Ecology of seed germination for broad-acre restoration of native vegetation on cracking clay vertosols

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Declaration

I certify that the substance of this thesis has not already been submitted for any degree and is not currently being submitted for any other degree or qualification.

I certify that any assistance received in preparing this thesis, and all sources used, have been acknowledged.

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Abstract

In Australia, substantial ecological restoration of farmland is undertaken in conjunction with community-based natural resource management agencies, with the objective of balancing agricultural land use and biodiversity conservation through revegetation with native species. Across the North-West Plains of New South Wales (NSW), both large-scale and small-scale restoration efforts are frequently required. However, fragmented agricultural landscapes, the lack of sufficient scientific information on the seed ecology of native species and the environmental conditions associated with the region's vertosol soils are major challenges for revegetation. This thesis investigated germination traits in 73 plant species that are important components of endangered vegetation communities in north-western NSW to produce information useful for plant propagation and ecological restoration.

In order to identify the optimum combination of environmental conditions to maximise the percentage and rate of germination in 14 *Eucalyptus* seedlots of ten species from north-western NSW, germination responses to seasonal temperature regimes and light were examined, and the relationship between these factors and seed size investigated. The effect of three alternating day/night temperature treatments (spring, summer, winter) and two light treatments (light/dark, dark) was investigated in growth cabinets. Germination patterns varied between species and seedlots. In general, the presence of light and winter and spring temperatures resulted in higher average germination than darkness and summer temperatures. However, some seedlots germinated consistently well under all treatments. Germination of small seeds was higher in the presence of light while larger seeds germinated better under continuous darkness. Time to germination was about threefold faster in response to summer and spring temperatures than winter temperatures.

The seeds of many acacias, which are important in ecosystem regeneration due to the ability of *Acacia* species to fix nitrogen, have a physical dormancy that must be broken prior to their use. In order to identify convenient methods to break seed dormancy in ten *Acacia* species from north-west NSW, seed response was investigated to three dormancy-breaking treatments and two incubation temperatures. Mechanical scarification (two intensities) and hot water were applied as seed pre-treatments and seeds incubated under two temperature/light-controlled treatments in germination cabinets. The results varied with species but the three dormancy-breaking treatments significantly increased germination percentage or reduced the time to germination in all but one species. Temperature had an effect on only one species. Germination was greater or more rapid after mechanical scarification than after hot-water treatment.

North-western NSW is one of Australia's biodiversity 'hotspots' due to the number of endemic plants and diversity of species, some of which lack seed ecology information for restoration and conservation purposes. Seeds of 49 species were investigated to identify limitations to germination, the pre-treatments needed to overcome such limitations, and determine the suitability of these species for direct seeding or propagation from seed. Seed viability, germination under different seasonal temperatures (winter, spring/autumn, summer) and a requirement for seed pre-treatment to promote germination were all assessed experimentally in germination cabinets. Seed viability varied widely among the 49 species; temperature determined germination success in 27 species and various seed pre-germination treatments were effective in increasing germination percentage in 22 species. The results will be useful in propagating these species in the nursery and for direct seeding in the field.

The decline in eucalypt-dominated woodlands across the wheat–sheep belt of southern and inland eastern Australia is of concern, and revegetation targets have been set to restore woody vegetation cover in over-cleared landscapes. In order to provide guidelines for direct-seeding eucalypts in large-scale revegetation of cracking clay soils in north-western NSW, seedling emergence was investigated in relation to moisture regime, sowing depth and seed size in six species of *Eucalyptus* in a glasshouse experiment. Seedling emergence was low despite high seed viability and provision of optimum temperatures and soil moisture. All six species exhibited greatest emergence when sown at 0–6-mm depth, with seed size being less important than moisture (except under dry conditions) and proximity to the surface. Species responded differently to the three watering treatments. Success in direct-seeding these species in vertosol soils in the region may be unreliable.

The findings of this research should contribute to seed management and direct seeding in large-scale revegetation projects on cracking clay soils in the agricultural districts of north-western NSW. The research variously examined the seed viability, quality, persistence and germination response of seeds in a range of species to light, temperature and sowing depth. It aimed to determine where release of germination constraints was necessary, as well as cost-effective techniques that can be applied to large quantities of seed required in broad-acre revegetation.

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Note to examiners

This thesis has been written in journal-article format. One chapter has been published in a scientific journal and three more are to be submitted in the near future.

I have attempted to minimise the duplication of material between chapters. However, some repetition remains, particularly in the methodology sections.

Publication arising from this thesis

Some content of this thesis has been published in a journal and presented in conferences:

Journal article

Ruiz Talonia Lorena, Reid Nick, Gross Caroline L., Whalley R. D. B. (2016) Germination ecology of six species of *Eucalyptus* in shrink–swell vertosols: moisture, seed depth and seed size limit seedling emergence. *Australian Journal of Botany* **65**, 22–30. – CHAPTER 6.

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Chapter 1. Introduction

Background

The link between human well-being and biodiversity[1] is complex. Agriculture has transformed land around the world in order to feed and clothe the burgeoning human population, but in so doing has induced large-scale biodiversity loss and land degradation (FAO 2015; Foley *et al.* 2005; Ramankutty *et al.* 2008). Nevertheless, agricultural landscapes contain a significant amount of biodiversity that can also support agricultural production through the provision of ecosystem services. It is therefore important to understand and plan effective strategies to retain, increase and manage biodiversity in farmland (Norris 2008). It is also necessary for science to

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[1] All technical terms are defined in the glossary

address trade-offs between biodiversity conservation and human development in agricultural landscapes, and to recognise that biodiversity is a key to sustainable agriculture, as it can increase the resilience of agriculture (Perrings *et al.* 2006). If agricultural ecosystems are degraded or damaged by injudicious management or natural disasters, ecosystem restoration – the recovery of the productivity and resilience of a system – is required (Tilman *et al.* 2002). Ecosystem restoration requires information from across several scales and from interdisciplinary perspectives with the vital support of policy makers, stakeholders, the scientific community and land-owners to align the efforts and goals of agriculture and biodiversity conservation (Banks 2004; Benayas *et al.* 2008).

In Australia, the extent and intensity of agriculture continues to increase. In 2013 agriculture accounted for approximately 55% of land use, with some level of agricultural activity being undertaken on 460 million hectares of Australia's total land area (ABS 2014). Another important factor that has an impact on revegetation in the study region is livestock grazing. In all, about 13% of Australia has been cleared for pastures and crops (ANRA 2002). However, investment in conservation and restoration of native vegetation on farmlands has been occurring since the early 1980s due to the recognition of the impacts of agriculture on native biodiversity and the importance of biodiversity in sustaining agriculture (Wells 2015). The objective of farmland rehabilitation programs has been to balance agricultural land use with biodiversity conservation and recovery by incorporating native biodiversity in farming and grazed landscapes (Close and Davidson 2003). Between 2011 and 2012, 68.9 million ha of farmed land comprised some type of vegetation management for land conservation (ABS 2014). Barson *et al.* (2012) estimated an increase of 2 million ha were set aside or restored to protect native vegetation by agricultural businesses on farms between 2007 and 2010. Revegetation is occurring in the agricultural districts of Australia for multiple purposes. These include: reducing losses of native biodiversity, provision of habitat for native species and shelter and fodder for livestock, reductions in soil salinisation and degradation, and to maintain water quality (Gibson-Roy *et al.* 2007). The Australian Government (2010) has a national framework for biodiversity conservation, *Australia's Biodiversity Conservation Strategy 2010–2030*, to counter threats to native species and ecological communities. These threats specifically include: (1) the loss, fragmentation and degradation of habitat; (2) the spread of invasive species; (3) the unsustainable use of natural resources; (4) climate change; (5) inappropriate fire regimes, and (6) changes to the aquatic environment and river flows. The aim of the strategy is to stop declines in biodiversity at multiple levels (genetic, species and ecosystem), engage Australians in biodiversity conservation and build ecosystem resilience in a changing climate with measurable results (EPBC Act 1999).

In New South Wales (NSW), Australia, considerable land rehabilitation is undertaken by natural resource management agencies such as the Local Land Services (LLS) through incentive programs that encourage community-based environmental management (Hallett *et al.* 2014; LLS 2014). The Border Rivers–Gwydir (BRG) catchment, where this study is based, comprises the jurisdictions of two state agencies, the Northern Tablelands LLS and the North West LLS, in northern NSW. The objective of these agencies is to deliver outcomes at a regional scale through actions implemented by individual landholders at the farm scale (Zerger *et al.* 2011). The Brigalow–Nandewar Biolinks Project (BNB) was funded by the Australian

Government Biodiversity Fund and is administered by the NTLLS and NWLLS, with targets to manage 25 000 ha for conservation and to re-establish 5000 ha of native vegetation through replanting and or natural regeneration (LLS 2014). The BNB targets include: (1) restoring native communities on farmland by increasing the area of suitable habitat for native biodiversity; (2) protecting and enhancing existing remnants of native communities on farmland, thereby retaining native species and populations and minimising fragmentation; (3) increasing connectivity across fragmented landscapes through the creation of vegetation corridors throughout the Northern Tablelands, and North West Slopes and Plains; (4) reconnecting farmland to the benefits supplied freely to agriculture by the environment (i.e. ecosystem services), and (5) building capacity among stakeholders for revegetation using agricultural equipment and vegetation management (BNB 2012). These are the efforts necessary to achieve revegetation of agricultural landscapes to meet regional land management objectives (Margules and Pressey 2000; Smith *et al.* 2013).

There are three main ways of returning native species to farmland through revegetation. These include natural regeneration, the planting of tubestock seedlings, and through direct seeding. Direct seeding is generally considered more convenient than the planting of tubestock due to reduced labour and material costs and the ability to sow a diverse seed mix (Dalton 1994; Gibson-Roy *et al.* 2007; Hallett *et al.* 2014; Knight *et al.* 1998). However, direct seeding for farmland restoration requires knowledge about site suitability, soil moisture conditions and other environmental factors required to create an optimal microenvironment for germination and early seedling growth (Moles and Westoby 2006). Additionally, direct seeding is constrained by a lack of information about the specific requirements of many species, including the collection, quality, viability, persistence, storage and germination requirements of most native plants species (Baskin and Baskin 2004a; Budelsky and Galatowitsch 1999; Hossain *et al.* 2014; Long *et al.* 2015).

This project addresses the germination ecology of native plant species that occur in Narrabri, Moree Plains and Gwydir Shires in north-western NSW and the species' responses to several environmental factors. Some of these species have proven difficult to germinate in the past, thus restricting their use in the restoration of native vegetation using both direct seeding or tubestock planting.

The remainder of this chapter consists of a literature review exploring the concept of ecological restoration and its relevance to modern agriculture in both grazing and cropping situations. It considers the need for broad-acre revegetation of native plant communities in heavily cleared landscapes; the means of restoring native vegetation to farmland with a special focus on direct seeding, and the information requirements about native seed ecology and germination for successful direct seeding. The chapter concludes with a statement of the aims and objectives of this thesis (section 1.6) and an explanation of the layout of the thesis (section 1.7).

Ecological restoration

Ecological restoration is the process of assisting the recovery of an ecosystem that has been degraded or destroyed and aims to improve the health, integrity and sustainability of the restored ecosystem (SER 2004; Wortley *et al.* 2013).

The scientific field of restoration ecology as well as practical ecological restoration programs such as erosion control, farmland revegetation, reforestation, minesite reclamation, habitat improvement and rangeland rehabilitation have been developed mainly in recent decades (Young *et al.* 2005). Restoration ecology has recently been incorporated into the biodiversity conservation policies of many countries through subsidies for native vegetation enhancement and conservation, but much more remains to be done (Benayas *et al.* 2009; Munro *et al.* 2009; Munro *et al.* 2007). Most restoration programs have been carried out in developed countries, with large areas of land having been successfully restored in North America, Europe, Australia, Asia and Costa Rica (Ruiz-Jaen and Mitchell Aide 2005; Wade *et al.* 2008). In North America, for example, restoration projects carried out in the last 20 years have restored 3 million ha of wetlands and cost US $70 billion dollars. In China, ecological restoration has become an industry with the revegetation of 37 million ha of forest across the northern provinces (Lü *et al.* 2012).

In Australia, examples of ecological restoration are known from the 19th century, but scientifically informed programs emerged only about five decades ago (Trigger *et al.* 2008). Initial restoration work was concerned with revegetating degraded land following mining (Cooke and Johnson 2002), but more recently restoration of other modified environments such as farmland has occurred (Ruiz-Jaen and Mitchell Aide 2005).

Why is ecological restoration in mixed farming environments necessary?

Agricultural ecosystems are critical to humanity for crop and livestock production as well as for a wide range of other environmental and ecosystem services (LaFevor 2014). Most of the world's biodiversity exists in human-managed landscapes and seascapes rather than in protected areas, and this biodiversity is vital for productive farming and forestry (Hobbs 1993). Many species of birds, insects and plants, some of them endangered, depend on mixed farmland for their survival (Kohler *et al.* 2011). Environments modified by agriculture are often essential for nature conservation due to the creation of landscape mosaics and environmental heterogeneity (Dornelas *et al.* 2009), and can sustain endangered species and retain important cultural attributes (Lindemann-Matthies *et al.* 2010). However, native vegetation in agricultural landscapes is commonly a threatened resource due to over-clearing and lack of recruitment and tree dieback (Bullock *et al.* 2007; Reid and Landsberg 2000; Wade *et al.* 2008).

As agriculture will need to provide the increasing human population with sufficient food and fibre at an acceptable environmental cost, revegetation projects are likely to become more common in future (Robertson and Swinton 2005). About 38.5% of the world's land surface is dedicated to agricultural production (FAO 2015). The advance of the farming frontier worldwide coupled with agricultural intensification, which commonly involves the increased use of fuel, fertilisers, herbicides, pesticides, water, overgrazing and injudicious logging, is a major cause of environmental degradation (Mulitza *et al.* 2010). Agriculture uses more fresh water than any other human activity worldwide, and relies mostly on non-native crop species for food production (FAOSTAT 2015). Accordingly, transformation of land for agriculture is generally accompanied by the removal of native vegetation, with its consequent fragmentation into small isolated patches, and commonly the invasion of non-native fauna and flora (Stoate *et al.* 2001). Agricultural systems therefore can degrade soil, water, air quality, and biodiversity. This degradation often affects agricultural production itself, for example through the loss of insects that provide pollination and pest control services that are typically less common in more intensively managed landscapes (Tscharntke *et al.* 2005).

Farmland in many areas is still in urgent need of ecological restoration and represents a significant challenge and an opportunity to re-establish native vegetation (Cramer *et al.* 2008; Robertson and Swinton 2005). Abandoned farmland that is no longer cultivated can be characterised by weed-dominated vegetation and associated with shrub encroachment and soil degradation with corresponding increased risk of wildfire and accelerated erosion (Stoate *et al.* 2009). Such land is often in need of significant rehabilitation, commonly showing symptoms of degradation such as a reduction in biodiversity and the numbers of plants and animals; pest outbreaks; plant epidemics such as dieback of native vegetation; diseased livestock; low yields, and soil erosion among others (Wade *et al.* 2008).

In agricultural landscapes, restoration through revegetation has attempted to reduce or reverse serious land degradation, with varied results (Munro *et al.* 2007). In general, the scale of modification of farmland is large, soil changes and degradation often difficult to address, and natural recolonisation by native species infrequent because the native organisms are typically constrained to small often distant habitat remnants, or consigned to isolated landscapes (Hobbs 1993). Therefore, restoration in farmland is likely to require a range of methods mostly based on sustained, repeated, long-term interventions (LaFevor 2014). Creating a variegated environment and restoring a wide diversity of plants, animals and ecosystem states in farmland can result in improvement of both agricultural and ecosystem productivity (Donald and Evans

2006). For example, the benefits of establishing trees in farmland potentially includes bioenergy production, soil nutrient cycling, carbon sequestration, erosion control, habitat restoration, increased water availability, wood production, as well as improved aesthetics and landscape conservation (McIntyre and Hobbs 1999; Vorosmarty *et al.* 2010). Ecological restoration of heterogeneous agricultural landscapes also benefits the conservation of other ecosystems services (Benayas and Bullock 2012). In the long term, carefully designed restoration can also offer yield financial benefits for farmers through improved yields or reduced costs (Benayas *et al.* 2008; Smith and Sullivan 2014). Revegetation also serves the immediate purpose of ameliorating the effects of agricultural expansion and intensification (Wade *et al.* 2008). Finally, some other functions that can be improved through restoration include: (1) genetic and population diversity for crop and livestock improvement and increased pest resistance; (2) biotic community assemblages that enhance crop quality; (3) a diversity of biophysical processes in ecosystems, and (4) interactions at landscape level between farmland and other ecosystems, which may improve resources for farming, and possibly resilience to climate change (Benayas and Bullock 2012).

Revegetation and grazing

Livestock and their managers are a part of complex grassy ecosystems (Whalley 2000) and livestock grazing accounts substantially for the variation in structure and composition of grassy ecosystems (Díaz *et al.* 2007; McIntyre and Martin 2001). Grazing can be used as a tool for conservation and regeneration of native grassland and associated biodiversity, as well as for weed control, but success is largely dependent on appropriate grazing strategies (Norton and Reid 2013). Many native grassland species, particularly perennials and tall palatable species, can disappear locally or decline in abundance and diversity after intense long-term livestock grazing; on the other hand, in the absence of grazing, annuals and weeds can invade and limit colonisation of some species due to excessive litter and the lack of soil gaps (Dorrough *et al.* 2004). Other effects produced by livestock are changes in above-ground and below-ground biomass, soil, water, the size of non-vegetated gaps, nutrients and temperature (Browning *et al.* 2014; DeMalach *et al.* 2014).

More specifically for this thesis, grazing can directly and indirectly affect seed dispersal, seedling emergence and survival and persistence, seed production, rates of plant colonisation and species richness and co-existence (Fuhlendorf *et al.* 2008; Whalley 2005). Livestock movement has been found to contribute to landscape connectivity (Rico *et al.* 2012). Therefore, this practice has potential importance for revegetation of grazed areas (Manzano and Malo 2006; Wessels *et al.* 2008), although the impact of this is difficult to estimate (Bullock *et al.* 2011). Ruminant livestock may also disperse seeds of hard-seeded species resistant to digestion, such as leguminous species (Gardiner *et al.* 2012). Studies of exotic species have shown that woody vegetation can become more abundant under grazing, probably due to a shift in the competitive balance between woody species and grasses (Johnston and Pickering 2001; Riginos and Young 2007; Sharp and Whittaker 2003). Based on these results, we would expect that appropriate grazing management has the potential to contribute to the dispersal of desired native woody species. The effects of ruminant livestock on the dispersal of native plants have been studied in European landscapes (Auffret 2011; Auffret *et al.* 2012), but rarely in Australia despite the fact that ruminants are major components of most contemporary Australian ecosystems.

Biodiversity conservation schemes developed for Australian grasslands suggest that the maintenance of a range of livestock grazing and rotational grazing regimes may be critical for the conservation of plant biodiversity in areas where mixed farming is one of the main land uses (Lunt *et al.* 2007).

It is estimated that up to 40% of semi-arid floodplain grasslands have been removed in NSW by grazing and cropping. However, in our study region of clay floodplains, the extent of intact grassland is unknown due to lack of data (Lewis *et al.* 2009). Grasslands in our study region have benefited from significant research in relation to livestock, defining management strategies that can direct grassland composition for desired outcomes (Lodge and Whalley 1985). However, the use of livestock for grassland restoration remains an important challenge from a science perspective (Whalley 2005).

The challenge of revegetating whole communities

Revegetation can involve restoration of whole communities and their ecological functions at both large and small scales over large areas. In practice, revegetation commonly consists of just putting trees back in the landscape (Bakker *et al.* 2000; Choi 2004; Ehrenfeld 2000; Lesica and Allendorf 1999; Young *et al.* 2001). While trees can improve the ecological functioning of the developing community, such as increasing water infiltration and soil surface cracking, understorey vegetation is also important to fully restore ecological processes (Colloff *et al.* 2010).

For sustainable restoration, multiple species with various ecological functions need to be incorporated into the community in the longer term, and environmental conditions should be consistent with: (1) individual species' requirements; (2) the fertility, abundance, dispersal and establishment capacity of the species; (3) the presence of dispersal agents, and (4) the germination and recruitment of new individuals (Bakker and Berendse 1999). Additionally, the availability of resources at the site, such as water supply, and geographical factors, such as erratic periods of drought and flooding, are critical for the success of revegetation (Audet et al. 2013).

Restoration projects worldwide are aiming to re-establish complex functional communities by combining a wide range of species from diverse ecosystems, including species with suitable traits that result in stable community states (Hérault *et al.* 2005; Honnay *et al.* 2002; Jonson 2010; Matthews *et al.* 2009; Wold *et al.* 2011; Young *et al.* 2001). Accordingly, it has been necessary to develop the expertise to manage a range of seed and seedling characteristics to meet these requirements (Gibson-Roy and Delpratt 2014). Other factors that must be considered include costs, specific management practices, and the involvement of revegetation practitioners whether they be from the private, government or community sectors (Qureshi and Harrison 2003). The capacity for seed collection sites to supply enough native seed of sufficient quality for revegetation at the scale required is another crucial consideration and often challenging (Mortlock 2000).

The scale of revegetation in farmland: is broad-acre revegetation important?

The restoration of ecosystems in highly fragmented agricultural landscapes frequently requires large-scale restoration practices, together with small-scale restoration efforts to fit into the larger context (Dorrough and Moxham 2005; Hobbs and Norton 1996). Small-scale restoration provides opportunities for survival or rescue of species of concern threatened by extinction through consideration of the autecology and habitat requirements, and usually its lower cost than large-scale restoration although growth rates can be slow and seedlings prone to damage (Holl *et al.* 2011). On the other hand, large-scale revegetation of farmland may enhance biodiversity and associated ecosystem services in order to support complex ecological interactions and long-term persistence of biodiversity (Ribeiro-Rodrigues *et al.* 2011). Therefore, flexibility and diverse practices are required to engineer appropriate conditions for various goals to be achieved and meet the manifold challenges of restoration (Ehrenfeld 2000; Thompson 2011). The scale of most restoration programs is commonly small, involving only a limited degree of recovery of the productive capacity of the landscape rather than the recovery of biodiversity at a scale approaching natural communities (Manning *et al.* 2006). Ideally, restoration programs should include the use of native species mixtures to replace exotics, the matching of species to the environment to encourage the re-establishment of understorey species, and the subsequent creation of self-perpetuating ecosystem functions (Manning *et al.* 2006).

In this particular research, large-scale restoration was crucial to meet the targets associated with the Brigalow–Nandewar Biolinks project (Chapter 1, Background).

Options for large-scale revegetation

Restoration can be achieved by a range of different methods. Large-scale, high-input revegetation can be advantageous if it increases the chances of plant survival and allows natural variability and population growth (Manning and Lindenmayer 2009; van Katwijk *et al.* 2016). Given the range of reasons for revegetating a given area, the choice of method will depend on the restoration objectives, the characteristics of the area to be restored, as well as on the timeframe and available (Ribeiro-Rodrigues *et al.*

2011). Above all, the most appropriate techniques should be selected and applied carefully to increase the chances of success, considering the labour inputs and resources required (Dorrough *et al.* 2008; Lindenmayer *et al.* 2012).

◆ Natural regeneration

Natural regeneration is the regrowth of plants from pre-existing propagules with minimal human interference at a site. It can involve specific manipulations to encourage revegetation from existing propagule sources, such as the soil seed bank, canopy-stored seeds or seed dispersed by wind, water or animals into or within the site (NSW Government 2015a).

