2	*40.0*	35.0	2.88	*0.1849*
		A. vesicaria	Autumn1	*28.6*	30.3	3.05	*0.9996*
		A. vesicaria	Spring1	*30.8*	30.8	0.31	*1.0000*
		M. sedifolia	Winter2	*33.3*	35.0	2.88	*0.9993*
Chamaesyce drummondii	Chd	*A. vesicaria*	Winter1	47.6	35.1	5.04	*0.0359*
		A. vesicaria	Spring1	42.9	37.2	5.90	*0.2297*
		A. vesicaria	Summer1	27.8	32.1	3.96	*0.9410*
		A. vesicaria	Autumn2	41.2	34.0	4.31	*0.1004*
		A. vesicaria	Summer2	48.5	34.9	4.89	*0.0195*
		M. sedifolia	Autumn1	36.0	33.6	4.35	*0.3332*
		M. sedifolia	Winter2	38.7	36.5	5.55	*0.3598*
		M. sedifolia	Spring2	50.0	35.8	5.14	*0.0156*
Chenopodiaceae seedlings that could not be identified to genus i.e. *Maireana* sp.*,* Sclerolaena *sp.* and *Enchylaena tomentosa*	Cheno	***A. vesicaria***	**Winter1**	**64.1**	**38.8**	**6.01**	***0.0005***
		A. vesicaria	**Autumn2**	**51.4**	**35.7**	**5.26**	***0.0099***
		A. vesicaria	**Winter2**	**50.0**	**35.2**	**4.89**	***0.0069***
		A. vesicaria	Summer2	38.1	39.1	4.48	*0.7494*
		M. sedifolia	Autumn1	37.5	34.3	3.82	*0.4057*
		M. sedifolia	Spring1	33.3	32.1	3.98	*0.4639*
		M. sedifolia	Summer1	30.0	33.6	4.27	*0.6860*
		M. sedifolia	Spring2	34.7	35.2	5.03	*0.5003*
Crassula colligata ssp. *lamprosperma* and *Crassula colorata* var. *colorata* seedlings combined	Crassula	***A. vesicaria***	**Autumn1**	**60.5**	**34.1**	**4.26**	***0.0001***
		A. vesicaria	**Winter1**	**64.4**	**35.0**	**4.86**	***0.0001***
		A. vesicaria	**Spring1**	**66.8**	**39.8**	**7.18**	***0.0007***
		A. vesicaria	**Summer1**	**51.4**	**32.1**	**3.53**	***0.0002***
		A. vesicaria	**Autumn2**	**51.2**	**32.1**	**3.49**	***0.0001***
		A. vesicaria	**Winter2**	**52.6**	**33.1**	**4.01**	***0.0004***
		A. vesicaria	Spring2	38.1	34.2	4.41	*0.1675*
		A. vesicaria	**Summer2**	**64.0**	**34.2**	**4.50**	***0.0001***
Eriochiton sclerolaenoides	Es	*A. vesicaria*	Summer1	28.6	30.3	3.09	*0.9045*
		A. vesicaria	**Autumn2**	**57.1**	**42.8**	**6.49**	***0.0079***
		A. vesicaria	Spring2	35.3	34.0	4.25	*0.1554*
		A. vesicaria	Summer2	30.8	30.8	0.31	*0.1689*
		M. sedifolia	Spring1	40.0	40.0	0.40	*1.0000*
Frankenia serpyllifolia	Fs	*A. papyrocarpa*	Winter1	*30.8*	30.8	0.31	*1.0000*
		A. papyrocarpa	Summer1	*30.8*	30.8	0.31	*1.0000*
		A. papyrocarpa	Autumn2	*28.6*	30.3	3.08	*1.0000*
		A. papyrocarpa	Winter2	*30.8*	30.8	0.31	*1.0000*
		A. vesicaria	Spring2	*47.1*	47.1	0.47	*0.5128*
		A. vesicaria	Summer2	*30.8*	30.8	0.31	*0.1851*
Grasses - only *Austrostipa nitida* positively identified	Grass	*A. papyrocarpa*	Autumn1	*35.3*	35.9	4.99	*0.4818*
		A. vesicaria	Spring1	*31.7*	34.5	4.29	*0.8125*
		A. vesicaria	Summer1	*30.5*	31.1	3.02	*0.5717*
		A. vesicaria	Autumn2	*37.9*	37.0	5.83	*0.4123*
		A. vesicaria	**Spring2**	***51.8***	**39.4**	**4.79**	***0.0048***
		A. vesicaria	Summer2	*35.5*	33.5	4.21	*0.3054*
		M. sedifolia	Winter1	*38.7*	32.3	3.68	*0.0904*
		M. sedifolia	Winter2	*40.0*	32.4	3.83	*0.0579*
Lepidium phlebopetalum	Lp	*A. papyrocarpa*	Autumn2	*38.9*	41.6	3.40	*0.4288*
		A. vesicaria	Autumn1	*30.8*	30.8	0.31	*0.6740*
		M. sedifolia	Summer1	*35.7*	30.3	3.09	*0.2466*
		M. sedifolia	Summer2	*28.6*	30.3	3.07	*0.7321*
Rhodanthe floribunda[2]	Rf[2]	*A. papyrocarpa*	Winter1	*28.6*	30.3	3.08	*0.4002*
		A. vesicaria	Autumn1	*35.0*	37.4	6.27	*0.5518*
		A. vesicaria	Spring1	*31.2*	32.0	3.59	*0.8909*
		M. sedifolia	Autumn2	*59.0*	48.3	9.88	*0.0695*
		M. sedifolia	Summer2	*36.8*	36.8	4.48	*0.6133*

Species or Taxa*	Label	*Maximum Group*	Collection Date	*IV*	Mean	S. Dev	*P***
Salsola australis	Sa	*A. vesicaria*	Autumn1	*35.3*	34.0	4.25	*0.6163*
		A. vesicaria	Winter1	*28.6*	30.3	3.10	*0.9996*
		A. vesicaria	Spring1	*30.8*	30.8	0.31	*0.1700*
		A. vesicaria	Summer1	*47.4*	36.7	4.40	*0.0413*
		A. vesicaria	Autumn2	*42.1*	33.8	4.34	*0.0979*
		M. sedifolia	Winter2	*26.7*	31.2	3.88	*0.9999*
		M. sedifolia	Summer2	*43.7*	31.9	3.55	*0.0143*
Sclerolaena obliquicuspis	So	*A. vesicaria*	Autumn1	*40.9*	38.5	4.57	*0.3468*
		A. vesicaria	Winter1	*30.8*	30.8	0.31	*1.0000*
		A. vesicaria	Spring1	*42.9*	36.2	5.26	*0.1948*
		A. vesicaria	Autumn2	*33.3*	35.0	2.89	*0.3744*
		A. vesicaria	Summer2	*50.0*	39.5	6.56	*0.0954*
		M. sedifolia	Summer1	*35.0*	36.6	5.39	*0.6619*
Stenopetalum lineare	Sl	*A. papyrocarpa*	Autumn1	*26.7*	31.2	3.88	*0.9735*
		A. papyrocarpa	Summer1	*41.2*	38.1	3.55	*0.0594*
		A. papyrocarpa	Spring2	*34.8*	34.0	4.52	*0.5691*
		A. papyrocarpa	Summer2	*33.3*	32.4	3.81	*0.5541*
		A. vesicaria	Winter1	*30.8*	30.8	0.31	*0.0195*
		M. sedifolia	Autumn2	*35.7*	35.7	0.36	*0.2877*
Tetragonia eremaea	Te	*A. vesicaria*	Autumn1	*30.8*	30.8	0.31	*0.5444*
		M. sedifolia	Summer1	*30.8*	30.8	0.31	*1.0000*
		M. sedifolia	Autumn2	*47.4*	48.6	2.31	*0.9548*
		M. sedifolia	Winter2	*33.3*	31.2	3.85	*0.6245*
		M. sedifolia	Summer2	*30.8*	30.8	0.31	*0.6820*
Vittadinia eremaea[1]	Ve[1]	*A. vesicaria*	Winter1	*30.8*	30.8	0.31	*0.1584*
		A. vesicaria	Spring1	*62.5*	53.1	5.40	*0.1743*
		A. vesicaria	Autumn2	*35.3*	38.2	3.58	*0.6967*
Zygophyllum sp. – only *Z. ovatum* was positively identified	Zygo	*A. vesicaria*	Autumn1	*38.9*	33.2	4.07	*0.1073*
		A. vesicaria	Winter1	*45.2*	34.8	4.81	*0.0350*
		A. vesicaria	Summer1	*27.3*	33.7	4.31	*0.9465*
		A. vesicaria	Autumn2	*37.5*	33.1	4.01	*0.1886*
		A. vesicaria	Spring2	*47.1*	35.7	5.22	*0.0309*
		M. sedifolia	Spring1	*41.0*	34.5	4.64	*0.1052*
		M. sedifolia	Winter2	*27.8*	32.8	3.90	*0.9751*
		M. sedifolia	Summer2	*38.9*	33.7	4.34	*0.1468*