◆ Brush matting

This process consists of laying branches of trees and shrubs laden with ripe fruits, directly on the revegetation site on a bare soil surface (NSW Government 2015a). This method can introduce seeds and act as a soil protection layer. It is generally used for small areas. An adaptation is the use of piles of branches without fruits but that sequester scarce resources in the soil for seeds to form a regenerating patch (Tongway and Ludwig 1996). Branches can also trap seeds moving across the soil surface (Bean *et al.* 2015).

◆ Transplanting

Seedlings can also occur naturally at very high densities following unusual environmental conditions such as large precipitation events that are irregular between years and within seasons (Arnold *et al.* 2014a). Excess seedlings can then be transported and transplanted to other areas where they are desired. Such transplants may include topsoil that can reintroduce other species as well as the target species. This method is known mainly for restoring plants growing in moist areas (NSW Government 2015a), but has been used successfully in deserts and for native grass restoration (Whalley 2012).

◆ Tubestock planting

Tubestock is a term used for seedlings raised in plastic nursery tubes and then transported to the revegetation site. For revegetation with tubestock, seeds are usually collected from natural populations and sown in seedling trays or directly into pots in the nursery or glasshouse. Seedlings are then transported to a shadehouse or outdoors before planting, bare-rooted and watered in the field (Morgan 1999). Where large-scale planting is required, an efficient team of planters or mechanical planters are required. Mechanical planters operate similarly to direct-seeding machines, by creating a hole in the soil for the seedling and then firming the soil around the seedlings with press wheels (Florabank 2014).

◆ Direct seeding

Direct seeding is the sowing of seeds directly into the soil. It is used for the establishment of many native species. There are two main approaches to direct seeding: (1) hand sowing is useful for small areas or in country inaccessible to machinery, and (2) mechanical direct seeding uses specialised sowing equipment (Florabank 2014). When successful, the benefits of direct seeding include the possibility of sowing large areas using multi-species mixtures of seeds. However, this assumes that the equipment can handle the seed of the chosen species and the seed pre-treatments, and that conditions are conducive to the establishment and survival of the new plants. In restoration projects in different types of ecosystem that compare direct seeding versus other restoration techniques, the total cost of direct seeding is at least half that of other propagation techniques (Bean *et al.* 2004; Cole *et al.* 2011; Vera and Parrotta 2001). However, successful germination and seedling establishment after direct seeding is variable and subjected mostly to the availability of appropriate microsites (Bonilla-Moheno and Holl 2010; Dalling and Hubbell 2002). It has proven challenging to restore a full complement of species at community level by direct seeding alone, and best results may be achieved from a combination of direct seeding and planting of seedlings (Doust *et al.* 2006).

Potential to use commercial seeders for broad-acre direct seeding

Various combinations of horticultural and agronomic techniques and machinery have made the reconstruction of complex native vegetation, particularly by direct seeding, technically feasible (Gibson-Roy and McDonald 2014; Rayburn and Laca 2013). Native plant seeds have been sown successfully using precision agricultural drill seeders with multiple boxes, allowing the seeds to be deposited at designated depths, rows, density, distance and wheeled pressure into the soil (Jonson 2010). Other cropping machinery such as fertiliser spreaders can be used in direct seeding to aerate and de-compact soils for seedbed preparation (Gibson-Roy *et al.* 2010).

Difficulties with species selection

Selecting plant species for revegetation projects requires planning to achieve ecosystem restoration (SER 2004). Native species generally must be deliberately reintroduced and exotic species eliminated or controlled (Kimball *et al.* 2014). Nevertheless, revegetation with direct seeding is suitable for only certain species because germination and seedling establishment are precarious stages in the life cycle of plants. Losses at these stages can vary among species, places and times (Doust *et al.* 2008; Geeves *et al.* 2008). Some of the factors that influence seed germination and seedling emergence and establishment are the conditions under which seeds are sown, such as the season of sowing, ambient light and moisture, seed traits such as seed size, persistence in the soil, time to germination, germination rate and the rate of seedling development (Jinks *et al.* 2006; Tunjai and Elliott 2012), and all should be considered in the selection of species for direct seeding.

Seed quality: purity and mass

Seed purity and mass are critical to direct seeding because they are used to determine how much seed of each species is required for the desired stocking density at establishment.

◆ Purity

Seed purity is obtained after the elimination of infected, dead and damaged seed, trash, inert material and seeds of other species (Kameswara *et al.* 2006). Seed purity is defined as the mass of pure clean seed expressed as a percentage of the mass of the whole sample (Langkamp 1987; Schmidt 2000). The percentage of pure seed is calculated as follows:

$$\text{Purity (\%)} = \frac{\text{mass of pure fraction}}{\text{total mass of sample}} \text{ x } 100 \qquad (1)$$

◆ Mass

Seed mass estimation is necessary to determine the amount of seed required for different uses (Gunn 2001), as well as to predict the relationships between seed size and ecological traits, such as seedling emergence from different soil depths (Bond *et al.* 1999).

◆ Viability

Before use, seed should be tested to establish if it is viable and germinable. A range of methods can be used to test viability, either by directly testing germination (Mortlock 1999), or by indirect tests such as seed cutting, tetrazolium (ISTA 2003), visual assessment and squash tests among others. These indirect tests are used to establish the quality of seeds that cannot germinate rapidly with a standard germination test (AOSA 2000; Schmidt 2000).

Germination

Information about seed germination and seedling establishment is essential for successful restoration (Broadhurst *et al.* 2008; Khurana and Singh 2001). Germination tests indicate the potential germination percentage, which with appropriate management should reflect the germination expected in the field (Schmidt 2000); ideally, germination percentages should be high.

Germination is a critical stage in the lifecycle of plants, and is one of the first physiological responses of the plant to environmental conditions (Bell 1999). It occurs when the environmental conditions match the seed germination requirements of a non-dormant seed (Boyd and Van Acker 2003; Vleeshouwers *et al.* 1995), and begins with the imbibition of water by the dry seed, followed closely by embryo expansion and elongation of the embryo axis (Finch-Savage and Leubner-Metzger 2006).

Different procedures can be used to germinate most species with similar results. In seed testing, germination is recorded when the seedling is judged as normal according to standard criteria specific to each species (AOSA 2014; Bányai and Barabás 2002; Gunn 2001; ISTA 1999; Schmidt 2000). However, many Australian plant species need a range of particular environmental conditions or pre-treatments for seeds to germinate and establish, as they are adapted to specific environmental cues (Bell 1999). These include dormancy-breaking treatments that stimulate germination (Bell *et al.* 1993; Cochrane and Probert 2006; Jurado and Westoby 1992; Vleeshouwers *et al.* 1995). Environmental factors such as exposure to flooding, fire or smoke from fires, ingestion or dispersal by animals, pH, and other soil conditions can also be important (Baskin and Baskin 2004a; Bell 1999).

Seed persistence

Seed persistence refers to the survival of seeds after they reach maturity. It is an important consideration for plant restoration programs where the survival of seeds in storage may affect the viability of the seed used in restoration and subsequently the potential presence and abundance of plants that establish at the restoration site (Long *et al.* 2015).

Seed dormancy

Dormancy is a property of some seeds, impeding germination under otherwise favourable physical environmental conditions (temperature, moisture light, etc.) for a period of time (Baskin and Baskin 2004b). Seed dormancy is broken when environmental conditions precipitate changes in the seed that then permit germination if seeds are subsequently exposed to the range of physical environmental conditions required by the seed's genotype (Finch-Savage and Leubner-Metzger 2006). However, conditions that effect breaking of dormancy can differ from those that allow seed germination after dormancy is relieved (Benech-Arnold *et al.* 2000). Dormancy helps extend the life of the soil seed bank so that the seed population is not exhausted after one germination event if the first cohort of seedlings does not survive to reproductive age (Lodge and Whalley 1981b).

With the exception of approximately 60% of taxa in tropical rainforests and 50% in tropical semi-evergreen forests, most plant species worldwide have dormant seed (Baskin and Baskin 2003). In Australia, seed dormancy is widespread, and there is much to be learnt about breaking dormancy in native plant species (Bell *et al.* 1993; Cochrane and Probert 2006; Jurado and Westoby 1992; Langkamp 1987). Five types of dormancy are widely distributed among different plant taxonomic groups (Baskin and Baskin 2014; Finch-Savage and Leubner-Metzger 2006). These are:

1. Morphological dormancy occurs when the embryo is differentiated but small and underdeveloped. Embryos in freshly matured seeds begin to grow (elongate) within a period of a few days to 1–2 weeks and seeds germinate within about 30 days.
2. Physical dormancy takes place when the seed coat or fruit is not permeable to water or oxygen but the embryo is fully developed. Germination occurs within about 2 weeks (or less) when the seed or fruit coat is scarified.
3. Physiological dormancy occurs when seeds are permeable to water, the embryo is differentiated and fully developed (elongated), but the embryo has a

physiological mechanism inhibiting germination. Thus, seeds require longer than 30 days to germinate and need a dormancy-breaking treatment.

4. Physical dormancy can be combined with physiological dormancy such as when the seed is not permeable and germination does not occur within about 2 weeks (or longer) after the seed or the fruit coat is scarified, although the seed becomes fully imbibed within a few hours following scarification.
5. A specialised type of morphological or morpho-physiological dormancy occurs when the seed or the fruit coat is permeable to water, but in addition to morphological dormancy there is a physiological component to seed dormancy.

Germination stimulation syndromes

In addition to seed dormancy, unfavourable environmental conditions can also limit germination (Thompson and Ooi 2010). Among these factors, the role of temperature for incubation and seed after-ripening, light and moisture (particularly in relation to seed burial) play a crucial role in the control of germination (Merritt *et al.* 2007). (Merritt *et al.* 2007). Various treatments that seeds would be unlikely to receive in nature are nevertheless effective in stimulating germination if the objective is to produce germinants in a rapid convenient manner (i.e. smoke water, sulphuric acid, nitrate, gibberellin) (Baskin *et al.* 2006a). However, most such treatments are beyond the scope of this thesis.

Research aims and objectives

The aims of this thesis are: (1) to quantify the germination of a wide range of native plant species from north-western NSW; (2) to investigate suitable techniques to break dormancy and permit germination of the seeds of species that are otherwise difficult to germinate, and (3) to determine suitable environmental conditions for the successful germination and establishment of selected species. The thesis results from the need for seed ecology research to assist with the large-scale restoration of native plant communities. The primary aim is to increase the success of revegetation through improving the knowledge of the plant establishment biology of native species.

The main objectives of the research in this thesis were:

1. To identify the effects of temperature, light, moisture, age of seed and seed storage and sowing depth of selected species on germination and seedling emergence under controlled and semi-controlled experimental conditions in germination chambers and the glasshouse.
2. To identify the type of seed dormancy present and to test the effectiveness of different treatments to break dormancy when present and so increase germination success.
3. To determine appropriate methods to break seed dormancy to be applied consistently and cost-effectively to bulk seed to achieve rapid, uniform and high germination percentages prior to direct seeding for restoration.
4. To determine the factors in relation to moisture regime, sowing depth and seed size that allow seedlings to emerge in vertosols. This is intended to provide guidelines for direct seeding practices that can enhance seedling emergence in large-scale revegetation of cracking clay soils in north-western NSW.

It is intended that the practical application of the work described in this thesis follows from these objectives by (1) determining whether direct seeding is suitable for the native species selected from the study region; (2) applying the knowledge obtained from revegetation of previously farmed land to assist with the restoration of large areas of native vegetation in a cost-effective manner, and (3) disseminating information on dormancy-breaking treatments for some of the species studied for use by practitioners and plant propagators.

Thesis outline

This thesis is divided into seven chapters. The chapters are organised according to increasing complexity of the experimental treatments, with a final Conclusions chapter.

Chapter 1 introduces the subject matter (section 1.1), provides a short literature review of ecological restoration (section 1.2) and discusses the options and challenges of large-scale revegetation in farmland (section 1.3) and the seed traits that should be considered before using species in restoration (sections 1.4 and 1.5). Chapter 1 also presents the research aims and objectives of the thesis (section 1.6).

Chapter 2 briefly describes the study region, the main vegetation types, land uses and the rationale for selection of the vegetation communities and species that are the focus of this thesis.

Chapter 3 reports the results of germination tests in growth cabinets of the non-dormant seed of ten eucalypt tree species in relation to light and temperature requirements.

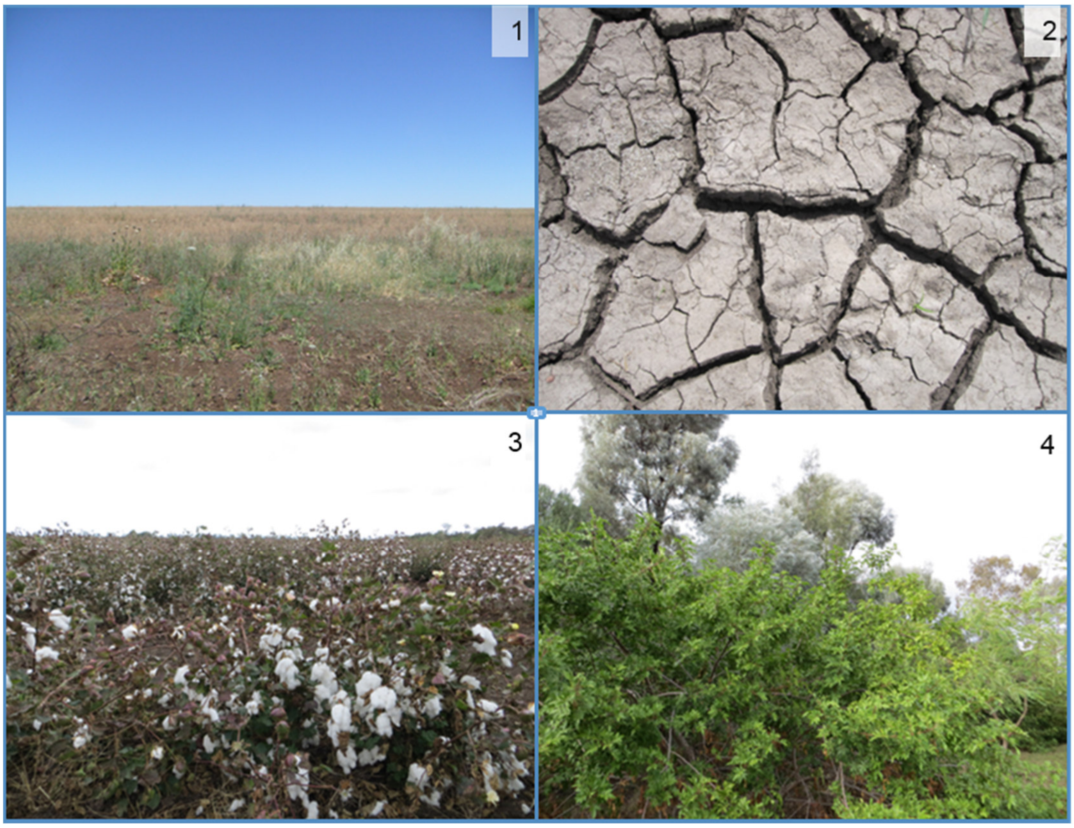
Chapter 4 documents viable pre-germination treatments for the seeds of *Acacia* species with physical dormancy.

Chapter 5 identifies the germination constraints present in the seeds of 49 plant species from the study region and describes methods to alleviate them.

Chapter 6 presents the seedling emergence germination responses of six *Eucalyptus* species in cracking clay soil, from a glasshouse experiment.

Chapter 7 summarises the main findings of the project as a whole and how these findings can contribute to management practice. This chapter also describes the limitations of the research and makes recommendations for future research.

Chapter 2. Study region and species



Typical scenes from the study region of the North-West Plains of NSW: (1) unutilised farm-land; (2) vertosol soil; (3) cotton crop, and (4) remnant native vegetation on a farm.

Introduction

This chapter consists of a brief overview of the study region in north-western NSW and the native vegetation communities and the species selected for study. The objectives of this chapter are to: (1) describe the geographical, physical and environmental conditions of the study region relevant to the project; (2) document the conservation status of the plant communities of interest, and (3) explain the selection of species from these communities, which are the focus of the germination studies in the remainder of the thesis.

Geography

The study region for this thesis comprises the plains and near slopes of the Border Rivers–Gwydir (BRG) catchment (Fig. 2.1). Some of the species studied also occur in the adjacent Namoi catchment, while others are more widely distributed. Both catchments are ecologically important because they include: (1) a diverse range of flora and fauna in communities listed as threatened under the *Environment Protection and Biodiversity Conservation Act 1999* (EPBC Act (1999)and the *Threatened Species Conservation Act 1995* (TSC Act (1995); (2) rivers, dams and wetlands that enable intensive irrigated agriculture on the plains and provide ecosystem functions and habitat for wildlife, and (3) plant species that are not classified as threatened but provide structural elements for regenerating ecosystems.

The North-West Plains of NSW are intersected by numerous waterways and rivers. The waterways and rivers of the BRG catchment are bounded in the north by the Macintyre River, which flows into the Barwon River, and by the Namoi River catchment to the south, which is formed by the Namoi, Manilla and Peel River Valleys (Australian Goverment). The BRG slopes region is characterised by hills and ranges, whereas the plains region is undulating to flat and constitutes nearly two-thirds of the catchment. In the BRG catchment, native vegetation covers less than 25% of the region with only 1% unmodified compared to its pre-1788 condition (Foster *et al.* 2016).

Fig 2.1 The administrative boundaries of the Murray-Darling Basin and its constituent catchments in eastern Australia.

Moree Plains, Gwydir and Narrabri Shires are located in north-western NSW (Fig, 2.2). The Moree Plains is bisected by the Mehi River in the centre of fertile black-soil plains. At Narrabri, topography varies from fertile floodplains to rugged mountain ranges, with the Namoi River meandering through the extensive plains. Gwydir Shire occupies the boundary between the North-West Slopes and Plains of NSW and consists primarily of alluvial plains and floodplains in the west, and hills and ranges in the east (NSW Government 2015b).

This study focused on the seed ecology of plants of the native vegetation communities of the North-West Plains, mainly of the grey cracking clay vertosols (colloquially known as ‘black soil plains’), in the western parts of the Moree Plains, Narrabri and Gwydir Shires in north-western NSW (Fig. 2.2). The Moree plains are administratively part of the Central Division of NSW, which is mainly freehold land with only 1.2% held in public conservation reserves and 3.9% in state forests. The Central Division is primarily alluvial plains and floodplains with few hills or ranges (Benson *et al.* 2006).

The field experimental site where soil collection and field experiments took place was the property ’Koiwon’, near Bellata, 42 km south-south-west of Moree, NSW, in the centre of the study region. Nevertheless, other plant species occurring on the slopes and plains in different soil types were also considered in some experiments.

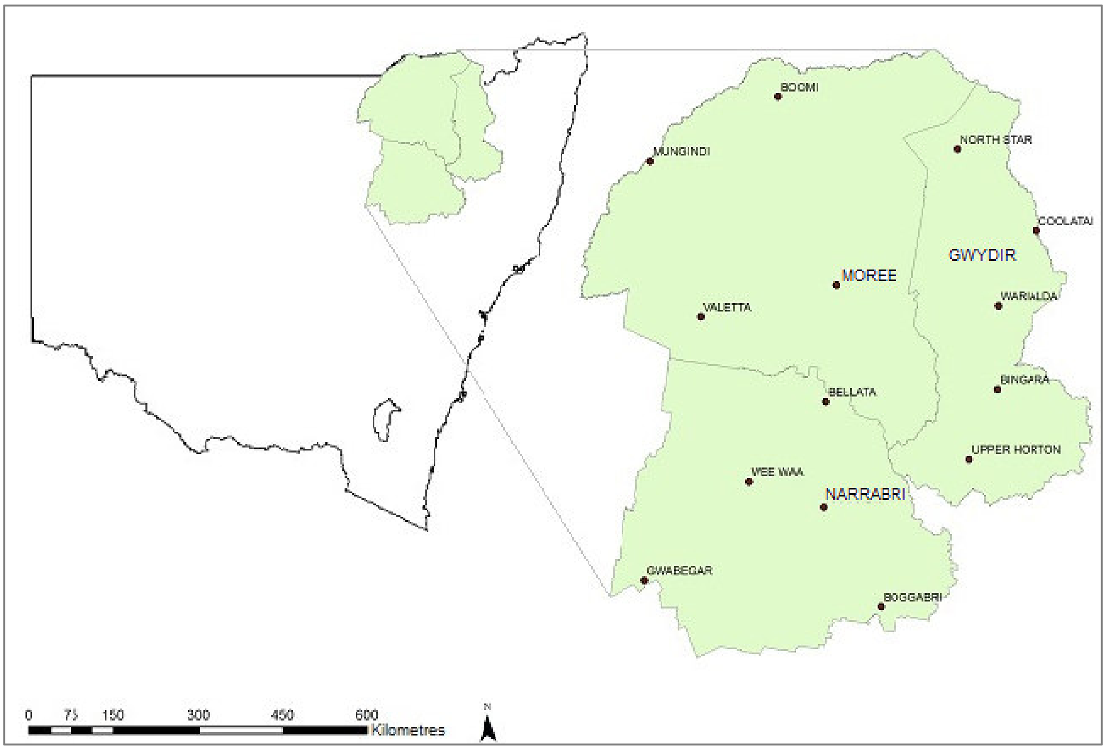


Fig. 2.2 Main study region for this project, comprising Moree Plains, Narrabri and Gwydir Shires

Climate

The climate of Bellata is warm temperate with a slight summer dominance of rainfall (Figs 2.3, 2.4). The average annual temperature (18.8°C) and the average annual rainfall (644 mm) were based on the last 100 years of climate statistics (BOM 2016). However, the region has a more variable and drought-prone climate than some other parts of the Australian wheatbelt (Chenu *et al.* 2013), meaning that rainfall may (or may not) occur in any month of the year (Fig. 2.3). The erratic rainfall means that water availability can often restrict seed germination, seedling emergence, establishment and plant survival of a diverse range of species (Arnold *et al.* 2014a).

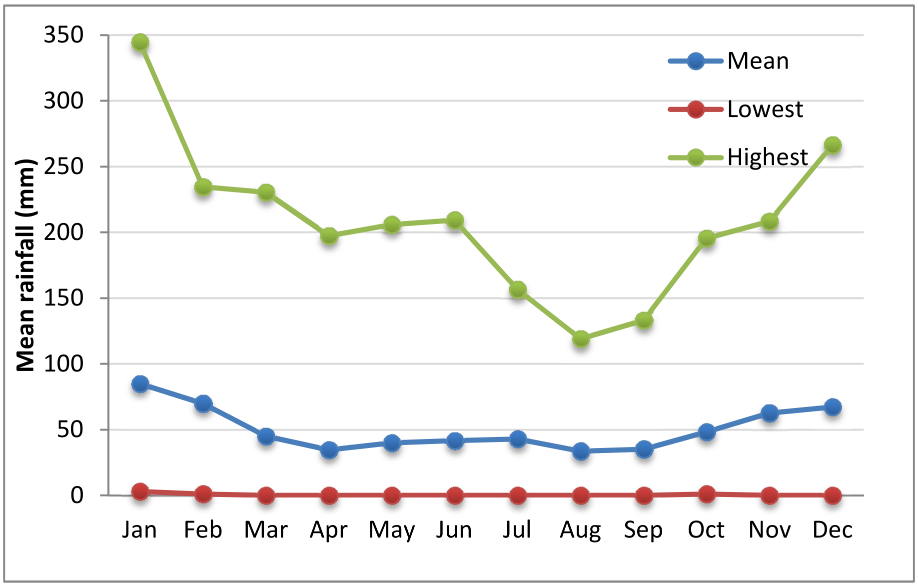


Fig 2.3 Mean monthly rainfall and lowest and highest mean monthly rainfall over 100 years (1914–2014) at Bellata, NSW. Station: Bellata Post Office, lat. 29.92° S, long. 149.79° E; elevation, 229 m a.s.l.