*Taxa with < 10 specimens have not been included in this analysis. Refer to Appendix 5 for a complete list of species or taxa found.

**Bold type indicates statistical significance (*P*<0.01).

[1] *Calotis hispidula* and *Vittadinia eremaea* seedlings were grouped when they could not be positively distinguished.

[2] *Cephalipterum drummondii* and *Rhodanthe floribunda* seedlings were group when they could not be positively distinguished.

Appendix 7

Soil Seed Bank Results: Indicator Species Analysis (Time)

Taxa	*A. Papyrocarpa*					*A. vesicaria*					*M. sedifolia*					Open				
	Maxgrp	**(IV)**	**Mean**	**S.Dev**	**p***	**Maxgrp**	**(IV)**	**Mean**	**S.Dev**	**p***	**Maxgrp**	**(IV)**	**Mean**	**S.Dev**	**p***	**Maxgrp**	**(IV)**	**Mean**	**S.Dev**	**p***
Av	Winter2	17.90	16.60	2.11	0.5800	Spring2	19.40	18.30	2.34	0.2970	Summer1	16.00	16.00	0.16	1.0000	Autumn2	16.00	16.00	0.16	0.6579
Bc	Autumn1	12.50	12.50	0.13	1.0000	Autumn2	19.20	21.40	3.24	0.8246	Autumn2	24.30	20.20	3.04	0.1653	Autumn2	19.20	15.90	1.28	0.1242
Ce	Summer1	27.50	23.00	4.01	0.1399	Spring1	18.30	20.30	3.03	0.7513	**Autumn2**	36.80	23.20	4.13	**0.0039**	Autumn2	40.50	31.20	3.58	0.0190
Ch[1]	Winter1	20.00	18.40	2.17	0.2546	Autumn2	19.30	20.80	3.23	0.6706	Autumn2	33.30	28.10	5.24	0.1866	Autumn1	12.50	12.50	0.13	1.0000
Ch/Ve[1]	Autumn1	12.50	12.50	0.13	1.0000	Autumn2	22.40	19.90	2.87	0.1780	Summer2	26.30	23.40	3.71	0.1542	Autumn2	21.90	21.00	3.06	0.3422
Cd[2]	Autumn1	12.50	12.50	0.13	1.0000	Autumn2	29.20	22.10	3.76	0.0462	Autumn1	27.00	22.30	3.53	0.0638	Summer2	32.50	23.50	3.47	0.0100
Cd/Rf[2]	Summer1	17.20	18.50	1.94	0.9588	Autumn2	23.60	20.30	3.00	0.1485	Summer1	25.50	20.70	3.12	0.0787	Summer1	22.90	21.70	3.05	0.3359
Chd	Winter2	14.80	16.10	1.86	1.0000	Summer2	20.30	19.00	2.47	0.2362	Spring2	26.00	20.40	3.02	0.0568	Winter2	15.60	17.40	2.08	0.9543
Cc	Spring2	18.20	18.40	2.43	0.7673	Autumn1	15.40	15.90	1.28	1.0000	Winter2	17.20	18.50	1.98	0.9993	Autumn1	12.50	12.50	0.13	1.0000
Cheno	**Spring2**	31.90	20.10	3.03	**0.0011**	Winter1	25.30	20.40	3.06	0.0633	Spring2	26.60	20.00	2.90	0.0257	Winter2	20.00	17.20	1.95	0.1345