Temperature is the most significant element influencing seed germination when water is not limiting, functioning to facilitate or constrain germination (Cochrane *et al.* 2014). Temperature interacts with hydric conditions to determine the amount and rate of germination, and perhaps to break dormancy (Benech-Arnold *et al.* 2000).

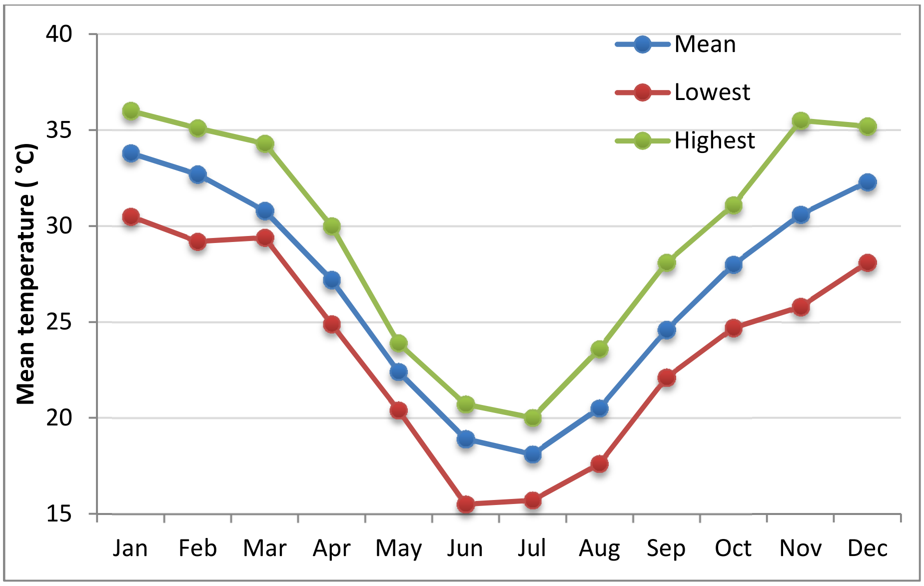


Fig 2.4 Mean monthly temperature, and lowest and highest mean monthly temperature, over 100 years (1914–2014) at Bellata, NSW. Station: Bellata Post Office, lat. 29.92° S, long. 149.79° E; elevation, 229 m a.s.l.

Soils

The floodplains in the study region consist of Quaternary alluvium comprising a range of soil types, most of which are cracking clays that have high fertility and have allowed agricultural development and farming, particularly since the second world war (SoE 2010; Ward 1999).

The grey clay vertosols that dominate the study region are the main focus of the project. They are mainly strongly alkaline, with pH increasing with depth down the profile, have low organic carbon and low total nitrogen concentrations, and carbon−nitrogen ratios lower than other soils in the region (Martin *et al.* 1988). Grey and black vertosols are called *cracking clays* because they shrink leaving deep soil cracks when dry, impeding water conductivity; they are notoriously difficult for germination and emergence of some species (Coughlan and Loch 1984). However, native tree and shrub communities dominated by *Eucalyptus coolabah*, *Acacia* *stenophylla, Acacia pendula* and *Acacia harpophylla* grow extensively on these fine-textured soils. Vertosol soils are self-mulching, which means that after wetting and drying or freezing and thawing, the clay re-aggregates forming a mulch of fine aggregates at the soil surface (Grant and Blackmore 1991). Furthermore, in the surface and subsurface, materials mix in a process known as churning. When the soil dries, many wide cracks open up from the surface downward; surface soil aggregates fall into the cracks and the subsurface soil is pushed upwards when the soil rehydrates and swells. This occurs as a result of the shrink–swell action of the soils in response to drying and wetting (Spaargaren 2008). Also, an excess of sodium attached to the clay particles (sodicity) makes the topsoil dispersive and prone to waterlogging, hard when dry and cloddy when tilled (Daniells 1989). The importance of these cracking clay soils lies in their favourable properties for the extensive dryland and irrigated farming enterprises in the region, as well as presenting difficulties for the re-establishment of native vegetation. Some of the beneficial properties of self-mulching clay soils are: (1) they provide an ideal seedbed environment when the soil is protected from mechanical compaction; (2) they are capable of storing considerable moisture, and (3) they are somewhat resistant to erosion because of their fine texture and strong structure as well as the generally flat topography (Yaalon and Kalmar 1978).

The vertosol soil used for the glasshouse experiments reported in this thesis was collected at Koiwon, near Bellata, NSW (Fig. 2.5). The location was at 29°48'28"S, 149°38'27"E, and was classified by Reid *et al.* (2009) as a grey to black vertosol with air-dry topsoil exhibiting variable self-mulching behaviour, from very prominent, where the surface has a crumb-like structure with large prominent cracks, to weak or no self-mulching and thin soil crusts present.

Land use

The North-West Plains are one of the most important areas in NSW for cropping and irrigation, with only about 20 to 30% of the native vegetation remaining, and less than 5% of some native plant communities (Benson 1991). The remaining native vegetation is highly fragmented with varying patch sizes and shapes resulting from extensive clearing (Benson *et al.* 2006). The North-West Slopes near Bingara and Warialda in the Gwydir Shire are generally used for extensive grazing of native grasses with small areas of grazing of sown pastures and crops (SoE 2012).

The tablelands, slopes and plains of the BRG catchment (Fig. 2.1) are subdivided into four Interim Biogeographic Regions (IBRA): New England Tablelands, Brigalow Belt South, Nandewar and Darling Riverine plains (ERIN 2012). Grazing (meat and wool production) is the main agricultural enterprise on the tablelands, with a combination of dryland cropping and grazing with some horticulture on the slopes, and more intensive irrigation and dryland cropping further west on the plains (BRG CMA 2013).

The primary land use in Narrabri, Moree Plains and Gwydir Shires is highly productive agriculture, mainly large-scale cropping of cotton, grains, fodder and oil seeds. Extensive grazing and small acreages of olive and pecan nut orchards are also found (SoE 2010). In Gwydir Shire, other major sources of rural production are beef and sheep (SoE 2012).

Vegetation and endangered ecological communities

Northern and north-western NSW is one of the most biodiverse parts of Australia, with endemic species of plant and animal, and many others at the limits of their distribution (Pressey and Taffs 2001). This region includes sections of two of the above bioregions: the Darling Riverine Plains and the Brigalow Belt South (Thackway and Cresswell 1995). The extant native vegetation varies from areas of extensive forest to more open forest, shrublands and grassy plains in the west (Benson *et al.* 2010; Carr and Atkinson 2013). ‘Native vegetation’ is defined in the NSW *Native Vegetation Act* 2003 as vegetation with >50% cover of native species at the peak of the annual growth cycle (NSW Government 2015c).

The main native vegetation types remaining in the Moree Plains Shire are: (1) woodlands covering approximately 15% of the Moree Plains, but consisting of only about 5% of tree canopy cover (open woodlands) interdigitating with grasslands, and (2) alluvial plains grassland, dominated by the Mitchell grasses *Astrebla lappacea* and *A. elymoides,* which are widespread throughout the region on cracking clay soils (Lewis *et al.* 2008). However, Mitchell grasslands are in decline due to overstocking and flooding (Lewis *et al.* 2010). The estimated distribution of the dominant vegetation communities across the Moree Plains, including those that are not listed in our priority list or are not endangered, is shown in Fig. 2.5.

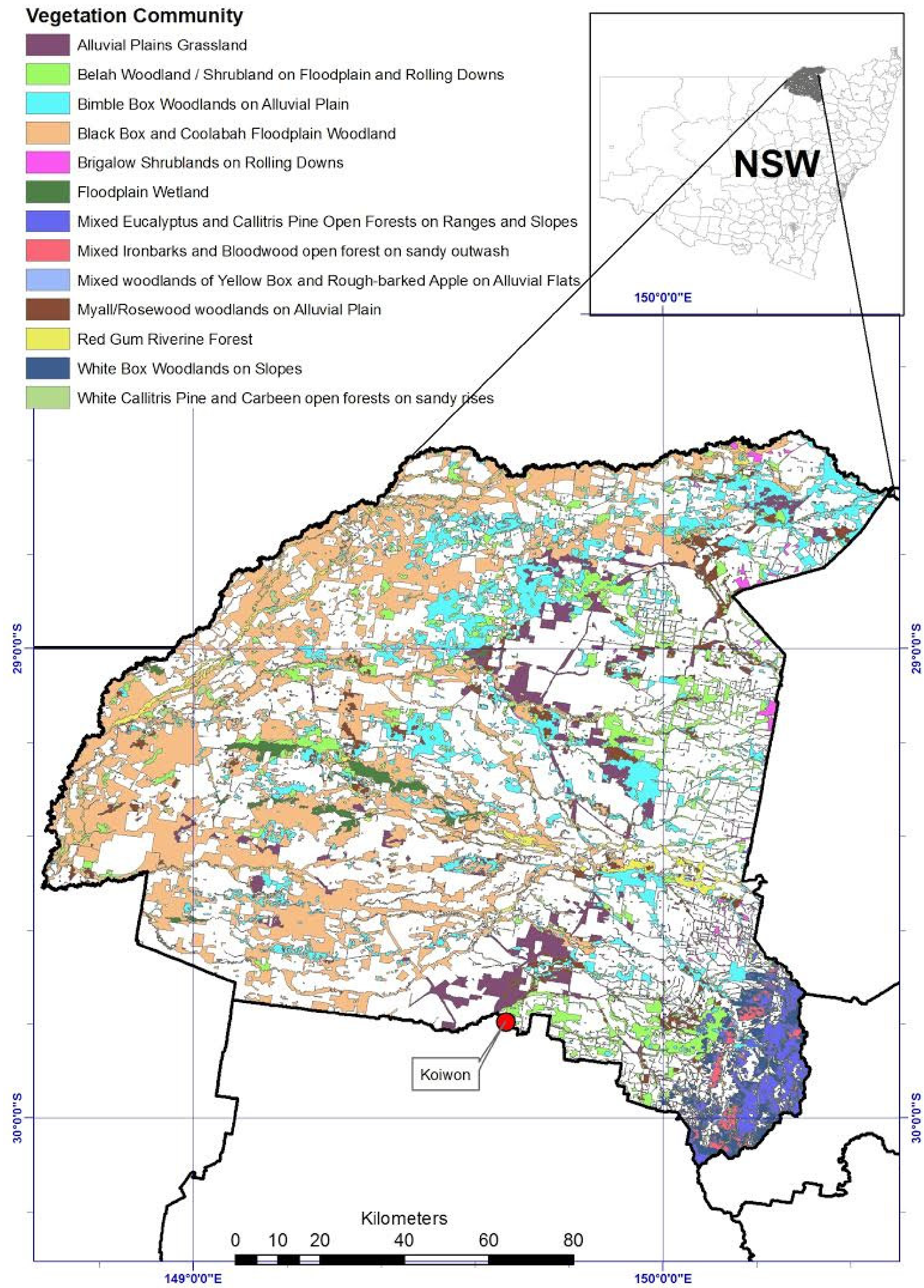


Fig. 2.5 Main native vegetation communities present across the Moree Plains Shire (White 2002). The white areas are the locations without available information and are probably cultivated and/or irrigated. The approximate location of 'Koiwon' where soil was collected for pot trials is indicated with a red dot. (The nomenclature of the plant communities differs from that in the Endangered Ecological Communities legislation.)

In the BRG catchment, there are currently 175 entities (taxa and communities) listed under state legislation in the NSW *Threatened Species Conservation 1995* (TSC Act) (Table 2.2) and in national legislation under the *Environment Protection and Biodiversity Conservation Act 1999* (EPBC Act). Once an ecological community is listed under these statutes, its recovery is promoted using conservation advice and recovery plans as specified in each Act (OEH 2006). An ecological community or species is considered threatened if: (1) its distribution has been significantly reduced; (2) it is susceptible to significant pressures, or (3) its ecological function is undergoing important decline (Department of Environment 2010). Listing implies a high risk of extinction in the immediate future, and recovery plans are intended to stop the decline of listed communities and maximise the probability of their long-term persistence in the wild. Seven threatened ecological communities in the study region are listed nationally as critically endangered or endangered (Department of Environment 2010) as well as endangered under NSW state legislation (OEH 2006) (Table 2.2). Six of these communities and their component species are priority targets for restoration in the Biolinks project (Chapter 1, section1.1; Table 2.1).

Table 2.1 Ecological communities in the study region listed under the *Environment Protection and Biodiversity Conservation Act 1999* and *Threatened Species Conservation Act 1995*.

Community name	Status	
	EPBC Act	TSC Act
***Brigalow (*Acacia harpophylla* dominant and co-dominant)**	Endangered	Endangered
***Coolibah – Black Box Woodlands of the Darling Riverine Plains and the Brigalow Belt South Bioregions**	Endangered	Endangered
***Natural Grasslands on Basalt and Fine-textured Alluvial Plains of Northern NSW and Southern Queensland**	Critically Endangered	Endangered
Grey Box (*Eucalyptus microcarpa*) Grassy Woodlands and Derived Native Grasslands of South-eastern Australia	Endangered	Endangered
***Semi-evergreen Vine Thickets of the Brigalow Belt (North and South) and Nandewar Bioregions**	Endangered	Endangered
***Weeping Myall Woodlands**	Endangered	Endangered
***White Box – Yellow Box – Blakely's Red Gum Grassy Woodland and Derived Native Grassland**	Critically Endangered	Endangered

*Priority target communities for the Biolinks Project.

Brigalow (*Acacia harpophylla* dominant and co-dominant)

This community is present in Queensland and NSW. In NSW it occurs on undulating plains, sand plains and on flat or gentle rises on alluvial plains or undulating plains in the Brigalow Belt South Bioregion from Narrabri to the Queensland border, with stands around Bellata, Biniguy and Croppa Creek (Benson *et al.* 2006). Other than *A. harpophylla*, this community has a high diversity of co-dominant trees and shrub species and a sparse ground layer. When the trees are widely spaced, the ground layer of grasses, chenopods and Brigalow forbs is much denser. Mistletoes are common, particularly when the trees are sparse. Brigalow is associated with gilgaied, heavy cracking clay or clay-loam soils (Department of Environment 2014). It was listed as nationally Endangered under the EPBC Act, because it has declined to approximately 10% of its former extent as a result of vegetation clearance for agriculture.

Coolibah − Black Box Woodlands of the Northern Riverine Plains in the Darling Riverine Plains and Brigalow Belt South Bioregions

The structure of this ecological community is determined by the frequency and duration of floods and area of the stand (patch size) (Capon 2005). It is typified by *Eucalyptus coolibah* as dominant, *E. largiflorens*, *Casuarina cristata, Acacia stenophylla, Acacia salicina* and *Eremophila bignoniiflora* as co-dominant species with other midstorey and understory species. It is present on grey, self-mulching clays of the floodplains, swamp margins, ephemeral wetlands and stream levees in Moree Plains, Narrabri and Walgett Shires, as well as elsewhere in the Darling Riverine Plains and Brigalow Belt South Bioregions. This community is habitat for fauna species of high conservation value (OEH 2006) and is listed as Endangered under the EPBC Act.

Natural Grasslands on Basalt and Fine-textured Alluvial Plains of Northern NSW

This community consists of a range of native grasses, forbs, low shrubs and sub-shrubs, with few or no emergent woody species. It can be extremely rich in herbaceous species including several that are threatened. The dominant grasses vary with soil type, flooding, recent rainfall and influence of tree cover, but commonly include *Dichanthium sericeum, Astrebla* spp., *Aristida leptopoda, Austrodanthonia bipartita, Austrostipa aristiglumis, Bothriochloa* spp., *Digitaria divaricatissima, Elymus plurinervis, Eriochloa crebra, Eulalia aurea, Panicum* spp., *Thellungia advena, Themeda* spp. and *Walwhalleya proluta*. The community is distributed on basalt and fine-textured alluvial plains from the Darling Downs in Queensland to NSW, and occurs in the Brigalow Belt South Bioregion, the Border Rivers−Gwydir catchment, and the Central West, Namoi, Condamine, Burnett, Mary and Fitzroy Basin Natural Resource Management Regions. However, its geographic distribution has declined severely, and the community is currently restricted to small remnants threatened by grazing, cropping, mining, weeds and dryland salinity (LLS 2014). It is listed as Critically Endangered under the EPBC Act and as Endangered in NSW under the TSC Act.

Grey Box (*Eucalyptus microcarpa*) Grassy Woodlands and Derived Native Grasslands of South-eastern Australia

The canopy of this community is dominated or co-dominated by *Eucalyptus microcarpa*, but it is usually associated with *Allocasuarina luehmannii, Brachychiton populneus, Callitris glaucophylla, E. albens, E. camaldulensis, E. conica, E. largiflorens, E. leucoxylon, E. melliodora* and *E. populnea*. The shrub layer composition is variable but can include *Acacia* spp., *Bursaria spinosa,* and *Cassinia*, *Dodonaea*, *Eremophila* and *Maireana* spp. The grassy layer commonly includes *Rytidosperma* and *Austrostipa* spp., *Elymus scaber,* and *Enteropogon*, *Dianella* and *Lomandra* spp. The community usually occurs in central NSW, northern-central Victoria and eastern South Australia in two forms, grassy woodland and derived grassland. The community is listed as Endangered in south-eastern Australia under the EPBC Act (Australian Government 2010).

Weeping Myall Woodland

Weeping Myall (*Acacia pendula*) trees are the sole or dominant canopy species in the low open woodlands and low woodlands that comprise this endangered community. The understorey often includes an open layer of shrubs, forbs, herbs or grasses. The community commonly occurs on plains, in alluvial shallow depressions or gilgais and on black, brown, red-brown or grey clay or clay-loam soils. Weeping Myall Woodland is present on the inland alluvial plains west of the Great Dividing Range in NSW. It also occurs in the Riverina, NSW South-Western Slopes, Darling Riverine Plains, Brigalow Belt South, Brigalow Belt North, Murray−Darling Depression, Nandewar and Cobar Peneplain Bioregions (LLS 2014). It was listed as Endangered in 2008 under the EPBC Act.

Semi−evergreen Vine Thicket

Semi−evergreen Vine Thicket is a low (<10 m high) dense form of dry rainforest, made up of vines, dry rainforest trees and shrubs. The main canopy is dominated by rainforest species such as *Cassine australis, Geijera parvifolia, Notelaea microcarpa, Ehretia membranifolia, Alstonia constricta, Alectryon subdentatus* and *Planchonella cotinifolia*. In NSW approximately 2500 ha remain, but generally as fragmented patches of <100 ha. In the Brigalow Belt North and South Bioregions, it has been fragmented, reduced in area and degraded through land clearing, inappropriate fire regimes, weed invasion and agricultural and grazing practices. It is listed as Endangered under the EPBC Act (OEH 2006).

White Box − Yellow Box − Blakely's Red Gum Grassy Woodland and Derived Native Grassland

This woodland, open woodland or derived grassland community can be open and is dominated by White Box *Eucalyptus albens*, Yellow Box *E. melliodora* and Blakely's Red Gum *E. blakelyi*, and a wide range of other tree species, shrubs, climbing plants, graminoids and many species of forbs. It is habitat for a range of animals including mammals, birds, reptiles, frogs and invertebrates. In NSW this community occurs throughout most of the Central Division of the state from the Queensland border in the north to the Victorian border in the south along the tablelands and western slopes, including the eastern part of the Great Dividing Range, and the adjacent inland plains (OEH 2006). It is listed as Critically Endangered under the EPBC Act (Department of Environment 2010).

Species selected

Seventy-three native species were selected from the six endangered ecological communities in the region for further study (Table 2.2). The selection was based on seed availability and the fact that the areas targeted for restoration by the Biolinks Project had these species and their constituent communities as part of the native vegetation that historically occurred in the vicinity (Smith and Reid 2011). This list contains representative species from the six main vegetation communities as priorities for revegetation and conservation in the study region (Table 2.2). These species were selected to provide the main structural elements in regenerating ecosystems and to impede the array of lifeforms of potential invasive colonisers.

Table 2.2 Species selected for this thesis on the basis of their occurrence in one or more of six endangered ecological communities. Botanical names correspond with those from the Australian National Botanic Gardens (ANBG 2016)

x = presence of the species in the communities marked
Letters indicate communities as follows:
B = Brigalow (*Acacia harpophylla* dominant and co-dominant)
C = Coolibah - Black Box Woodlands
G = Natural Grasslands on Basalt and Fine-textured Alluvial Plains of Northern NSW
V = Semi-evergreen Vine Thickets of the Brigalow Belt and Nandewar Bioregions
M = Weeping Myall Woodlands
W = White Box – Yellow Box – Blakely's Red Gum Grassy Woodland and Derived Native Grassland
TC = Thesis chapter where the species was researched

Scientific name	Family	Life Form	B	C	G	V	M	W	TC
Acacia deanei	Fabaceae	Shrub						x	4
Acacia decora	Fabaceae	Shrub						x	4
Acacia excelsa	Fabaceae	Tree		x		x			4
Acacia harpophylla	Fabaceae	Tree	x			x			5
Acacia leucoclada	Fabaceae	Tree						x	4
Acacia ligulata	Fabaceae	Shrub/tree							4
Acacia melanoxylon	Fabaceae	Shrub/tree						x	4
Acacia oswaldii	Fabaceae	Shrub/tree		x			x		4
Acacia pendula	Fabaceae	Tree	x	x	x		x		4
Acacia salicina	Fabaceae	Tree		x		x	x		4
Acacia stenophylla	Fabaceae	Tree		x	x	x	x		4
Alectryon oleifolius	Sapindaceae	Tree	x	x	x	x	x		5
Allocasuarina littoralis	Casuarinaceae	Tree	x						5
Alstonia constricta	Apocynaceae	Tree		x		x			5
Angophora floribunda	Myrtaceae	Tree						x	5
Apophyllum anomalum	Capparaceae	Shrub	x	x		x	x		5
Atalaya hemiglauca	Sapindaceae	Tree	x	x	x		x		5
Atriplex nummularia	Chenopodiaceae	Shrub		x			x		5
Atriplex semibaccata	Chenopodiaceae	Shrub			x	x	x		5
Brachychiton populneus	Malvaceae	Tree				x		x	5
Cadellia pentastylis	Surianaceae	Tree	x			x			5
Capparis lasiantha	Capparaceae	Vine	x	x	x	x	x		5
Capparis mitchellii	Capparaceae	Tree	x	x	x	x	x		5
Casuarina cristata	Casuarinaceae	Tree	x	x	x	x	x		5
Casuarina cunninghamiana	Casuarinaceae	Tree				x			5
Clematis microphylla	Ranunculaceae	Vine				x		x	5
Corymbia tessellaris	Myrtaceae	Tree							5
Cullen tenax	Fabaceae	Herb			x		x	x	5
Cymbopogon refractus	Poaceae	Grass			x			x	5
Desmodium brachypodum	Fabaceae	Forb						x	5
Dodonaea viscosa	Sapindaceae	Shrub				x		x	5
Ehretia membranifolia	Boraginaceae	Shrub	x			x			5
Einadia nutans	Chenopodiaceae	Forb	x	x	x	x	x	x	5

Scientific name	**Family**	**Life Form**	**B**	**C**	**G**	**V**	**M**	**W**	**TC**
Elaeodendron australe	Celastraceae	Tree	x			x			5
Enchylaena tomentosa	Chenopodiaceae	Shrub	x	x	x	x	x		5
Eremophila debilis	Scrophulariaceae	Shrub		x	x		x	x	5
Eremophila maculata	Scrophulariaceae	Shrub		x			x		5
Eremophila mitchellii	Scrophulariaceae	Shrub/tree	x	x		x	x		5
Eucalyptus albens	Myrtaceae	Tree			x	x		x	3
Eucalyptus blakelyi	Myrtaceae	Tree						x	3, 6
Eucalyptus camaldulensis	Myrtaceae	Tree		x				x	3, 6
Eucalyptus chloroclada	Myrtaceae	Tree						x	3
Eucalyptus dealbata	Myrtaceae	Tree				x			3
Eucalyptus melanophloia	Myrtaceae	Tree				x		x	3, 6
Eucalyptus melliodora	Myrtaceae	Tree			x			x	3, 6
Eucalyptus pilligaensis	Myrtaceae	Tree	x					x	3, 6
Eucalyptus populnea	Myrtaceae	Tree	x	x	x	x	x	x	3, 6
Eucalyptus sideroxylon	Myrtaceae	Tree						x	3
Eulalia aurea	Poaceae	Grass			x				5
Flindersia maculosa	Rutaceae	Tree	x			x			5
Geijera parviflora	Rutaceae	Shrub	x	x	x	x	x	x	5
Glycine clandestine	Fabaceae	Vine			x			x	5
Grevillea striata	Proteaceae	Tree							5
Hardenbergia violacea	Fabaceae	Vine						x	5
Jasminum lineare	Oleaceae	Vine	x			x			5
Jasminum suavissimum	Oleaceae	Vine						x	5
Lomandra longifolia	Asparagaceae	Grass		x				x	5
Maireana brevifolia	Chenopodiaceae	Shrub	x					x	5
Melaleuca bracteata	Myrtaceae	Shrub							5
Melaleuca trichostachya	Myrtaceae	Shrub		x					5
Myoporum montanum	Scrophulariaceae	Shrub	x	x	x	x	x	x	5
Notelaea microcarpa	Oleaceae	Shrub	x			x		x	5
Owenia acidula	Meliaceae	Tree	x		x	x			5
Pandorea pandorana	Bignoniaceae	Vine				x			5
Pittosporum angustifolium	Pittosporaceae	Shrub				x	x		5
Rhagodia spinescens	Chenopodiaceae	Shrub	x	x		x	x		5
Santalum lanceolatum	Santalaceae	Tree				x	x		5
Senna artemisioides	Fabaceae	Shrub							5
Solanum cinereum	Solanaceae	Forb							5
Spartothamnella juncea	Lamiaceae	Shrub				x			5
Sporobolus elongatus	Poaceae	Grass			x			x	5
Ventilago viminalis	Rhamnaceae	Shrub	x			x			5
Xerochrysum bracteatum	Asteraceae	Herb						x	5

Chapter 3. Effect of temperature and light on seed germination of ten *Eucalyptus* species

Abstract

The germination responses to seasonal temperatures and light/darkness of 14 seedlots of ten *Eucalyptus* species important for revegetation of native communities in north-western New South Wales were examined. We investigated the influence of three alternating day/night temperatures (15/5, 25/15 and 35/25°C) representing winter, spring and summer conditions, respectively, and of two light treatments (light/dark or dark) on the germination of *E. albens* Benth, *E. blakelyi* Maiden*, E. chloroclada* L.A.S.Johnson & K.D.Hill*, E. dealbata* A. Cunn. ex Schauer*, E. camaldulensis* Dhehn*, E. melanophloia* F. Muell, *E. melliodora* A. Cunn. ex Schauer*, E. pilligaensis* Maiden, *E. populnea* F. Muell and *E. sideroxylon* A. Cunn. ex Woolls in growth cabinets. Overall germination was high, germination patterns varied significantly between species and seedlots within species, but these differences were small. In

general, the presence of light and either winter or spring temperatures resulted in higher average germination (96%) than darkness and summer temperatures (93%). Seedlots of *E. chloroclada*, *E. blakelyi, E. sideroxylon, E. melliodora* and *E. melanophloia* germinated consistently well under all treatments, whereas germination in seedlots of *E. albens, E. dealbata*, *E. melliodora*, *E. pilligaensis* and *E. populnea* was affected by the treatments imposed. Germination of small seeds was higher in the presence of light while larger seeds germinated better under continuous darkness. Time to germination was about threefold faster in response to summer and spring temperatures than winter temperatures.

Introduction

Eucalypts are the dominant trees across a third of the Australian continent (Australian Government 2007; Hill 2009). They are adapted to a wide range of environments from humid to arid and are the dominant species in many Australian ecosystems (Slee *et al.* 2006). Previous research on *Eucalyptus* species indicates that some species have a specific set of temperature, moisture and light requirements for emergence and establishment (Bell 1994; Bell *et al.* 1999; Boland *et al.* 1980; Dorrough and Moxham 2005; Merritt *et al.* 2007; Orscheg *et al.* 2011; Schütz *et al.* 2002; Yates *et al.* 1996). However, there are many *Eucalyptus* species for which there is little or no information about the effect of these factors on their germination. Furthermore, environmental requirements for germination vary among species, populations and seedlots within species (Cochrane and Probert 2006; Salazar *et al.* 2011).

Temperature and light are amongst the dominant environmental factors regulating seed germination and seedling establishment (Baskin and Baskin 2014; Hoyle *et al.* 2013).

In most species, maximum germination and seedling establishment occur across a range of temperatures (the ‘germination temperature niche’), outside which both the germination rate and the number of germinants decline (Azam *et al.* 2012). The germination niche is the environmental range optimal for germination. Theoretically, species with wider germination temperature ranges should be able to exploit a broader spatio-temporal array of opportunities (Fried *et al.* 2010). The range of temperatures in which germination occurs is an expression of niche width and is an important consideration for plant propagation from seed (Thompson and Ceriani 2003).

Light can control germination as well as affecting seedling survival, growth and fitness in subsequent stages of the life cycle of plants (Smith 2000). Light is a requirement for germination in positively photoblastic species, and it affects the persistence of seeds in the soil (Saatkamp *et al.* 2011). Positively photoblastic seeds fail to germinate when seeds are maintained in dark (Fenner and Thompson 2005). Negatively photoblastic seeds, on the other hand, only germinate in the absence of light (Maze and Whalley 1992). Germination can also depend on the interaction between light and temperature, as seeds may require light to germinate at one temperature but not at another (Baskin and Baskin 2014; Koutsovoulou *et al.* 2013; Li *et al.* 2003).

Seeds of various sizes germinate differentially in response to environmental conditions such as light (Leishman *et al.* 2000). In general, small-seeded species are light-demanding while the large seeds of forest tree species are shade-tolerant (Milberg *et al.* 2000; Poorter and Rose 2004). Another hypothesis, however, proposes that selection should favour germination in response to light regardless of seed size (Fenner 2000; Koutsovoulou *et al.* 2013). Research on a wide range of species shows that small-seeded species often have a light requirement, germinating only at or close to the soil surface where emergent seedlings are able to persist (Saatkamp *et al.* 2011; Xia *et al.* 2015). Large seeds can successfully germinate from deeper in the soil and can remain there longer than small seeds due their greater mass of seed reserve (Kitajima 2002).

Plant species and populations vary in the rate and percentage of germination (Baskin and Baskin 2014), and this variability can be measured using attributes such as the time to commencement of germination, germination rate and the uniformity in timing or synchrony of germination (Soltani *et al.* 2015). Because seeds in nature respond to numerous interacting environmental factors, it is useful to examine two or more factors simultaneously. The effect of the interaction between light and temperature on germination has been documented in a range of native Australian species (Bell *et al.* 1995; Clarke *et al.* 2000; Schütz *et al.* 2002; Willis and Groves 1991), but information is lacking on most species and on the specific traits that are useful in providing clues as to how a given species interacts with particular environmental stimuli (Pywell *et al.* 2003). Measuring seed responses under controlled conditions that approximate the breadth of the germination niche should allow predictions of plant behaviour under a range of conditions including those that seeds will encounter under future climate change (Bykova *et al.* 2012; Thuiller *et al.* 2008). Such information is necessary to inform seed management practices, plant propagation strategies and broad-acre revegetation.

The aim of this study was to determine the most favourable conditions for germination of multiple *Eucalyptus* seedlots to inform strategies for their propagation in restoration projects in north-western NSW. The objective of the study was to identify the optimum combination of temperature and light conditions to maximise the percentage and rate of germination of seeds of 14 *Eucalyptus* seedlots belonging to ten species, and investigate the relationship between these factors and seed size.

Methods

Seeds of 14 seedlots of ten species of *Eucalyptus* were collected during the summer of 2009–2010 in native vegetation communities in north-western New South Wales, Australia, where *Eucalyptus albens*, *E. blakelyi, E. camaldulensis, E. chloroclada*, *E. dealbata*, *E. melliodora, E. melanophloia, E. pilligaensis*, *E. populnea* and *E. sideroxylon* are common. Seven of these ten species were represented by a single seedlot, *E. blakelyi* and *E. melliodora* each had two seedlots (SL1 and 2) and *E. populnea* had three seedlots (SL1, 2 and 3). Seeds for each seedlot were collected from different populations of at least ten trees during summer (Table 3.1). Clean seeds were stored in dark air-tight containers inside maintained at 3°C until their use in this experiment in January 2015.

Table 3.1. Location, date of collection and habitat of 14 seedlots (SL) of ten eucalypts in north-western New South Wales, Australia (Harden 1990).

Species	SL	Latitude	Longitude	Date	Habitat
E. albens		-30.0929	150.2822	Feb-10	Grassy or sclerophyll woodlands, fertile, well-drained soils
E. blakelyi	1	-30.2213	151.0311	Dec-09	Grassy woodlands, moderately fertile soil
	2	-30.0627	150.0865	Feb-10	
E. camaldulensis		-29.4853	150.0791	Dec-09	Grassy woodland or forest adjacent to permanent water on deep rich alluvial soils
E. chloroclada		-29.6688	150.6059	Dec-09	Dry sclerophyll woodlands on deep sand
E. dealbata		-31.1757	149.063	Jan-10	Grassy woodland on skeletal soils usually on basic rocks
E. melanophloia		-29.6367	150.5425	Dec-09	Grassy or sclerophyll woodland, light soils
E. melliodora	1	-29.3821	150.4748	Feb-10	Grassy woodland, moderately fertile, sandy or alluvial soils
	2	-29.5304	150.5609	Dec-09	
E. pilligaensis		-30.3474	149.8115	Dec-09	Sclerophyll woodland, sandy or light loamy soils
E. populnea	1	-29.3009	149.5553	Jan-10	Grassy woodland, moderately fertile loamy soils
	2	-29.8984	149.748	Feb-10	
	3	-29.3658	149.7672	Feb-10	
E. sideroxylon		-30.137	151.0379	Nov-09	Sclerophyll woodland on lighter, poorer soils

Experimental design

Seeds were placed in 9-cm glass Petri dishes on top of filter paper Whatman No. 1, overlying a sponge (to keep seeds moist for the duration of the experiment) with 10 mL of tap water added at the start of the experiment. Four replicates per temperature treatment, consisting of 100 seeds per Petri dish, were randomly placed throughout three temperature-controlled growth cabinets at three day/night alternating temperatures (15/5°C, 25/15°C and 35/25°C) based on the mean seasonal temperatures likely to occur in the seed collection region during winter, spring and summer, respectively (BOM 2016). Seeds were incubated either in constant darkness (dark treatment) or under a 12-h light/12-h dark daily cycle (light/dark treatment) in each growth chamber. The white light from the fluorescent bulbs (36 W) in the growth chambers had an intensity of ≈50 µmol m^{-2} s^{-1}. Dishes in the dark regime were wrapped in aluminium foil to eliminate light. Cumulative germination of seeds exposed to the alternating cycle of light and dark was recorded every day for a maximum of 30 days, germination being defined as the emergence of a healthy radicle (AOSA 2014). Only one seedlot of each of nine species was incubated under light/dark conditions due to lack of seed. Total germination of seeds in the dark treatments was scored only once at the end of the incubation period (30 days) to avoid light exposure. Immediately after the experiment, non-germinated seeds were cut or squashed to confirm endosperm presence and estimate viability (Mortlock 1999). Viability was always ≥95% so no adjustments were made. Eight samples of 100 seeds per seedlot were weighed and the average mass recorded per species. There was no significant difference in seed mass between seedlots of the same species.

Statistical analysis

In order to compare total emergence among treatments, we analysed the cumulative number of germinated seeds expressed as a proportion of the total number of seeds sown in each dish (100) using generalised linear models (GLM). The seedlot, temperature and light treatments were treated as fixed effects. Not all seedlots could be included in all combinations of treatments due to limited availability of seeds of some seedlots. Data were analysed in three models: (1) all three seasonal temperature regimes with eight seedlots from five species; (2) spring and summer temperatures for 13 seedlots of nine species, and (3) only the spring temperature regime with all 14 seedlots of the ten species. A logit function was used with a quasibinomial error structure for the three analyses to account for over-dispersion in the data (O'Hara and

Kotze 2010). All analyses were carried out using R 3.3.1 (R Core Team 2016). A full model was fitted initially in each case, from which non-significant terms were dropped, producing the following generalised linear models:

$$Y_{ijkl} = \mu + S_i\,(T_j + L_k) + \varepsilon_{ijkl},\ (1)$$

$$Y_{ijl} = \mu + (S_i * T_j\ ) + \varepsilon_{ijl},\ (2)$$

$$Y_{ikl} = \mu + S_i + \varepsilon_{il},\ (3)$$

where Y_{ijkl} was the percentage of germinated seeds, μ the overall mean, $S_i\,(T_j + L_k)$ was the interaction between the *i*th seedlot, S, with the sum of the *j*th temperature, T, and *k*th light condition, L, and ε_{ijkl} was the error term. In model 2, $(S_i * T_j)$, was the interaction between the *i*th seedlot, S, and the *j*th temperature, T. An additional model was fitted substituting the mean seed weight for each species. We used a least significant difference test (LSD) to identify groups of similar mean germination in relation to seedlot, temperature and light treatments. Individual analyses of each species were used to compare total germination in relation to temperature and/or light.

Results

Seed germination of the 14 *Eucalyptus* seedlots was generally high and averaged 95% with maximum and minimum values of 100% and 74% per dish, respectively. *Eucalyptus chloroclada* had the highest germination and *E. populnea* SL1 the lowest (Figs 3.1–3.3; Table 3.5). Germination was not inhibited completely in any seedlot under the conditions used in this study, indicating that the temperature threshold for germination of these species was below 5/15°C and above 25/35°C.

Three different seasonal temperatures and light vs dark

Eight seedlots of five species were tested in relation to all three temperature regimes and the two light conditions (Table 3.2). The most parsimonious generalised linear model of seed germination contained two significant two-way interactions: seedlot × temperature and seedlot × light (*P* < 0.01 and *P* < 0.05, respectively).

Table 3.2. Analysis of deviance results for seed germination of eight seedlots of five species of *Eucalyptus* in relation to season (temperature) and light (light/darkness) treatments.

Source of variation	df	Deviance	Residual deviance	*F*	*P*	
Null			514			
Seedlot (SL)	7	133	381	9.5	3.6E-08	***
Temperature (Temp)	2	61	319	15.2	3.3E-06	***
Light	1	11	308	5.6	0.021	*
SL * Temp	14	136	172	4.8	4.4E-06	***
SL * Light	7	33	139	2.3	0.034	*

df: degrees of freedom; **P* ≤ 0.05, ****P* ≤ 0.001

Average germination percentage of all 14 seedlots of the ten species was higher under spring and winter temperatures (97% and 96%, respectively) than summer temperatures (93%). Considering the seedlot × temperature interaction, *E. chloroclada* (98–99%) and *E. sideroxylon* (96–98%) had uniformly high germination percentages regardless of temperature (Fig. 3.1a). Four seedlots, *E. blakelyi* SL2, *E. populnea* SL2 and SL3 and *E. albens*, had similarly high germination percentages in winter and spring (97–98%) but significantly reduced germination in response to summer temperatures (89–94%). *Eucalyptus populnea* SL1 had a similar seasonal pattern to these four seedlots but its germination percentages were significantly lower (81–94%). *Eucalyptus blakelyi* SL1 showed the reverse seasonal pattern with reduced germination under winter temperatures (85%) but high germination in spring and summer (97–98%). The two seedlots of *E. blakelyi* exhibited opposite seasonal germination patterns: SL1 had a significantly lower response to winter temperatures

(85%) than to summer (98%) or spring (97%) temperatures, whereas SL2 had least germination in summer. All three seedlots of *E. populnea* exhibited a similar seasonal germination pattern with greatest germination in winter and spring, and least in summer. However, *E. populnea* SL1 performed poorly with significantly lower germination in spring and summer than other seedlots (Fig. 3.1a). In the seedlot × light interaction, all seedlots exhibited the general pattern of higher germination in the light than in the dark by 1–7% except for *E. albens*, which germinated better in the dark (96%) than the light (91%). However, there were few significant differences (Fig. 3.1b). All seedlots exhibited similarly high germination percentages (94–99%) except for *E. blakelyi* and *E. populnea* SL1 in the dark (92% and 87%, respectively) and *E. albens* in the light (91%).

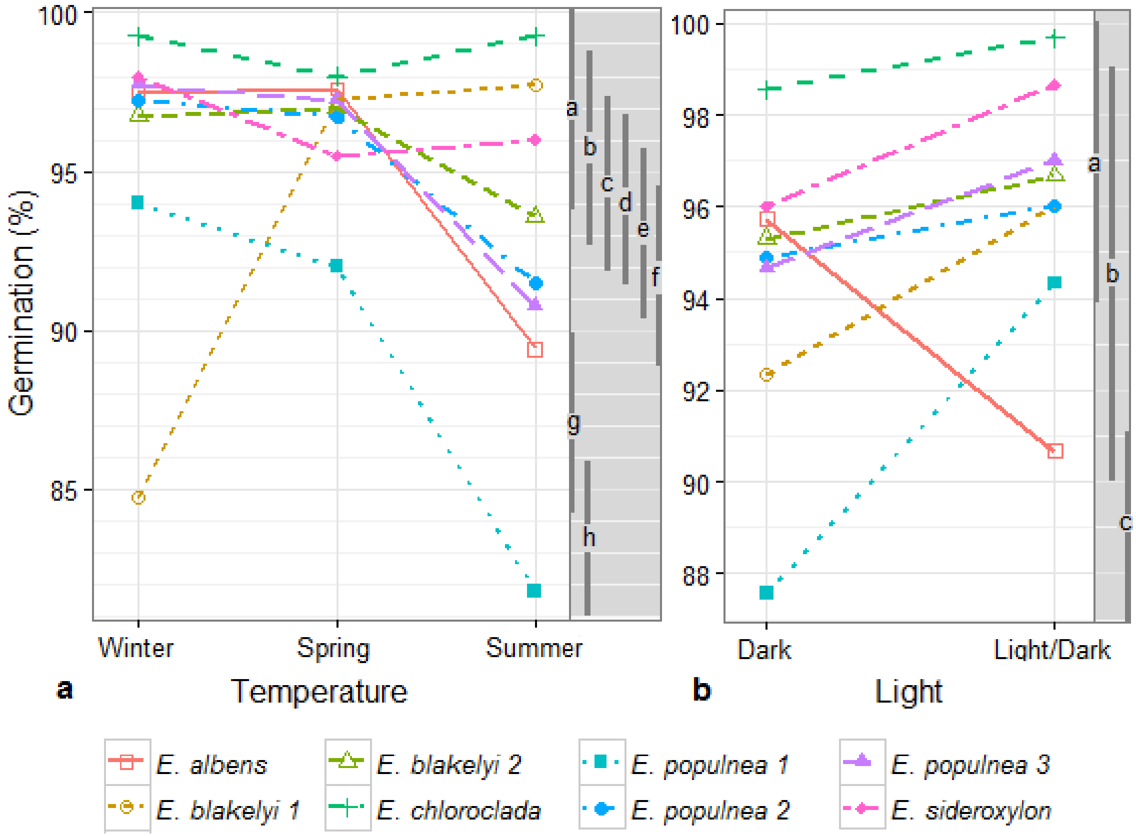


Fig. 3.1. Mean seed germination (%) of the eight seedlots of five species of *Eucalyptus* under (a) three seasonal temperature regimes (15/5°C, 25/15°C and 35/25°C) representing winter, spring and summer, respectively, and (b) two light conditions. Letters with lines on the right hand side of each graph indicate significantly different groups of means (LSD, $P \leq 0.05$).

Two seasonal temperatures and light vs dark

In the analysis of the germination of 13 seedlots of nine species comparing two seasonal temperatures (spring and summer) and light/dark vs dark conditions, the seedlot × temperature interaction was significant but the effect of light was not (Table 3.3, Fig. 3.2). Seedlots exhibited high germination in response to spring temperatures except for *E. melliodora* SL1 and *E. populnea* SL1, which had significantly lower germination (92–94%) than the seedlot with the highest germination in spring (*E. melanophloia,* 99%; Fig. 3.2).

Table 3.3. Summary of analysis of deviance of seed germination of 13 seedlots of nine species of *Eucalyptus* in relation to the effects of two seasonal temperatures (spring and summer) and light vs dark (not significant, and so removed from analysis).

Source of variation	df	Deviance	Residual deviance	*F*	*P*	
Null			516			
Seedlot	12	59	457	26.7	2.2E-09	***
Temperature	1	203	254	7.7	1.6E-06	***
SL * Temp	12	54	200	2.0	0.031	*

df: degrees of freedom; **P* ≤ 0.05, ****P* ≤ 0.001

Germination was more variable in response to summer temperatures. Four seedlots (*E. populnea* SL1 and SL3, *E. albens*, *E. pilligaensis* and *E. populnea*) had significantly lower germination in summer (82–91%) than in spring (92–97%), respectively. Only two seedlots (*E. melliodora* SL1 and *E. populnea* SL1) performed significantly worse in both spring and summer (88–93% and 82–92%, respectively) than the best performing seedlots (*E. chloroclada* in summer and *E. melanophloia* in spring, both 99%, Fig. 3.2). Four seedlots had higher germination percentages in response to summer temperature than spring by 1–3%, although not significantly so (*E. chloroclada*, *E. dealbata*, *E. blakelyi* SL1 and *E. sideroxylon*). Five seedlots had lower germination in summer than spring by 1–5%, but not significantly so (*E. melanophloia*, *E. melliodora* SL1 and SL2 and *E. blakelyi* SL2).

Spring temperature and light vs dark

In the analysis of all 14 seedlots of ten species in response to spring temperatures only, seedlot had a significant impact on germination but light did not (Table 3.4), that is both light and dark treatments averaged similar germination percentages (96 and 95%, respectively).