Crassula	**Autumn2**	29.30	18.20	2.16	**0.0002**	Autumn2	20.30	17.50	1.86	0.0812	**Autumn1**	23.60	17.60	1.91	**0.0060**	**Autumn2**	35.10	21.10	3.30	**0.0011**
Es	Spring2	19.20	19.20	0.19	1.0000	Autumn2	31.60	24.00	3.29	0.0178	Spring1	21.40	21.90	1.18	1.0000	Autumn1	12.50	12.50	0.13	1.0000
Fs	Winter1	14.30	16.60	2.13	1.0000	Spring2	25.80	26.60	1.51	1.0000	Autumn1	12.50	12.50	0.13	1.0000	Autumn1	12.50	12.50	0.13	1.0000
Grass	Autumn1	18.20	20.00	2.86	0.6888	Summer2	22.80	19.80	2.72	0.1635	**Summer2**	34.10	20.00	2.82	**0.0001**	Summer1	18.90	21.90	2.95	0.8246
Lp	Autumn2	24.10	24.60	1.15	0.8903	Autumn1	16.00	16.00	0.16	0.9400	Autumn2	17.20	16.90	2.04	0.6983	Autumn1	12.50	12.50	0.13	1.0000
Rf[2]	**Winter1**	16.00	16.00	0.16	**0.0001**	Autumn2	22.50	25.60	3.22	0.9997	Autumn2	43.40	32.20	5.18	0.0344	Spring1	15.40	15.80	1.25	0.2519
Sa	Autumn2	15.40	15.80	1.26	0.7434	Summer1	22.00	19.50	2.70	0.2732	Summer2	20.60	18.40	2.39	0.1318	Autumn1	12.50	12.50	0.13	1.0000
So	Summer2	20.00	18.40	2.16	0.1788	Summer2	29.10	23.00	3.98	0.0816	Summer1	18.40	20.50	3.07	0.8118	Autumn1	12.50	12.50	0.13	1.0000
Sl	Spring2	21.10	20.10	2.97	0.3522	Summer2	21.20	17.50	2.17	0.0903	Autumn2	16.70	18.40	2.19	0.5634	Spring2	15.40	15.90	1.27	1.0000
Te	Autumn1	12.50	12.50	0.13	1.0000	Winter2	14.80	16.10	1.86	0.8982	Autumn2	26.50	27.90	1.94	0.6544	Autumn1	12.50	12.50	0.13	1.0000
Ve[1]	Autumn1	12.50	12.50	0.13	1.0000	Spring1	37.50	32.00	3.50	0.2231	Autumn2	19.20	15.90	1.27	0.1210	Autumn1	12.50	12.50	0.13	1.0000
Zygo	Spring1	19.40	18.10	2.11	0.2657	Spring2	19.80	19.20	2.56	0.2989	Spring1	21.70	18.50	2.28	0.1048	Autumn2	19.60	19.60	2.71	0.4912

*Taxa with < 10 specimens were not included in this analysis. Refer to Appendix 5 for a complete list of species or taxa recorded in the soil seed bank study.

**Bold type indicates statistical significance (P<0.01).

[1] *Calotis hispidula* and *Vittadinia eremaea* seedlings were grouped when they could not be positively identified.

[2] *Cephalipterum drummondii* and *Rhodanthe floribunda* seedlings were grouped when they could not be positively identified.

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