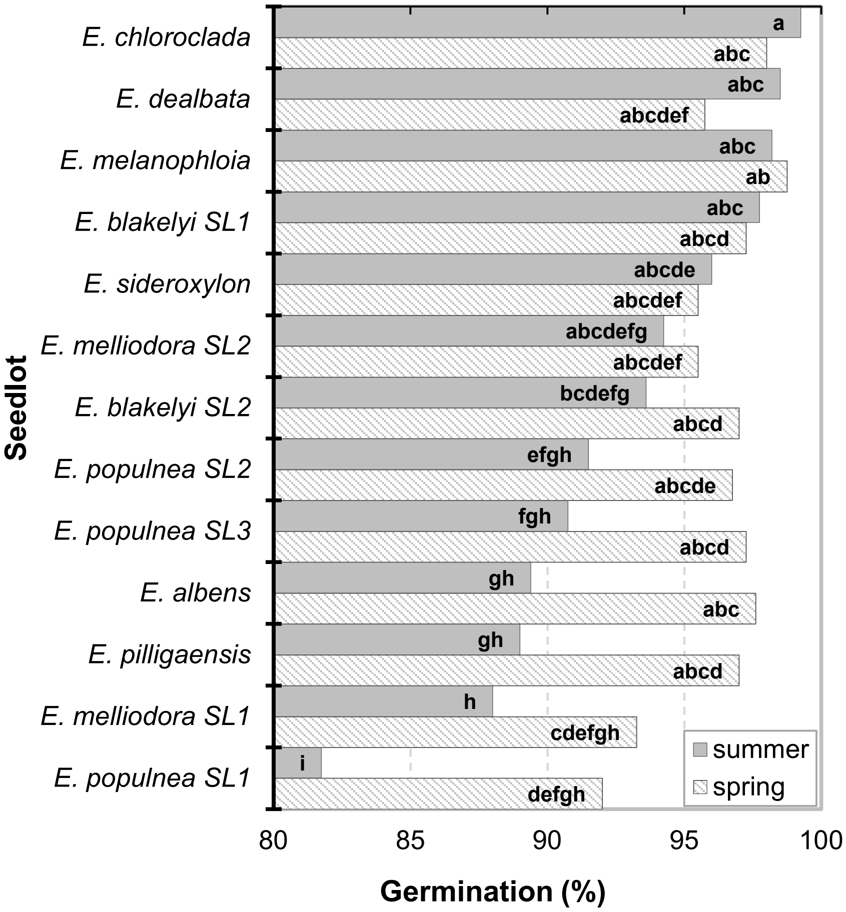


Fig. 3.2 Mean seed germination (%) of 13 seedlots of nine species of *Eucalyptus* in response to two seasonal temperature regimes (25/15°C spring and 35/25°C summer, respectively). Letters indicate significantly different groups of means (LSD, $P \leq 0.05$).

Table 3.4. Summary of analysis of deviance of seed germination of 14 seedlots of ten species of *Eucalyptus*. The effect of light vs dark was not significant and was dropped from the model.

Source of variation	df	Deviance	Residual deviance	F	*P*	
Null			159.7			
Seedlot (SL)	13	91.7	68.0	5.4	1.3E-05	***

df: degrees of freedom; ***$P \leq 0.001$

When each seedlot was analysed individually (Table 3.5), differences in germination between light and dark treatments were significantly different for *E. populnea* SL1 ($P \leq 0.01$) with higher germination in the light treatment (Fig. 3.1). *Eucalyptus choloroclada* and *E. melanophloia* had the highest average germination percentage (99%), and *E. camaldulensis* the lowest (89%). Analysis of the germination of each individual seedlot yielded similar results to the three combined analyses, with temperature having a greater influence on germination than light (Table 3.5). The interaction between temperature (all three seasons) and light was not significant in any of the analyses of individual seedlots and was consequently dropped from the models.

Table 3.5. Species average weight per 100 seeds and summaries of two analyses of deviance per seedlot in relation to three temperatures (Temp) and two light conditions, and two seasonal temperatures.

Species	Weight (mg)	Three seasons (Spring, Summer Winter)		Two seasons (Spring, Summer)
		Temp	Light	Temp
E. albens	1317	0.05 *	ns	0.04 *
E. blakelyi SL1	202	1.6E-04 ***	ns	ns
E. blakelyi SL2		ns	ns	ns
E. camaldulensis	511	–	–	–
E. chloroclada	230	ns	ns	ns
E. dealbata	345	–	–	ns
E. melanophloia	854	–	–	ns
E. melliodora SL1	358	–	–	0.02 *
E. melliodora SL2		–	–	ns
E. pilligaensis	209	–	–	0.04 *
E. populnea SL1	120	4.7E-04 ***	2.2E-03 **	4.9E-03 **
E. populnea SL2		4.0E-03 **	ns	0.06 .
E. populnea SL3		0.04 *	ns	0.06 .
E. sideroxylon	476	ns	ns	ns

**P* ≤ 0.05, ***P* ≤ 0.01, ****P* ≤ 0.001; ns = not significant, '–' = not tested; weight = species mean value in milligrams (mg) per 100 seeds.

Species seed-size effect

When mean seed weight was substituted for seedlot in the three-season analysis, a significant seed-weight × light interaction was obtained (Table 3.6). Although germination was generally high in both the light and dark treatments (96% and 94%, respectively), Seed weight had a weak positive effect on germination in total darkness, but a strong negative effect in the light (Fig. 3.3). In other words, larger seeds germinated better than smaller seeds in darkness although not significantly so, while small seeds germinated significantly better than large seeds in the light. Intermediate-sized seeds germinated to the same degree in the light or dark (Fig. 3.3).

Table 3.6. Summary of analysis of deviance of seed germination after substituting each seedlot by their mean species seed weight in relation to the effect of light. The interaction of seed weight with temperature was not significant and was dropped from the model.

Source of variation	df	Deviance	Residual deviance	*F*	*P*	
Null			513.8			
Seed weight	1	1.1	512.6	0.2	0.628	
Light	1	11.2	501.5	2.3	0.133	
Temperature	2	58.1	443.3	6.0	0.004	**
Seed weight * Light	1	25.7	417.7	5.3	0.024	*

df: degrees of freedom; ***P* ≤ 0.01, **P* ≤ 0.05

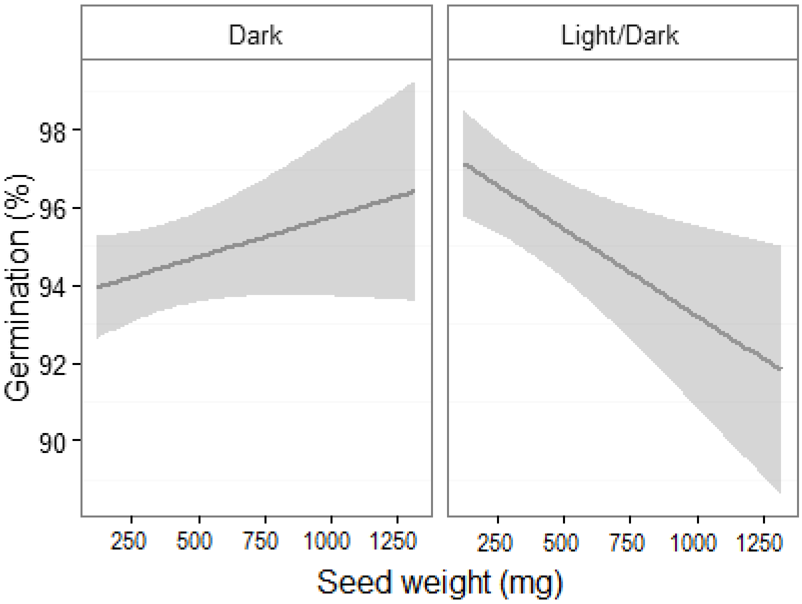


Fig. 3.3 Predicted germination percentage of *Eucalyptus* species of different seed size in relation to light conditions. The shading indicates the 95% confidence intervals.

Timing of germination

Germination in the warmer temperatures (spring and summer) began early, around day 2–4, and had ceased by day 10 in all species in the light/dark treatment. Under winter temperatures, the commencement of germination was delayed until day 12–20 and continued up to day 30 (Fig. 3.4).

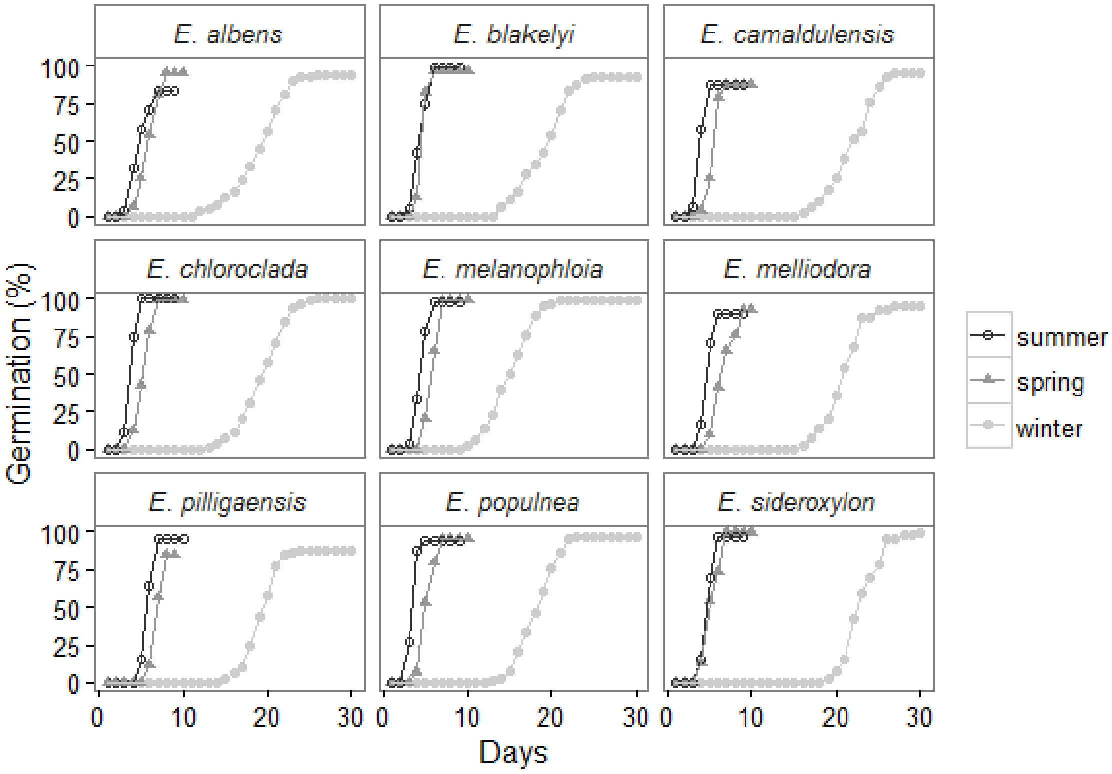


Fig. 3.4 Average cumulative germination (%) through time of nine species of *Eucalyptus* in relation to three seasonal temperatures in the light/dark treatment.

Discussion

Seeds of the species examined in this study do not require specific pre-treatments, but they do require an appropriate microenvironment for optimal germination (Stoneman 1994). Differences in germination response to temperature and light conditions among the 14 seedlots were evident in each experiment. In general, our temperature results showed that winter or spring is better for obtaining high germination (96% in both seasons) than summer (93%), or that all three temperature regimes are equally good. Significant differences in germination between temperature and light treatments per were small for any given seedlot (ranging from 1–13%, average ≈6%), and factors such as the timing of germination may be of greater consequence for successful germination and establishment.

Stoneman (1994) reported that temperatures for maximum germination of *Eucalyptus* species range between 20°C and 30°C (fixed temperatures), but that alternating temperature regimes are most favourable. Our results agree with others who report that canopy species tend to germinate over a wide range of temperatures but with declines in germination at sub-optimal and supra-optimal temperatures (Cochrane *et al.* 2014; López *et al.* 2000). Such variation in germination response is usual between and within species and even from tree to tree, and there is no overall consensus about the optimum conditions for germination of *Eucalyptus* species (Battaglia 1997; Burrows 2000; Clifford 1953). The different germination responses of seedlots of the same species could be due to factors such as time of seed collection and site or seed storage conditions (Bell *et al.* 1995) as well as to adaptation of provenances to local environmental conditions (Grose and Zimmer 1958; Li *et al.* 2003). Information about the optimal conditions for germination for *E. albens, E. chloroclada, E. dealbata, E. pilligaensis E. populnea* and *E. sideroxylon* in relation to light and temperature is unavailable or ambiguous and based on responses to constant temperatures and continuous light rather than alternating conditions more typical in nature (Boland *et al.* 1980; Clifford 1953). Our results for the temperature and light requirements of *E. blakelyi* coincide with those of Li *et al.* (2003), with a spring daily temperature regime (15/25°C) and the presence of light optimal for germination. These results differ from the constant 30°C reported to be optimal by Boland *et al.* (1980). For *E. camaldulensis,* Grose and Zimmer (1958) reported that a constant temperature of 35°C in the light is optimal for germination, but that similarly high results can be obtained under varying light conditions depending on the temperature. Boland *et al.* (1980) reported that temperatures between 15°C and 30°C are optimal for *E. camaldulensis*. We obtained modest germination (89%) for *E. camaldulensis* under spring temperatures (25/15°C). In our study, *E. melliodora* germinated better under spring temperatures (25/15°C), similar to Burrows (2000), who reported that a constant temperature of 25°C in darkness was optimal for germination of this species. Our results suggest that either winter or spring is optimal for the germination of *E. albens*, with summer sub-optimal. Semple and Koen (2005) found that *E. albens* does not germinate in winter, but their study was conducted under field conditions and the temperatures recorded were between −1 and −5ºC. Their results suggest that they may have reached the temperature threshold for the germination of this species. In relation to light requirements for germination, Clifford (1953) commented that *E. melliodora* and *E. sideroxylon* require darkness and *E. camaldulensis* requires light, although our results show greater variability in the range of light conditions required for high germination.

The seedlots tested in this study can be examined according to their response and germination niche within the habitat specialist–generalist concept. This concept posits that specialist species should succeed in homogeneous environments whereas generalists should succeed across a broader gradient of environmental conditions (Devictor *et al.* 2008). We infer that *E. chloroclada*, *E. blakelyi* SL 2, *E. sideroxylon, E. melliodora* SL2 and *E. melanophloia* are germination generalists because they germinated consistently well under all treatments, while the remaining species are specialists, because their germination was affected by particular treatments. Seeds that germinate under a broad range of environmental conditions or have a wide germination niche have more chances for propagation, establishment and survival in restoration projects (Cochrane *et al.* 2011; Luna and Moreno 2010). Seedlots sensitive to environmental conditions can be further subdivided by their specific type of response: (a) seedlots whose germination declined in response to particular temperatures (*E. albens, E. dealbata*, *E. melliodora* SL1, *E. pilligaensis* and *E. populnea* SL2 and 3), or (b) seedlots whose germination was affected by both temperature and light, viz. *E. populnea* SL1 (Fig. 3.1, Table 3.5). Note, however, that the latter seedlot had the lowest germination overall due to poor seed quality. Germination under only the most favourable environmental conditions may protect seedling from sudden death following germination and allow emergence only under suitable conditions for survival and establishment (Benvenuti *et al.* 2001). However, provenances with narrow requirements are at higher risk from seasonal changes and are less likely to succeed under altered conditions associated with climate change (Aitken *et al.* 2008).

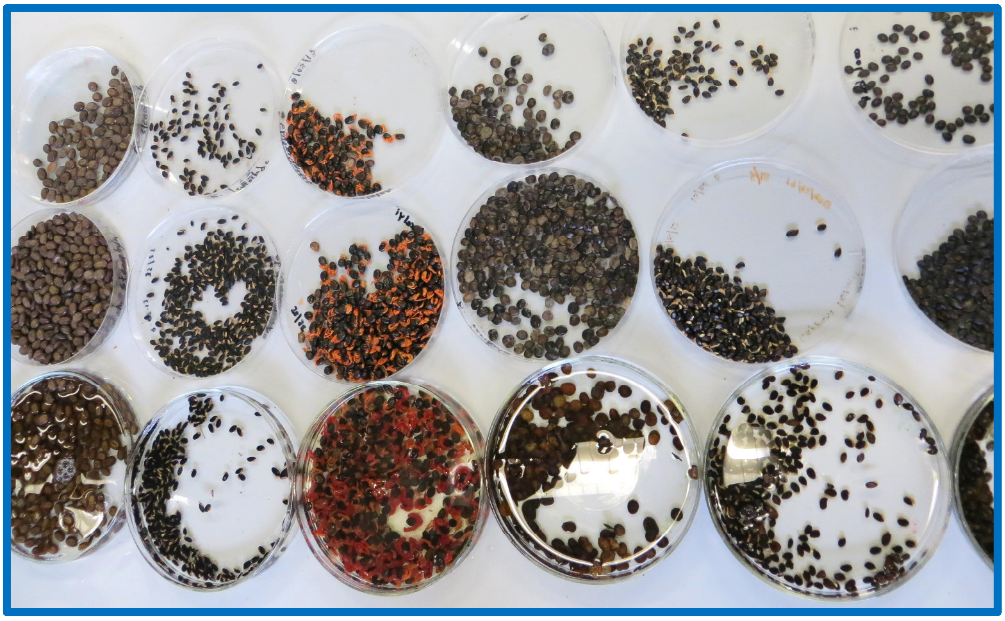
Germination of small seeds was higher in the presence of light while large seeds germinated better in continuous darkness. This may be because continuous darkness relates to shading or burial in the soil in nature. Small seeds are reliant on light as it indicates their position in or near the soil surface, where it is safe to germinate for small seeds but not necessarily for large seeds (Milberg *et al.* 2000; Pons and Fenner 2000). Our results agree with this hypothesis, as only the species with the largest seeds (*E. albens*) had higher germination in darkness although not significantly greater than in the light, while the smallest seeds (*E. populnea*) had least germination in continuous darkness (Fig. 3.1b).

The chances of seed survival in nature rely upon rapid germination in the face of risks such as predation, desiccation or being transported to sites unsuitable for germination and seedling survival (Bochet 2015). Small-seeded *Eucalyptus* species are expected to germinate at a high rate once environmental conditions are suitable (Swanborough and Westoby 1996). We found that spring and summer temperatures were more favourable for rapid germination as germination commenced in about one-third of the time taken in winter. These results agree with previous research that recorded more rapid germination at higher temperatures up to the limit at which high temperatures are fatal to seed (Grose and Zimmer 1958; Li *et al.* 2003). However, in nature, *Eucalyptus* germination does not commonly occur under summer temperatures at the latitudes of the species of interest for this study (Bell 1999). Thus, it can be inferred that spring temperatures are most favourable for germination for the seedlots tested in this experiment.

Conclusion

Although we did not find a single optimum temperature or light treatment for germination, relatively high germination percentages were obtained with multiple *Eucalyptus* species and seedlots across a range of temperatures and light conditions. The conditions provided in these experiments were close to optimum so that overall germination percentage was high. However, not all seedlots had a similarly wide range of environmental conditions for maximum germination, with some treatments resulting in small but significant reductions in germination. Nevertheless, none of the seedlots tested reached the threshold at which germination did not occur. Information about the temperature and light requirements for maximum germination, as well as the relationship between seed mass and rate of germination may be useful to (a) avoid unsuitable environmental conditions for direct seeding, and to (b) understand the contribution of these factors in explaining germination results in the field.

Chapter 4. Effective methods to overcome physical dormancy and enhance germination of *Acacia* seeds for direct seeding



Abstract

Acacia species (Fabaceae) have an important role in ecosystem regeneration due to their ability to enrich the soil through nitrogen fixation. However, the seeds of many species have a physical dormancy that must be broken prior to their use in direct seeding for revegetation. We measured the effects of mechanical scarification (two intensities) and hot water seed pre-treatments on the percentage germination and time to germinate of ten *Acacia* species from north-western New South Wales (*A. deanei* M. B. Welch, Coombs & Mc Glynn, *A. decora* Rchb*, A. excelsa* Benth*, A. leucoclada* Tindale*, A. ligulata* A. Cunn. ex Benth.*, A. melanoxylon* R. Br.*, A. oswaldii* F. Muell, *A. pendula* A. Cunn. ex G. Don*, A. salicina* Lindl. and *A. stenophylla* A. Cunn. ex Benth) under two temperature and light-controlled conditions (35/25°C and 25/15°C in alternating 12-h light and darkness) in germination cabinets. The three dormancy-breaking treatments significantly increased the germination percentage and/or decreased the time to germination in all but one species (*A. melanoxylon*) compared to the untreated controls. However, the results varied with species. Temperature had an effect on only one species (*A. decora*). Germination was greater and/or more rapid after mechanical scarification than after the hot-water treatment. Mechanical scarification provided a practical method for improving germination in nine species.

Introduction

The continuing decline of native vegetation in Australian agricultural landscapes across southern and inland eastern Australia is of concern to land managers and scientists alike, with revegetation occurring in the most heavily affected landscapes (Ansell *et al.* 2016; Vesk and Mac Nally 2006). In north-western New South Wales (NSW), the Namoi, Gwydir and Macintyre River catchments have been cleared of substantial areas of woodland, grassland and shrubland for dryland and irrigated cropping in the past 60 years (Reid *et al.* 2007). Large-scale revegetation through direct seeding or replanting of seedlings with native species has been proposed in areas with restricted recruitment of native vegetation following a long history of agriculture (Azam *et al.* 2012; Munro *et al.* 2009). Direct seeding, if successful, is more cost-effective than planting seedlings as it requires a lower investment in time and effort over large areas (Gibson-Roy and Delpratt 2014).

Nitrogen-fixing species such as *Acacia* are important in pioneering revegetation programs, conservation management and sustainable agriculture in diverse ecosystems due in part to their ability to restore soil nitrogen levels (Adams and Attiwill 1984; Hosseini Bai *et al.* 2014; Ma *et al.* 2015). The nitrogen fixed by these species interacts with soil organic carbon to enhance soil structural stability; largely determines the species composition and structural diversity of the resultant plant communities, and stimulates, increases and diversifies the soil microflora in degraded ecosystems (Murray *et al.* 2001).

Acacia species have a pantropical distribution, and almost 1000 species are widespread across most habitats in Australia, ranging from coastal to subalpine and from high-rainfall areas to the semi-arid and arid inland regions (ANBG 2015; Le Maitre *et al.* 2011). Furthermore, *Acacia* species have proved to be convenient for direct seeding due to large seed size, low cost of seed collection, tolerance of adverse environments and ease of establishment in diverse ecosystems (Bartle *et al.* 2002; Knight *et al.* 1998). However, most *Acacia* species from arid and semi-arid regions have seeds with physical dormancy produced by a water-impermeable seed coat and the legume lens that prevents water uptake (Baskin *et al.* 2000; Tran and Cavanagh 2013). This lens opens in response to the right environmental signals, allowing dormant seeds to imbibe water and germinate (Baskin 2003). It is hypothesised that, in nature, mechanisms such as fire, abrasion, seed movement across the ground surface, microbiological attack, high or fluctuating soil temperatures and passage through the digestive tracts of animals could have the effect of breaking the physical dormancy of seeds (Tran and Cavanagh 2013).

Artificial methods that have proven successful in relieving seed-coat-imposed dormancy in a wide range of genera are fire, dry or wet heat, temperature fluctuations, precise nicking of the seed coat and mechanical or acid seed scarification (Jayasuriya *et al.* 2009; Teketay 1998; Venier *et al.* 2012). Methods to break dormancy such as microwave energy (Nguyen Tran 1979; Tran 1981) and hot-wire scarification have also been studied (Masamba 1994). Of the widely used methods, mechanical scarification and hot-water treatments are popular and effective in breaking the physical dormancy of large quantities of seeds, and they are logistically manageable in terms of skills, safety, time and costs (Sandif 1988; Teketay 1998). Mechanical scarification in legumes damages random areas of the seed coat, whereas hot water disrupts the structure of the seed coat around the lens, which is the primary site of water entry (Baskin *et al.* 2000; Morrison *et al.* 1998).

Once imbibition occurs, germination becomes responsive to the range of temperatures to which seeds are exposed (Baskin and Baskin 2014). Most legume species of temperate zones germinate better at temperatures coincident with those in spring in their place of origin, as an adaptation to maximise seedling establishment (Funes and Venier 2006; Turner *et al.* 2005), and higher germination occurs at fluctuating rather than constant temperatures (Baskin 2003). Further, some species are known to respond better to a combination of environmental signals such as temperature and moisture rather than a single stimulus (Cochrane and Probert 2006).

The specific requirements for optimum germination of most Australian *Acacia* species sown directly in situ is not well known (Carr *et al.* 2004). However it is likely that many seeds do not germinate because of physical dormancy. Research is needed to obtain a better understanding of the specific germination requirements, including the time taken for seeds of different species to germinate. A better knowledge of these requirements will allow better preparation of seed for direct seeding and propagation. Once a seed has been sown in a suitable environment, a longer period to germinate implies greater exposure to predation, aging, damage, transportation to less favourable conditions or the likelihood of seed death if the seed cannot withstand exposure to variable environmental conditions or extremes (Bochet 2015; Long *et al.* 2015).

The aim of this research was to identify convenient methods to break seed dormancy in ten *Acacia* species drawn from native communities in north-western NSW. The methods chosen were those that: (1) may be applied consistently to bulk seed, at low cost with no safety issues; (2) achieve a rapid, uniform and high germination percentage that matches the seed viability percentage, and (3) can easily be used prior to direct seeding for restoration. Specifically, we investigated the response of seeds of ten species to three dormancy-breaking treatments and two incubation temperature regimes.

Methodology

Seeds of ten native species (*Acacia deanei, A. decora, A. excelsa, A. leucoclada, A. ligulata, A. melanoxylon. A. oswaldii, A. pendula. A. salicina* and *A. stenophylla*) were collected from at least ten trees of each species from natural populations in north-western NSW, Australia (Table 4.1). Seeds of each species were collected in one summer (between December 2013 and January 2014), and dry-stored in air-tight plastic containers in a walk-in cold room at 3°C at UNE, until their use in this experiment in March 2014.

Treatments

To overcome the physical dormancy imposed by the hard seed coats, seeds of all species were subjected to one of the following four treatments: control (no pre-treatment), hot water treatment, one pass through a mechanical scarifier and three passes through the scarifier. For the hot water treatment (HW) we experimented with several combinations of time of exposure and temperature, before deciding to pour 120 mL of boiling water on to seeds in 150-mL glass containers. The seeds were left in the water for 5 minutes to cool to room temperature (~22ºC), after which the water was poured off. The seed scarifier used was a Kimseed® (Kimseed International). This appliance scarifies seeds by using rotating abrasive-coated discs that abrade the hard surface of the seeds. Scarification treatments were defined after trials that revealed that batches of seed with hard seed coats passed three times through the scarifier (Sc3) provided better results compared to those passed a single time (Sc1) through the scarifier (Bush *et al.* Unpublished). For this treatment, the second fastest machine speed was used. Untreated control and treated seed samples were incubated at one of two day/night alternating temperatures 25/15°C or 35/25°C, based on spring seasonal temperatures in the seed collection sites and potential restoration areas (BOM 2016).

Table 4.1. Occurrence of ten species of *Acacia* in north-western NSW, Australia, coordinates of seed collection sites and viability (V) percentage.

Species	Collection site Longitude	 Latitude	V (%)	Occurrence	Potential Utility in restoration
A. deanei	144.5788	−29.5194	54	Sclerophyll communities on plains, slopes and tablelands, near watercourses, in gullies or on stony hillsides	Provides low-level cover in windbreaks, erosion control. Drought and cold tolerant, regenerates on disturbed sites. Browsed by stock but can be toxic if heavily browsed. Valuable habitat for native birds and insects and in apiculture
A. decora	144.5779	−29.5194	50	Woodlands, dry sclerophyll forest, scrub and grassland, rocky hillsides and ridges, plains and lower slopes; in sandy and stony soils	Drought and frost tolerant
A. excelsa	149.7079	−28.7415	78	Savanna or eucalypt woodland, in red sandy loams, or in yellow or brown gravelly, sandy or clay soils	Provides shelter and shade. Tolerant to frost and drought. Used for its stable hard and dark timber
A. leucoclada	144.9733	−29.7339	73	Hilly undulating terrain, sclerophyll communities or along creek banks in a variety of soil types including gravelly, sandy and clay loams	Used in windbreaks and erosion control. Wood used for posts, poles or rails, firewood, charcoal and pulpwood. Provides shelter and shade for stock. Valuable in apiculture and has potential for floriculture. Moderate to fast growing. Frost tolerant
A. ligulata	149.0036	−29.093	68	Widely distributed in arid and semi-arid Australia, floodplains, lower hill slopes and fringing salt lakes, on inland and coastal sand dunes and in woodland	On sand-hill sites acts as an important soil stabiliser and controls erosion, germinates readily in disturbed soils
A. melanoxylon	145.6998	−31.2144	61	Tablelands and coastal escarpments, in an extensive range of soils and climatic conditions	Fast-growing successional species. Important host for a number of native butterfly larvae. Slight to moderate tolerance of salt and frost. Valuable timber
A. oswaldii	149.7868	−29.8802	85	Arid, semi-arid and subtropical mainland Australia in eucalypt or mulga country, mainly in calcareous sands or loam	Can be used as fodder where other edible trees are scarce
A. pendula	149.9129	−29.655	98	Major river floodplains, in alluvial and heavy clay soils	Frost and drought tolerant. Provides good shelter and windbreak. Attracts native birds. Foliage is palatable to livestock and is sometimes used as drought fodder
A. salicina	144.0318	-29.658	93	Along the banks of rivers and creeks, on gentle slopes, alluvial plains and floodplains on a wide range of soils including heavy-textured alluvial clays	Can be drought tolerant, adapted to clay alkaline soils
A. stenophylla	149.8817	−29.7373	86	Extensively distributed in inland arid areas, common in watercourse-fringing vegetation on cracking clays, loams or sand	Shelterbelt and windbreak species. Tolerant of saline, alkaline and waterlogged soils and survives periodic flooding, but with substantial provenance variation

Experimental design

Random samples of seeds of each species were tested for viability prior to the experiment with a cut-test and recorded as viable if the endosperm was yellow-cream, plump and turgid with a healthy-looking embryo. A minimum of three replicates of 50 seeds each were placed in 9-cm glass Petri dishes. Seeds were placed on top of Whitman No. 1 filter paper above a sponge; in order to maintain seed moisture during the experiment, 10 mL of tap water were added at the start of the experiment and whenever required. Dishes were placed randomly in one of two temperature-controlled germination cabinets for a maximum of 7 weeks. The light intensity in the cabinets was about ≈50 µmol m^{-2} s^{-1} with a 12-h light/12-h dark photoperiod. Germination was recorded every second day and defined as the emergence of a visible healthy radicle (AOSA 2014). Germinants were removed from dishes. Immediately after the final record was taken, non-germinated seeds were cut-tested to confirm seed viability, and the number of empty seeds subtracted from the total number of seeds sown to estimate germination percentage.

Statistical analysis

In order to compare total emergence among species and treatments, we analysed the cumulative number of seeds germinated expressed as a proportion of the total number of seeds sown in each replicate, using generalised linear models (GLM). Species, temperature and treatment were modelled as fixed effects. A logit function was used with a quasi-binomial error structure to account for over-dispersion in the data (O'Hara and Kotze 2010). All statistical analyses were carried out using R 3.3.1 (R Core Team 2016). A full model was fitted initially, but the final model chosen after removal of non-significant terms was:

$$Y_{ijk} = \mu + (S^*T)_{ij} + \varepsilon_{ijk},$$

where Y_{ijk} was the percent of germinated seeds, μ was the overall mean, $(S^*T)_{ij}$ the interaction between the *i*th species, S, and the *j*th dormancy breaking treatment, T, and ε_{ijkl} was the error term. Analyses for each species compared total germination in relation to seed treatment and temperature.

Results

Average seed germination of all species in the experiment was 42% with maximum and minimum values of 100% and 0% per replicate respectively. *Acacia oswaldii* had the highest mean germination (68.6%) and *A. melanoxylon* the lowest (20.6%). Seed viability was variable among species, ranging from 50% to 96% for *A. decora* and *A. pendula*, respectively.

The analysis of species, scarification treatment and temperature yielded a significant species × treatment interaction (Table 4.2). The three-way interaction (species × treatment × temperature) was not significant ($P = 0.07$) in the full model, which was fitted initially, and so was removed. Subsequently, temperature was not significant and was also removed.

Table 4.2. Summary of analysis of deviance of the final model of seed germination of ten species of *Acacia* subject to three different pre-treatments and a control (Treatment) and incubated under two different temperature regimes (Temperature).

Source of variation	Df	Deviance	Resid. Dev	F	P	
Null			3933			
Species (Sp)	9	1247	2686	15.6	1.2E-17	***
Treatment	3	852	1835	31.9	6.4E-16	***
Sp x Treatment	27	414	1412	1.8	0.02	*

df: degrees of freedom; *$P \leq 0.05$, ***$P \leq 0.001$

Germination in the control was generally lowest in all species except for *A. oswaldii* (germination 65.9%). Two species did not respond to any treatment: *Acacia melanoxylon*, with low germination (18.6–23.2%), and *A. oswaldii* with high germination, regardless of treatment (65.9–73.2%; Table 4.3, Fig. 4.1). Only the *A. oswaldii* control (65.9%) and to a lesser extent the *A. leucoclada* control (26.9%) achieved germination percentages approaching those obtained with pre-treatments (Fig. 4.1). Germination of eight of the ten species were significantly greater than their respective control with at least one treatment, but responses to specific treatments varied among species. Germination of seeds with three passes of mechanical scarification (Sc3) was on average greater (56.5%) than that of seeds treated with one pass in the scarifier (46.1%) or with heat (39.1%). *Acacia pendula* germination was greater than that of other species in response to mechanical scarification and highest in response to three passes (Sc3, 87.7%) which was also the highest germination recorded in all treatment–species combinations (Fig. 4.1). The mechanical scarification treatments were generally more effective in increasing seed germination than the hot-water treatment in the eight species exhibiting a significant pre-treatment response. *Acacia ligulata* was an exception, exhibiting greater germination in response to hot water and Sc3 than one pass (Sc1).

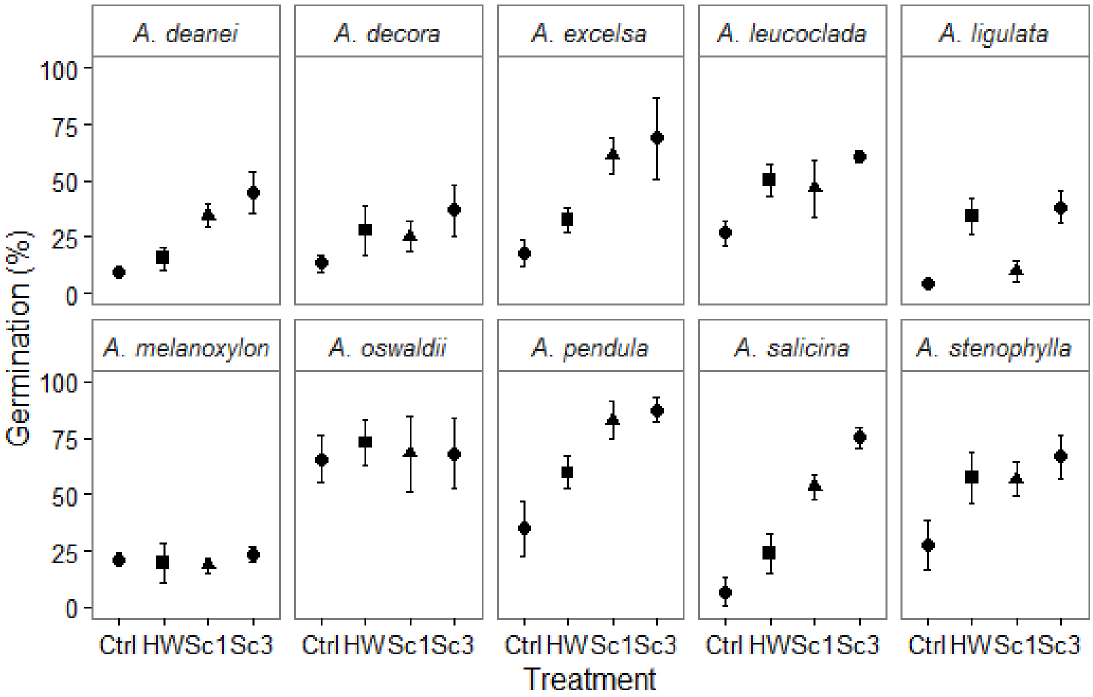


Fig. 4.1 Mean seed germination (%) of ten species of *Acacia* in response to three treatments (HW = hot water, Sc1 = one pass in the scarifier, Sc3 = three passes in the scarifier, Ctrl = control). Error bars are ±1 S.E.

Individual analyses of the effects of treatment and temperature for each species detected germination differences among treatments in eight species, as well as a treatment × temperature interaction in *A. decora* (Fig. 4.2). In all other species, germination did not vary significantly with temperature (Table 4.2). In *A. decora*, the germination in response to Sc3 was significantly greater at the lower (25/15°C) than the higher (35/25°C) temperature but the response to other treatments was similar regardless of temperature (Fig. 4.2). In *A. pendula*, germination in the control treatment was marginally greater at the lower than the higher temperature, but not significantly so.

Table 4.3. Overall percent germination (Germ), and analyses of deviance of each species in relation to three different treatments (Treat) and control at two incubation temperatures (Temp).

Species	Germ	Temperature		Treatment		Treat * Temp	
	(%)	(%)	*P*	(%)	*P*	(%)	*P*
A. deanei	26.0	6.5	0.362	86.5	< 0.001	7.0	0.470
A. decora	25.9	19.1	0.149	40.5	0.003	40.4	0.003
A. excelsa	45.1	16.2	0.293	61.3	0.006	22.5	0.204
A. leucoclada	46.1	3.8	0.675	57.9	0.043	38.2	0.146
A. ligulata	21.7	3.8	0.504	94.1	< 0.001	2.1	0.851
A. melanoxylon	20.6	38.4	0.441	27.8	0.74	33.8	0.680
A. oswaldii	68.6	49.8	0.262	6.8	0.92	43.4	0.360
A. pendula	66.9	8.5	0.335	71.1	< 0.001	20.3	0.085
A. salicina	40.7	8.1	0.171	87.0	< 0.001	4.9	0.332
A. stenophylla	52.3	1.9	0.721	80.7	< 0.001	17.4	0.341

Percentage contribution to total variance (%) in germination percentage given for Temperature, Treatment and their interaction, as well as the statistical probability (*P*).

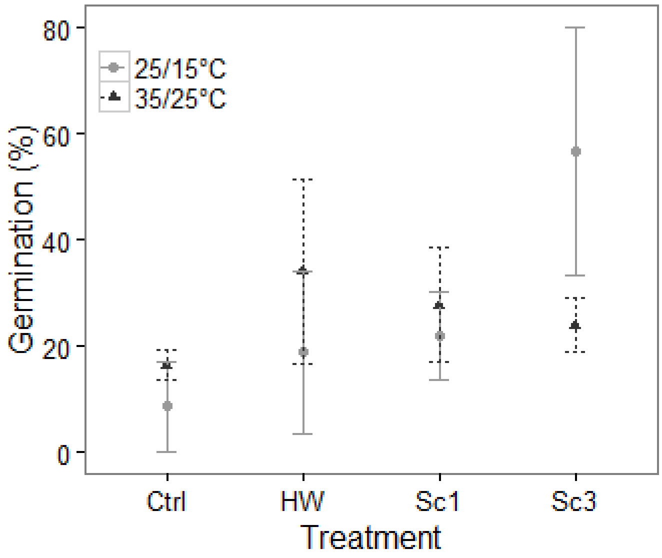


Fig. 4.2 Mean germination (%) in *Acacia decora* in which both treatments (HW = hot water, Sc1 = one pass in the scarifier, Sc3 = three passes in the scarifier and Ctrl = control), and temperature (25/15°C and 35/25°C) had a significant effect. Error bars are ±1 S.E.

Additionally, scarification treatments shortened the time to germination in some species. Germination in all species commenced by the first or second week, with an increase in germination rate between weeks two and three (Fig. 4.3). Most of the germination of treated seeds occurred during the first week, with the most rapid germination occurring in response to scarification treatment Sc3, followed by Sc1. This trend was clearest in *A. oswaldii* and *A. pendula*, and not detected in *A. melanoxylon*. Germination of untreated seeds generally occurred more gradually (Fig. 4.3).

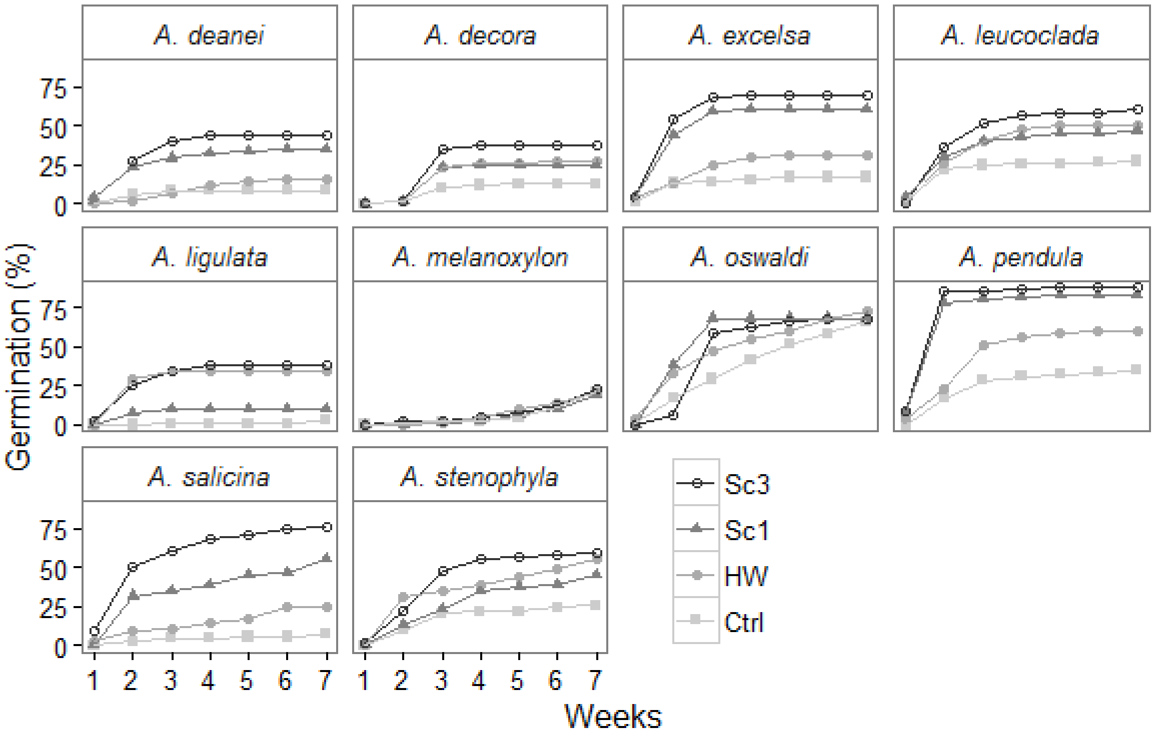


Fig. 4.3 Mean cumulative germination (%) over time of ten species of *Acacia* in response to three different seed treatments and the control.

Discussion

The *Acacia* species in this experiment showed varied responses to dormancy-breaking treatments, and all except *A. oswaldii* exhibited physical dormancy imposed by the seed coat. These results were expected as *Acacia* species are widely distributed with large morphological and physiological differences and with varying sensitivities to seed pre-treatments within and between species, populations and individual plants within populations (Doran and Gunn 1987).

Eight of the ten species in this study had higher germination following mechanical scarification than control, and higher or similar germination to that achieved with the hot-water treatment. Germination percentages in treated seeds were always higher than those of control seeds except for *A. melanoxylon* and *A. oswaldii.* Other studies have similarly reported higher germination after pre-treatment with mechanical scarifiers compared with other conventional methods such as heat and acid scarification (Dittus and Muir 2010; Jones *et al.* 2016). Where germination was greater after hot-water treatment than mechanical scarification, it could be that, aside from breaking dormancy, heat had a further positive effect as has been recorded with other legume species (Clemens *et al.* 1977).

Seeds of *A. oswaldii* were not affected by any treatment. However, the seed of this species had visible seed coat fissures prior to treatment, probably caused during collection, handling and cleaning and due to the comparatively thin seed coat of this species (Pound *et al.* 2015), which in all likelihood is fragile. The thin seed coat could also be responsible for the high germination percentage of untreated seeds of this species. In the case of *A. melanoxylon*, it is likely that the treatments or incubation environment were suboptimal (either harsher or softer than required) or the seed had viability issues not related to seed fullness, such as seed age. This species generally occurs in cooler and wetter climates than the temperatures used in this experiment (Florabank 2010), and incubation at these temperatures could have been responsible for the poor response. Germination of this species was <25% regardless of treatment in our study, but was much higher in other studies : 74% in Neilsen and Brown (1997) and 67–90% in Burrows *et al.* (2009) in response to similar treatments. Germination did not differ significantly between the two incubation temperatures in most species, which implies considerable thermal tolerance (except in *A. decora*). *Acacia* species normally germinate under a wide range of temperatures once physical dormancy is relieved (Teketay 1996).

Empty and incomplete seeds, suggestive of insect damage, were the main causes of low seed viability, particularly in *A. decora, A. deanei* and *A. leucoclada*. Seed quality played an important role in these results. Species with lower viability also had lower germination, even after viability was adjusted by discarding empty seeds from the calculations of germination percentage. Seed infestation by insects is a major problem in propagation of *Acacia* species; the negative impact on seed germination and seedling recruitments is well documented (Auld 1983; Or and Ward 2003), and should be a consideration in any work with seeds of these species.

An advantage of pre-treating seeds of *Acacia* species is that germination occurs more rapidly and uniformly, even when the final cumulative germination of treated seed does not differ significantly from the control, as for instance with *A. oswaldii*. Rapid germination of viable seeds after physically breaking the seed coat was anticipated, as it allows immediate water uptake to initiate germination. This allows revegetation practitioners to take advantage of favourable rainfall conditions before surface soil moisture is lost, a particularly important consideration in areas with low or sporadic rainfall (Knight *et al.* 1998). The seed of some of these *Acacia* species has required more time to germinate in other experiments than recorded here, which may be due to the inadequacy of the treatments for breaking physical dormancy in the other experiments (Pound *et al.*

2015). For successful revegetation under field conditions, a uniformly short time to germination is desirable and plays a crucial role in direct-seeding success by reducing the time that seeds are exposed to potentially lethal factors (Woods and Elliott 2004). Whenever rapid germination is required, the combination of species and treatment that generates the highest germination percentage should be used. Alternatively, if germination is required over a longer period, treatments that produce more gradual germination can be selected.

Conclusion

Although the best method for breaking physical dormancy in *Acacia* seeds varies among species, it is essential to identify a standard treatment, or a limited number of treatments, that enhance germination in order to be able to process large quantities of seed. From the practical perspective of direct seeding, the present experiment indicates that mechanical scarification is the most suitable option for nine out of ten species compared to the hot-water treatment, in terms of mean germination percentage and time to germination. At least some seed of eight of the *Acacia* species in this i study should be triple-scarified before direct seeding, to increase germination and improve the efficiency of seed use. However, more detailed knowledge about individual species' responses to specific scarifier machines, scarification intensity and number of passes would be useful to refine this method prior to embarking on extensive direct seeding.

Chapter 5. Seed germination constraints of 49 plant species native to north-western New South Wales, Australia

Pictures in clockwise order from top left: seed in storage; germinants of *Geijera parviflora*; seed loculi of *Owenia acidula*, and bare seed of *Alectryon oleifolius.*

Abstract

Three attributes—seed viability, germination under three different seasonal temperatures (winter, spring/autumn and summer) and the requirement for seed pre-treatment to promote germination—were assessed for 49 native plant species from north-western New South Wales, most of which are poorly understood with regard to seed ecology. Seedlot viability, optimum season for germination and germination constraints such as seed dormancy were determined in order to assist with the propagation of these species from seed. Percentage seed viability varied widely among the 49 species. It was >75% in 38 of the species, and <50% in seedlots of only seven species, which were discarded prior to conducting further germination tests. Seasonal temperature affected germination success in 27 species, while various seed pre-germination treatments were effective in increasing germination in 22 species. The most frequent seed treatment required was rupturing the seed coat. This resulted in approximately a two-to-three-fold increase in germination percentage. Seed stratification, seed coat removal and seed leaching increased germination in some species. The results will contribute to determining seed pre-treatments for germination prior to direct seeding in both the nursery and field, and contribute to the understanding of the ecology of the germination of a range of native species in the study region.

Introduction

The vegetation of north-western NSW varies from areas of dry rainforest and sclerophyll forest in the east through to woodlands and open woodlands, shrublands and grasslands in the west (Benson *et al.* 2010; Carr and Atkinson 2013). The area is one of Australia's biodiversity 'hotspots' with respect to the number of endemic plant species (Joseph *et al.* 2014). It also encompasses many species and communities that are listed as threatened, and their recovery and conservation are promoted through national and state legislation (OEH 2006). There is a large deficit of tree cover in the region owing to past over-clearing of native vegetation, principally for agriculture (Donaldson 1996; Donaldson and Heath 1997; Peasley 1995). Revegetation by direct-seeding or planting seedlings is required for the re-establishment of native vegetation in the area (McDonald and Williams 2009; Windsor and Clements 2001). Revegetation with direct-seeding requires less investment in time and effort over large areas and results in a more natural dispersion of plants than other methods of plant propagation

(Gibson-Roy and Delpratt 2014). However, direct-seeding is only suitable for certain species because germination constraints are common and vary with species, geography and season (Doust *et al.* 2008; Geeves *et al.* 2008). The factors that influence seed germination and seedling emergence and establishment include the environmental conditions under which seeds are sown and stored, the persistence of seeds in the soil seed bank, and the occurrence of environmental stimuli or conditions that may be required to break seed dormancy and elicit germination, predation and seedling growth rate (Jinks *et al.* 2006; Tunjai and Elliott 2012). Seed attributes such as viability, quality, dormancy, size and other physical or physiological characteristics are also fundamental determinants of germination success (Baskin and Baskin 2014). Difficulties in eliciting germination have been recorded in many Australian species but numerous species have not been to date tested, and the potential causes of germination failure in direct seeding trials have often not been identified (Bell 1999; Clarke *et al.* 2000; Jurado and Westoby 1992; Merritt *et al.* 2007).

Temperature is an important stimulus that enhances or limits seed germination. Germination responses to temperature vary from species to species and are adaptive in part to the seasonal environmental conditions under which plants have evolved (Probert 2000). Usually, the temperature range associated with high germination percentage corresponds to the seasonal temperature just before emergence occurs in the field (Baskin and Baskin 2014). Seed dormancy, however, is widespread and limits the conditions under which germination and seedling establishment can occur (Thompson and Ooi 2010; Vleeshouwers *et al.* 1995). Dormancy inhibits germination of viable seed under environmental factors that would be otherwise favorable for germination (Baskin and Baskin 2004b; Finch-Savage and Leubner-Metzger 2006).

Dormancy has been reported in a wide range of Australian species in many different families (Bell *et al.* 1993; Cochrane and Probert 2006; Jurado and Westoby 1992). Several common methods are used to deal with different dormancy types. Breaching the seed coat through heat or mechanical scarification breaks physical dormancy (Cochrane and Probert 2006). Stratification is another method used to relieve physiological dormancy and promote germination of many species by exposing seeds to summer or winter-like temperatures (Baskin *et al.* 2004). Lastly, low seed viability is common in many Australian species and is important for managing seeds and understanding seed persistence through time, either in the soil or in storage (Sawma and Mohler 2002). Seed longevity, storage conditions and the activity of contaminants such as insects, fungi, and other microorganisms also commonly affect viability (Bewley and Black 1982). The behaviour of stored seeds through time can be recalcitrant, orthodox or intermediate, and varies from species to species (Baskin and Baskin 2014).

The objectives of this research were to (1) identify limitations to the germination of 49 plant species native to north-western NSW; (2) investigate suitable pre-treatments to overcome any germination constraints, and (3) contribute to deciding which species can be used for direct seeding or propagation in revegetation projects.

Methodology

Seed preparation and viability

Seeds of the 49 species (Appendix 5.1) were collected from at least ten plants of a single population of each species, from natural stands in the study region. Seeds were either tested within a few days of collection (seeds with age 0) or dry-stored until testing in air-tight containers at constant 3°C in a walk-in cold room at UNE. Random samples of 30 seeds of each species were tested for viability using indirect tests such as the seed-cutting test, tetrazolium test (ISTA 2003), visual assessment or squash tests depending on seed and embryo size and surrounding structures as indicated in the appendix. Percent viability of each species was ranked in quartiles: high (H, 76–100%), medium (M, 51–75%), low (L, 26–50%) and insufficient viability (I, 1–25%) and unviable (U, 0%). Unless seed viability was ≥ 51%, seedlots were considered suitable for investigating germination constraints. If seed viability was ≥ 51% the seedlot was considered suitable for investigating germination constraints. If seed viability was consistently ≤50%, the seedlot was discarded and a new seedlot of the same species was tested. Seeds of various ages in some species were tested in order to identify samples with ≥ 51% viability.

Seed pre-treatments

Wherever physical dormancy was suspected after verification of seed impermeability or suggested by the literature, seeds were subjected to potential dormancy-breaking treatments such as hot water or mechanical scarification. THE HOt water treatment consisted of pouring boiling water on to seeds in glass containers, leaving them in the water for 5 minutes and pouring the water off to allow the seed to cool to room temperature (~22ºC). Mechanical scarification consisted in the puncturing or chipping the seed coat. Seeds of some species were placed on a moist substrate under either summer (35/25°C) or winter (5/15°C) temperatures in germination cabinets to effect warm-moist and cold-moist stratification treatments, respectively. Stratification treatment lasted a minimum of four and a maximum of eight weeks, depending on the development of the embryo. When a physical or chemical component of the seed coat or fruit coat was suspected of inhibiting germination, the endosperm and embryo were completely removed from the seed coats or fruit before testing and categorised as ‘bare seed’. Finally, seeds of species in which it was supposed that germination was prevented by inhibitors in the seeds were leached before testing. The leaching treatment consisted of subjecting seeds to five cycles of soaking and rinsing (soaking seeds for 24 h once and rinsing, then repeating four times but soaking for only 1 h followed by rinsing). Rinsing was conducted with water in the case of *Lomandra longifolia* and a 1:10 liquid detergent–water solution mix for *Pittosporum angustifolium*.

Germination

Treated and non-treated seeds (control) were placed in Petri dishes in light and temperature controlled germination cabinets. Seeds were placed on top of filter paper (Whatman No. 1), over a sponge cloth to maintain moisture over several days. Alternatively, large seeds of *Eremophila* and *Owenia* species were sown and covered by sand in 120- cm^2 square by 4 cm deep plastic transparent dishes. Unless stated otherwise, seeds of each species were incubated under three daily alternating temperature regimes in 12-h light/dark conditions, using at least three replicates of 30 seeds per treatment. Incubation temperatures were chosen based upon the records of seasonal means likely to occur naturally in the seed collection sites and target areas for sowing: 15/5°C (winter), 25/15°C (spring or l), and 35/25°C (summer), (BOM 2016). Some species were only tested under one or two temperature regimes where insufficient seeds were available. Only seeds that appeared healthy were chosen for germination tests. The light intensity within the cabinets was about ≈50 µmol m^{-2} s^{-1}. Seeds were watered at the start of the experiment (10 mL for dishes) and as required during the experiment. Dishes were placed randomly throughout each temperature-controlled cabinet and germination recorded every second day. Germination was defined as the emergence of healthy radicle (AOSA 2014), or when shoots emerged from the sand. Recording of germination ceased when no new germinants appeared for three consecutive records. Germination was calculated after discarding empty seed coats and adjusting the number of viable seeds in each dish.

Statistical analysis

Individual analyses of each species were used to compare total germination between temperature and seed pre-treatment levels. The cumulative number of seeds germinated was analysed as a proportion of the total number of viable seeds sown in each treatment using generalised linear models (GLMs). A logit function was used with either a binomial or quasi-binomial error structure, this last used when the data were over-dispersed (O'Hara and Kotze 2010). When seed pre-treatment was required, a full model was fitted to account for all factors and their interactions. Temperature and seed pre-treatment were treated as fixed effects. All statistical analyses were carried out in R 3.3.1 (R Core Team 2016).

The model for seed species incubated at different temperatures was:

$$Y_{ij} = \mu + S_i + \varepsilon_{ij},$$

where Y_{ij} was the percent of germinated seeds, μ was the overall mean, S_i the ith seasonal temperature, S, and ε_{ij} the error term. The model for species with treated seeds incubated at different temperatures was:

$$Y_{ijk} = \mu + (S*T)_{ij} + \varepsilon_{ijk},$$

where Y_{ijk} was the percent of germinated seeds, μ was the overall mean, $(S*T)_{ij}$ the interaction between the ith seasonal temperature and the jth seed treatment, T, and ε_{ijk} was the error term.

Results

Seed viability was variable within and between species when more than one seedlot was tested (Table 5.1). Seeds of 27 species were not dormant and did not require any specific pre-treatment for germination. The seeds of the other 22 species were dormant or required pre-treatment to allow or enhance germination at a level commensurate with each species' respective seed viability percentage. A problem was encountered with the viability of the first seedlot tested in eight species: six with non-dormant seeds and two with dormant seeds. Further seedlots of these species were tested for viability until a suitable seedlot was identified. Germination of two species was <20% regardless of treatment.

Seed viability

The highest viability recorded was close to 100% in freshly collected seeds of *Acacia harpophylla* and *Flindersia maculosa* (Appendix 5.1). The viability of the first seedlot of nine species was <55%, and so additional seedlots were tested (Fig. 5.1). In each of these nine species, the difference in viability between the seedlot with the lowest and highest values was significant ($P \leq 0.05$). There were four main potential causes of low viability. Seed age was the main determinant of viability, with low viability being associated with older seedlots (several years in storage), followed by seedlot quality. Fungal infection of seeds and physical damage were the other two factors contributing to low seed viability (Table 5.1), with physical damage associated with insect herbivory or incomplete, malformed, dry or empty seed coats. These factors were responsible for about 25% of the seeds of *Alectryon oleifolius* and *Capparis* spp. being rejected for seed testing, although germination of these and most other species was high once unviable and unhealthy-looking seeds were discarded.

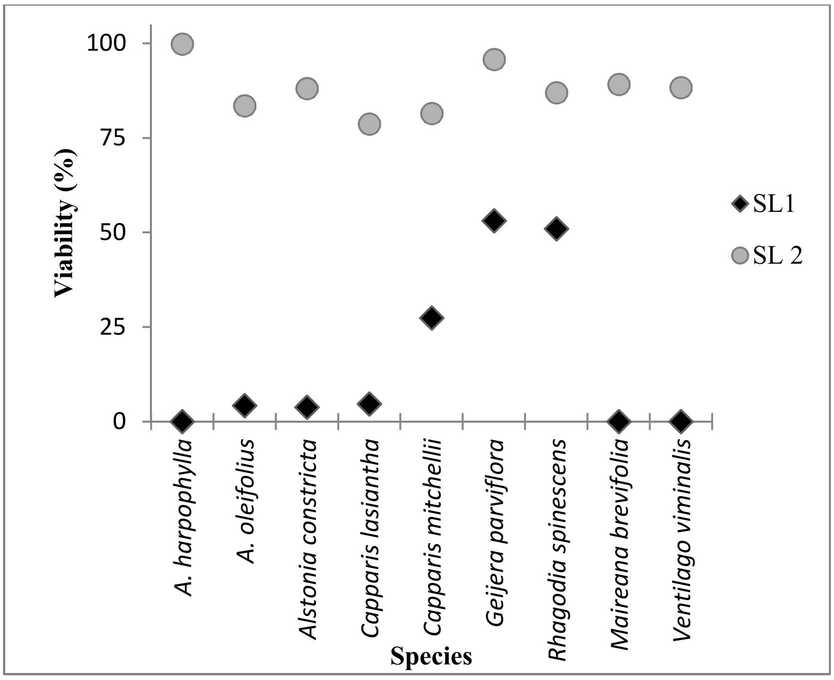


Fig. 5.1 Seed viability of two seedlots (SL1 and SL 2) in nine species in which the viability of SL 1 was <60%.

Table 5.1. Range in seed viability between seedlots of eight species and problems associated with low viability.

Scientific name	Viability range	Viability issues
Acacia harpophylla	U – H	Age, fungus
Alectryon oleifolius	N – H	Age, seedlot
Alstonia constricta	L – H	Age, seedlot
Capparis lasiantha	N – H	Age, empty
Capparis mitchellii	L – H	Age
Geijera parviflora	M – H	Age
Maireana brevifolia	N – H	Age
Rhagodia pinescens	M – H	Age
Ventilago viminalis	U – H	Age, damage

Seed viability class: U = 0%; N = 1–25%; L = 26–50%; M = 51–75%; H = 76–100%

Temperature

Of the 27 species without seed dormancy or germination constraints, nine germinated equally well in response to each temperature treatment. Seeds of 14 species in this group germinated significantly better at a specific temperature and four species exhibited reduced germination in response to one of the three temperature treatments (Table 5.2). Temperature of spring−autumn conditions (25/15°C) were associated with the highest germination in most species, while winter temperatures (15/5°C) were less favourable for most species. Seeds of *Grevillea striata* and *Flindersia maculosa* were only tested at a single temperature, but germination was high in both cases (96.7% and 80.8%, respectively).

The optimal temperature for germination varied among the 22 species exhibiting dormancy or in which germination of untreated seeds was less than optimal. Eleven of these species responded significantly to temperature. In four of these species, there was a significant treatment × temperature interaction. Seeds of *Alectryon oleifolious, Notelaea microcarpa* and *Owenia acidula* germinated only at a single temperature (Table 5.3). The 25/15°C treatment promoted highest germination in most species requiring a seed pre-treatment. Seeds of four of these species were were only tested at a single temperature (Fig. 5.3).

Table 5.2. Germination percentage in species that did not require seed pre-treatment, and the optimum temperature for germination based on analysis of deviance and seed viability.

Scientific name	Mean	±	SE	Optimum Temperature	*P*	Viability

				(°C)		
Acacia harpophylla	99.4	±	0.2	All three	ns	H
Allocasuarina littoralis	58.7	±	3.5	25/15	***	M
Alstonia constricta	82.5	±	2.7	35/25	***	H
Angophora floribunda	43.4	±	2.5	35/25, 25/15†	ns	M
Atalaya hemiglauca[s]	83.1	±	8.6	35/25, 25/15†	ns	H
Atriplex nummularia[b]	81.3	±	2.3	25/15, 15/5	***	H
Atriplex semibaccata[b]	64.8	±	2.7	35/25	***	H
Brachychiton populneus	76.7	±	7.3	25/15	**	H
Cadellia pentastylis	85.9	±	8.1	35/25, 25/15†	ns	M
Casuarina cristata	69.2	±	11.4	35/25, 25/15†	ns	H
Casuarina cunninghamiana	41.3	±	1.8	35/25	*	M
Clematis microphylla	80.8	±	3.6	25/15	***	H
Corymbia tessellaris	82.9	±	6.0	35/25†	*	H
Ehretia membranifolia	97.3	±	1.8	25/15,15/05	***	H
Einadia nutans	73.5	±	3.1	35/25	***	H
Enchylaena tomentosa	90.8	±	2.3	All three	ns	H
Eremophila mitchellii	53.8	±	2.4	25/15	**	M
Flindersia maculosa	96.7	±	1.4	25/15	ns	H
Grevillea striata	80.8	±	7.7	25/15	ns	H
Maireana brevifolia[b]	85.4	±	1.4	25/15	**	H
Melaleuca bracteata	92.0	±	1.2	All three	ns	H
Melaleuca trichostachya	53.0	±	2.6	All three	ns	H
Pandorea pandorana	80.0	±	3.8	All three	ns	H
Rhagodia spinescens	73.3	±	2.1	25/15, 15/05	***	H
Sporobolus elongatus	89.7	±	1.8	25/15	***	H
Ventilago viminalis[s]	80.0	±	7.4	35/25, 25/15	***	H
Xerochrysum bracteatum	63.1	±	3.0	25/15	***	M

† tested only at 25/15°C and 35/25°C; [b] seeds sown in their bracteoles; [s] seed samara removed; *$P \le 0.05$; **$P \le 0.01$; ***$P \le 0.001$; ns, $P \ge 0.09$; viability: M = 51–75%, H = 76–100%

Germination pre-treatments and dormancy

Seeds of 22 species were pre-treated. Germination differences between treated and control seeds were significant in 18 species. Most of the germination constraints in these species were tentatively related to dormancy. Physiological dormancy (PD) imposed by low growth potential of embryo was the most frequent germination constraint. Seeds of nine species required stratification to increase germination, with seven responding to warm stratification and two to cold stratification. In six species physical dormancy (PY) was identified and relieved by scarification or hot water treatment (Table 5.3). Seeds of four species presented an underdeveloped embryo related to morphological dormancy (MD) and did not require seed pre-treatment. Three species presented a germination constraint presumably due to an inhibitor not related to dormancy, as seed leaching or removing the entire seed coat allowed germination.

Table 5.3. Analysis of deviance of the germination response of 22 species to seed pre-treatment (Treatment), temperature (Temp) and their interaction (Temp × Tr); the type of germination constraint (Dorm) and pre-treatment applied, and seed viability (V).

Species	Treatment		Temperature		Temp x Tr		Dorm	Pre-treatment	V
	F	*P*	F	*P*	F	*P*			
Alectryon oleifolius[a]	52.5	***	–		–		X	B	H
Apophyllum anomalum	161.7	***	616.8	***	3.1	ns	PD	SC	H
Capparis lasiantha	492.0	***	1.8	ns	43.5	***	PD	SC	H
Capparis mitchellii	79.9	***	0.9	ns	1.7	ns	PD	SC	H
Cullen tenax	1229.7	***	1.4	ns	0.4	ns	PY	HW	H
Desmodium brachypodum	62.2	**	20.3	***	3.4	ns	PY	SC	M
Dodonaea viscosa	37.5	***	13.7	***	4.3	ns	PY	HW	H
Elaeodendron australe[s]	23.9	**	–		–		PY	SC	M
Eremophila debilis	184.8	***	48.0	***	2.3	ns	PD	B	H
Eremophila maculata	401.2	***	124.3	***	10.7	**	PD	B	H
Geijera parviflora	299.4	***	39.4	***	–		I	B	H
Glycine clandestine	74.5	***	6.7	*	0.0	ns	PY	HW	H
Jasminum lineare[s]	ns		–		–		MD	N	H
Jasminum suavissimum[s]	ns		–		–		MD	N	M
Lomandra longifolia	323.0	***	21.3	***	1.5	ns	PD, I	Le, St	H
Myoporum montanum	153.4	***	23.4	**	11.1	**	PD	SC	H
Notelaea microcarpa	ns		–		–		MD	N	M
Owenia acidula	21.3	**	–		–		PD	St	H
Pittosporum angustifolium	212.8	***	0.3	ns	0.7	ns	MD, I	Le	H
Santalum lanceolatum[s]	ns		–		–		MD	N	M
Senna artemisioides	10.7	**	14.8	***	0.0	ns	PY	SC	H
Solanum cinereum	102.2	***	148.6	***	8.0	*	PD	St	H

[a] aril removed; [s] tested only at spring temperature (25/15°C); *$P \le 0.05$; ** $P \le 0.01$; *** $P \le 0.001$; $P \le 0.09$; ns, $P \ge 0.09$; germination constraints: PY = physical dormancy; PD = physiological dormancy, MD = morphological dormancy; I = inhibitor, X = non-dormant. Treatments: SC = seed coat or fruit coat scarification, St = stratification, HW = Hot water, Le = leaching, B = bare seed, N = no treatment. Viability: M = 51–75%, H = 76–100%.

Lomandra longifolia germinated better after a combination of treatments than a single treatment. Seeds of *Alectryon oleifolius* did not appear to exhibit dormancy but with the seed coat removed seeds had higher germination (86.5%) than untreated seeds (69.8%). In *Owenia acidula* the source of germination inhibition was not identified. After seed pre-treatment and incubation at optimal temperatures, germination increased from generally low percentages to >50% in 20 species but remained low (<20%) in *Elaodendron australe* and *Owenia acidula* (Fig. 5.2). Seeds of *Geijera parviflora* had zero germination when pre-treatment was not applied.

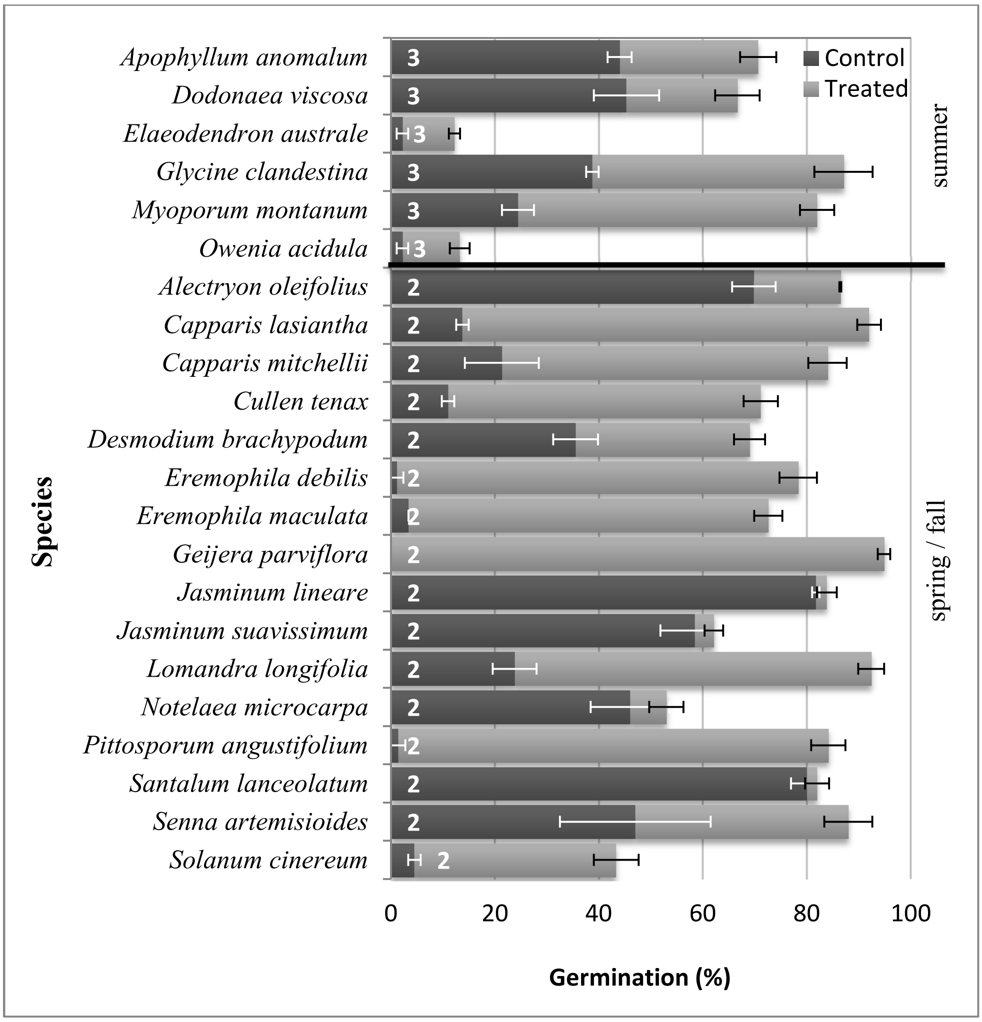


Fig. 5.2 Mean germination of untreated and pre-treated seeds of 22 species incubated only at the temperature producing maximum germination. Mean temperature of incubation: 1 = 15/5°C (w = winter), 2 = 25/15°C, 3 = 35/25°C. Error bars are ±1 SE.

Discussion

This research identified germination constraints in 49 species of a range of plant life forms in north-western NSW. Germination constraints were mainly due to: (a) the need for seed pre-treatment in almost half of the species tested, due mainly to seed dormancy and inhibitors; (b) suboptimal environmental conditions, constraining germination below the potential indicated by viability tests, and (c) low seed viability (a frequently disregarded issue) due to a variety of causes. We verified that temperature is an important cue for optimal germination in many species regardless of whether a seed pre-treatment was required or not. In some cases, inappropriate temperatures led to suboptimal germination suggesting that extreme temperatures are likely to inhibit germination. The response to pre-treatments and temperature was not uniform across plant life forms or taxonomic families. The most effective seed pre-treatments required for germination in order of frequency were: (a) rupture of the seed coat, (b) stratification, and (c) leaching of compounds from the seed. The selection of species for direct-seeding needs to be based on the seed viability, environmental conditions at the sowing site and the cost-effectiveness of treatments. Additionally, species and appropriate seed-treatments should be matched. In this discussion, we propose explanations for the germination constraints revealed and discuss the methods that produced maximum germination, emphasising the novel aspects of this research and issues to be resolved in the direct-seeding of these species in revegetation projects in north-western NSW.

Seed quality and viability

Seed viability was variable among the species tested, but most species had high seed viability (50–100%). Seeds lacking dormancy are considered to remain viable for less time than species with seed dormancy (Hendry *et al.* 1994). However, in this experiment various combinations of seed persistence and dormancy types were observed. Low viability in at least one seedlot was recorded in six species with non-dormant seeds (*Acacia harpophylla, Alectryon oleifolius, Alstonia constricta*, *Maireana brevifolia Rhagodia spinescens* and *Ventilago viminalis*). Seeds of other seedlots of these species germinated readily without pre-treatment under appropriate environmental conditions. The viability of seeds of both *Capparis* spp. and *Geijera parviflora*, which required pre-treatment for germination, also determined their germination success. Viability of all eight species was high (>75%) within 2 years of seed collection and after empty seeds had been discarded. Only 52–70% of seeds of *Alectryon oleifolius*, *Apophyllum anomalum, Capparis* spp. and *Cadellia pentastylis* were filled. Empty seeds of the latter two genera were caused by insect larvae present in seeds when collected and discarded from germination tests. The observed low viability in seeds of the eight species related to long periods of storage (>2 years), with molecular oxidative processes presumably causing the loss of viability (Hendry 1993). Short duration of seed longevity is typical of seeds with weak seed coats (Yasseen *et al.* 1994), such as the six species that did not require treatment as well as the *Capparis* spp. that required only a mild pre-treatment. Recalcitrant seeds are characteristically intolerant to desiccation (Berjak and Pammenter 2013). Thus a certain level of recalcitrance could be responsible for viability loss over time in these species. Other factors responsible for loss of seed viability and that occur commonly in nature include exposure to extreme environmental conditions, microbial pathogens and insect herbivory (Anderson *et al.* 2004), some of which were observed in this experiment.

Temperature

Although germination of some species can occur in any season, in other species germination is greatly affected by seasonal temperature (Roche *et al.* 1998). Seeds of 31 species in this study depended on specific temperatures for maximum germination. Germination under specific ambient conditions is a strategy to avoid seedling death when the environment for survival and establishment is unsuitable (Benvenuti *et al.* 2001). However, species that are not adapted to a wide range of environmental conditions are at higher risk from climate variability and climate change (Aitken *et al.* 2008). Spring/autumn incubation temperatures stimulated maximum germination in 24 species (*Alectryon oleifolius, Allocasuarina littoralis, Brachychiton populneus, Clematis microphylla, Desmodium brachypodum, Eremophila* spp., *Geijera parviflora, Maireana brevifolia, Senna artemisioides, Solanum cinereum Sporobolus elongatus* and *Xerochrysum bracteatum*). Conversely, summer temperatures were optimal for nine species (*Alstonia constricta, Apophyllum anomalum, Atriplex semibaccata, Casuarina cunninghamiana, Dodonaea viscosa, Einadia nutans, Glycine clandestine, Myoporum montanum* and *Owenia acidula*). Temperature conditions are typically optimal under spring and autumn conditions providing moisture is adequate (Bell *et al.* 1995; Leck and Brock 2000; Roche *et al.* 1998).

The germination of four species was significantly greater at two temperature levels but not at the cooler (*Ventilago viminalis*) or warmer extremes (*Atriplex nummularia, Ehretia membranifolia* and *Rhagodia spinescens*). Suboptimal temperatures did not impede germination completely in most species but did so in *O. acidula* and *N. microcarpa*, in which germination was recorded only at a single temperature.

Temperature did not affect germination in 12 species either alone or in combination with a pre-treatment, seeds of these species germinating consistently well under all temperatures. Germination in these 12 species is evidently adapted to a broad range of temperatures and seeds that germinate under a wide range of environmental conditions have a greater likelihood of propagation, establishment and survival (Cochrane *et al.* 2011; Luna and Moreno 2010).

The interaction between seed pre-treatment and temperature was significant in four species (*Capparis lasiantha, Eremophila maculata, Myoporum montanum* and *Solanum cinereum*). This is not unexpected as once dormancy is relieved, seeds can respond to environmental signals for germination (Cochrane and Probert 2006).

The germination response to temperature is particularly important when undertaking direct-seeding activities in regions with an unpredictable climate (Jurado and Westoby 1992), as is the case in north-western NSW (Chenu *et al.* 2013). Exposure of a seed to variable environmental conditions prior to and during germination can determine the fate of the plant and influence later stages such as flowering (Rubio de Casas *et al.* 2012). Adaptive plasticity has the potential to synchronise the response of otherwise susceptible plants with favourable environmental windows for survival, establishment and subsequent life stages (Montesinos-Navarro *et al.* 2012). However, environmental extremes common in the study region (BOM 2016) likely limit germination and seedling emergence and survival.

Dormancy

Seeds of *Apophyllum anomalum, Capparis* spp., *Elaeodendron australe* and *Santalum lanceolatum* presented physiological dormancies manifested by limited development of the embryo and an inability to overcome mechanical constraints imposed by the seed coat or fruit (Baskin and Baskin 2003). This was easily relieved by puncturing or partially removing the seed coat, although harsher treatments were detrimental to the seeds of these species. On the other hand, seeds of *Eremophila debilis* and *E. maculata*, which also exhibited physiological dormancy, required harsher treatments to disrupt the fruit and seed coat, with complete removal of the endosperm and embryo promoting maximum germination.

The physiological dormancy of seeds may be relieved in the field in summer or winter (Baskin and Baskin 2014) owing to a requirement for temperature stratification. Of the species with physiological dormancy, seeds of seven species responded positively to stratification treatments (Table 5.3). Species of *Lomandra*, *Myoporum,* and *Owenia* responded to moist–warm stratification, while *Solanum cinereum* responded to moist–cold stratification. Response to stratification is characteristic of seeds with physiological dormancy, and the short period (4–8 weeks) required to overcome this dormancy in the current experiment suggests that it was either intermediate or non-deep dormancy (Baskin *et al.* 2004). However, it is important to stress that seeds of all seven species had been dry-stored at 3°C for at least 6 months and that after-ripening during dry storage can sometimes relieve dormancy or contribute to it (Baskin *et al.* 2004; Finch-Savage and Leubner-Metzger 2006). In *O. acidula*, the difficulty in extracting complete embryos and endosperm from the loculi of the hard endocarp and removing the outer endocarp prevented us from determining accurately the type of dormancy or a suitable treatment to increase germination.

Several species with physical dormancy (water impermeable seed coats or fruits) in this research lacked documented information about their germination, or the literature was focused on technical issues rather than the practical purpose of plant propagation. The literature pertaining to related species in the same genus or family is generally consistent with our findings (Baskin and Baskin 2004b). Our results indicate that germination clearly improves when physical dormancy is relieved by rupturing the seed coat (Baskin *et al.* 2000) mechanically or with hot water. Seeds in this study that had a physical dormancy were *Cullen tenax, Desmodium brachypodum, Dodonaea viscosa, Glycine clandestina* and *Senna artemisioides*. These five species required treatment to disrupt the seed or fruit coat to achieve high germination; thus, it is unlikely that these species have additional embryo dormancy (Bahrani *et al.* 2008). However, additional unnoticed dormancy could have been relieved by seed after-ripening (Finch-Savage and Leubner-Metzger 2006) in seeds that were stored and not tested fresh. It was also evident that the intensity or duration of the treatments needs to be applied differently among species to achieve maximum germination, as responses were not uniform among species. For instance, seeds of *Cadellia pentastylis* had high germination without pre-treatment under the right temperature conditions, although it is related to taxa that typically present a water-impermeable seed coat causing physical dormancy (Baskin *et al.* 2006b). Similar results were obtained to a lesser extent in *Senna artemisiodes.* High germination without seed pre-treatment in these species is probably explained by the fact that seeds of these species were freshly collected and used, and seeds of some species do not need a dormancy-breaking treatment if they are collected and germinated fresh before the seed coat or fruit dries and becomes water-impermeable (Baskin *et al.* 2006a).

Maximum germination in *Elaeodendron australe* and *Owenia acidula* was low (12.2%, 13.3%, respectively) despite pre-treatments and apparently high viability. The low germination percentages obtained in these species could have been due to: (1) dormancy-breaking treatments being either excessive or insufficient; (2) incubation time being too short to allow germination; (3) temperatures being above or below the optimum required to promote germination; (4) incorrect combinations of treatments and/or environmental conditions to break dormancy, or (5) low seed viability despite apparent evidence to the contrary.

Fresh seeds of *Notelaea microcarpa*, *Jasminum* spp. *Pittosporum angustifolium* and *Santalum lanceolatum* presented underdeveloped embryos indicative of morphological dormancy (Baskin and Baskin 2003). These species were not affected significantly by treatments to relieve dormancy and started germination in a maximum of 30 days from the sowing date.

Other germination constraints

The seeds of three species (*Geijera parviflora, Lomandra longifolia* and *Pittosporum angustifolium*) required standard pre-germination treatments (ISTA 1999) in addition to dormancy-breaking treatments to promote germination, and this was due to morpho-physiological dormancy in the two latter species. The seeds of these species apparently contain natural germination inhibitors that were eliminated by removal of the complete seed coat in *Geijera parviflora* as found by Mole (1996); removal of the inner pericarp by means of leaching in addition to stratification in the case of *Lomandra longifolia*, as found by Plummer *et al.* (1995) in another species of the same genus (*L. sonderi*), and leaching of the viscid resin surrounding the seeds of *Pittosporum angustifolium*. Endogenous substances that inhibit or delay seed germination are present in different parts of the seeds or fruits of various plants and their removal is necessary to stimulate germination (Debeaujon *et al.* 2000; Wareing 1965).

Conclusions

Information about differences in seed viability, optimum temperatures and pre-germination treatments to promote germination in these 49 species will be useful to guide their use in direct seeding for revegetation purposes in north-western NSW. The pre-treatment information for germination is also relevant to the nursery production of seedlings of these species. However, the results confirm the necessity for rigorous testing of the viability of individual seedlots and of age-related germination cues prior to the use of the seeds of these species. Issues that prevent native plant propagation and seed dormancy mechanisms in native species are still poorly understood. Seed of species that obtained high germination percentages in this experiment (>75%), with or without pre-germination treatment, can be used in direct seeding, providing all other environmental requirements are met for the germination of seed and plant propagation. These external ecological factors as well as seedling growth rate and hardiness, the timing of germination and intraspecific and interspecific competition are important determinants of the success of direct seeding in the field.

Chapter 6. Germination ecology of six species of *Eucalyptus* in shrink–swell vertosols: moisture, seed depth and seed size limit seedling emergence

Abstract

We examined the potential of direct-seeding *Eucalyptus* species to revegetate the vertosol ('cracking clay') soils that characterise the floodplains of north-western New South Wales. We investigated the influence of sowing depth (0, 6, 12 and 20 mm) and three soil-moisture scenarios (dry, moist and flooded) on seedling emergence of six species of *Eucalyptus* with a range of seed sizes (*E. blakelyi, E. camaldulensis, E. melanophloia, E. melliodora, E. pilligaensis* and *E. populnea*). We used cracking clay soil from the region in a glasshouse environment. Seedling emergence was low despite high seed viability and provision of optimum temperatures and soil moisture conditions. All six species exhibited greatest emergence when sown at 0–6-mm depth, with seed size being less important than moisture (except under dry conditions) and proximity to the surface. Species responded differently to the three watering treatments. *Eucalyptus melanophloia* exhibited greatest emergence in the 'dry' watering treatment. The floodplain species, *E. camaldulensis*, *E pilliganesis* and *E. populnea*, had the greatest emergence under flood conditions. *Eucalyptus blakelyi* and *E. melliodora* exhibited intermediate emergence in relation to all three soil-moisture regimes. Although the direct seeding of these species in vertosol soils in the region may be successful on occasion, windows of opportunity will be infrequent and the planting of seedling tubestock will be more reliable for revegetation.

Introduction

Large-scale revegetation is a means of reversing land degradation and increasing habitat for biodiversity conservation (Hallett *et al.* 2014; Kell 2011) as well as providing a tree resource for uses such as forestry. Although the customary goal of ecological restoration has been to return ecosystems to their historical state, the pace of environmental change means that current and future conditions are increasingly likely to favour novel ecosystems involving new combinations of species better suited to the altered conditions (Hobbs *et al.* 2011; Perring *et al.* 2013). In such circumstances, it has been suggested that plant species from the general region but not necessarily from the site to be revegetated should be tested for establishment, growth and persistence in novel associations, so as to expand options and increase the likelihood of success of rehabilitation efforts (Perring *et al.* 2013). In Australia, the continuing decline of *Eucalyptus*-dominated vegetation formations across the wheat–sheep belt of southern and inland eastern Australia is of concern to land managers and scientists, and ambitious revegetation targets have been set to restore woody vegetation to the most heavily cleared landscapes (Ansell *et al.* 2016; Reid and Landsberg 2000). In north-western New South Wales (NSW), the alluvial plains of the Namoi, Gwydir and Macintyre River catchments have been cleared of eucalypt woodland and smaller areas of acacia and casuarina scrub and shrubland for dryland and irrigated cropping in the past 60 years (Reid *et al.* 2007). Clearing of the heavy shrink–swell vertosols ('cracking clays') of the floodplains has proceeded to such an extent that natural-resource management agencies have estimated a shortfall in tree cover across the North-West Plains of NSW of at least 280 000 ha to sustain land management and reverse land degradation (Donaldson 1996; Donaldson and Heath 1997; Peasley 1995). Recruitment of eucalypts and other native woody perennials is limited in agricultural districts with a long history of agriculture on clay soils (Azam *et al.* 2012; Weinberg *et al.* 2011). Thus, to restore eucalypts rapidly to farmland in this region, direct seeding or replanting of seedlings is required (Dorrough and Moxham 2005). Compared with seedling planting, direct seeding requires little investment in time and effort over large areas and results in a natural dispersion of plants (Gibson-Roy and Delpratt 2014). However, successful establishment may be inhibited on heavy clay soils. Although only large-seeded species are recommended for the direct seeding of farmland (St-Denis *et al.* 2013), sowing small-seeded species such as eucalypts can be effective if high seed volumes are used (Geeves *et al.* 2008).

The floodplains of north-western NSW are dominated by shrink–swell vertosols, which are notoriously difficult soil environments for the germination and emergence of small-seeded species. For germination, seeds are reliant on the soil hydraulic properties to imbibe sufficient moisture (Arnold *et al.* 2014b; Evans and Etherington 1990). Optimum soil moisture for plant growth and survival is limited to the range in soil-water content between field capacity and wilting point (Osman 2013) and seed germination ceases or is dramatically reduced and slowed at water potentials nearing wilting point (say –0.7 to –1.0 MPa) for some grass (Whalley 1987) and eucalypt species (Arnold *et al.* 2014b). Although increasing clay content in the soil profile is associated with greater water-holding capacity, this does not mean that more water is available to plants, because the smaller pore size of the soil matrix holds water at greater suction pressure (Rab *et al.* 2011). Moreover, after large rainfall events, vertosols rapidly become plastic, leading to waterlogging, anaerobic conditions and increased denitrification (Balogh *et al.* 2011). Maintaining sufficient soil moisture for the germination of small seeds in shrink–swell vertosols is challenging because the surface dries out rapidly after wetting (Watt and Whalley 1982b), and the low hydraulic conductivity of heavy clay soils means that water lost at the surface is not replaced from below. The shrinking and cracking of the drying surface soil damages the fine roots of small seedlings and results in large variations in water content near the surface. Watt (1972) and Harper *et al.* (1965) documented this extremely rapid and uneven drying and crusting that occurs in the surface layers of heavy clays following cessation of rainfall, impeding seed germination and seedling emergence. These adverse soil conditions can become harsher for germination and seedling survival after intensive farming (Arndt 1965).

Eucalyptus species have specific moisture requirements for germination and establishment, and are sensitive to minor variations in soil micro-environment (Schütz *et al.* 2002). For successful direct seeding, seeds need to be sown at the appropriate depth for maximum germination and seedling survival (Lambert *et al.* 1990). Small seeds germinate better at or near the soil surface, whereas larger seeds can emerge from greater depths (Milberg *et al.* 2000; Xia *et al.* 2015). A small increase in seed depth below the optimum reduces germination considerably (Boyd and Van Acker 2003; Saatkamp *et al.* 2011). In addition, surface-sown seed may dry out or be predated by ants (Yates *et al.* 1994). In field studies of the fate of *Eucalyptus*, 90–96% of seed on the soil surface were predated after 1 day (Stoneman and Dell 1994; Yates *et al.* 1995).

Understanding how depth in the soil profile and varying moisture contents affect germination and seedling emergence increases our ability to design successful direct-seeding operations for large-scale revegetation (Boyd and Van Acker 2003). Accordingly, the aim of the present study was to examine the potential of several species of *Eucalyptus* common in north-western NSW for revegetation of vertosol soils by direct seeding. Given the difficulties associated with vertosols, and the dry El Niño conditions that have prevailed in the region in recent years, we undertook a glasshouse study to examine questions related to germination and seedling emergence. We hypothesised that (1) there should be an optimum combination of moisture regime and sowing depth for eucalypt species of differing seed size or weight to maximise seedling emergence; (2) regardless of seed size (all eucalypt species generally have small seeds), emergence should be maximal close to the surface, and (3) specialist floodplain eucalypts adapted to germinate in floods should exhibit maximum emergence under simulated flood conditions. Our primary objectives were to (1) determine the effect of sowing depth, moisture regime and seed weight on the germination of a range of eucalypts in cracking clay soils; (2) identify the factors that allow eucalypts to establish in vertosols, and (3) use these results to provide guidelines for the direct seeding of eucalypts in relation to moisture regime, sowing depth and seed size to enhance seedling emergence in large-scale revegetation of cracking clay soils in north-western NSW. The species selected were mean to represent the full span of available seed sizes of eucalypt in the study area.

Materials and methods

We examined the influence of different sowing depths (0, 6, 12 and 20 mm) and three soil-moisture scenarios (dry, moist and flooded) on the emergence of seedlings of six species of *Eucalyptus* with a range of seed sizes. *Eucalyptus blakelyi*, *E. camaldulensis*, *E. melanophloia*, *E. melliodora*, *E. pilligaensis* and *E. populnea* are widespread in north-western NSW and are common or occasionally occur on the cracking clay floodplains of the region. Seeds were collected from at least 10 trees in natural populations of each species from the Moree Plains, Narrabri and Gwydir Shires, north-western NSW (Table 6.1). Seeds were stored until use in air-tight containers in a cold room (3°C).

The top 10 cm of topsoil of a self-mulching grey to black vertosol was collected from a cultivation paddock on a farm (29°48′28″S, 149°38′27″E) near Bellata, NSW, in April 2013. The soil was watered, and allowed to dry out in the glasshouse three times over 7 weeks to remove weeds. The dry soil was then sieved through a 2-mm sieve, and 650 g of soil was added to plastic square pots (height 150 mm, base 6.5 × 6.5 cm, top 8.5 × 8.5 cm to about three-quarters full). These procedures were necessary despite their possible effect on soil characteristics such as pore size, aeration, moisture levels, water movement, crusting and aggregation, which can affect seedling emergence and development (Bronick and Lal 2005; Cotching *et al.* 2002). The drainage holes in the bottom of the pots were covered with absorbent paper to prevent soil loss.

Table 6.1. Seed-collection details of six species of *Eucalyptus* in northern NSW

Date, collection date of seeds, weight of the mean value (cg) per 100 seeds. Viability percentage was tested *ad hoc* in germination cabinets

Species	Latitude	Longitude	Date	Weight (cg)	Viability %	Occurrence
E. blakelyi	–30.2213	151.0311	Dec-09	20.2	93	Grassy woodland, moderately fertile soil
E. camaldulensis	–29.4853	150.0791	Dec-09	51.1	91	Grassy woodland or forest adjacent to permanent water on deep fertile alluvial soil
E. melanophloia	–29.6367	150.5425	Dec-09	85.4	98	Grassy or sclerophyll woodland, light soil
E. melliodora	–29.3821	150.4748	Feb-10	35.8	91	Grassy woodland, moderately fertile, sandy or alluvial soil
E. pilligaensis	–30.3474	149.8115	Dec-09	20.9	93	Sclerophyll woodland ,sandy or light loamy soil
E. populnea	–29.3009	149.5553	Jan-10	12.0	88	Grassy woodland, moderately fertile loamy soil

Experimental design

The experiment consisted of sowing seed of six species of eucalypt at four depths (0, 6, 12 and 20 mm) under three moistures regimes (dry, moist and wet). Each combination of factors was replicated five times (with three blocks in one glasshouse bay and two blocks in another (Fig.6.1). Each block contained one pot representing each combination of species, moisture and depth, arranged randomly. Twenty-five seeds were sown per pot either at the surface or buried at varying depths. Immediately before the experiment, seeds from the same batch of each species were tested for viability and germinability in climate-controlled germination cabinets. The seeds were viable (≥88% germination) so no adjustments were made for viability.

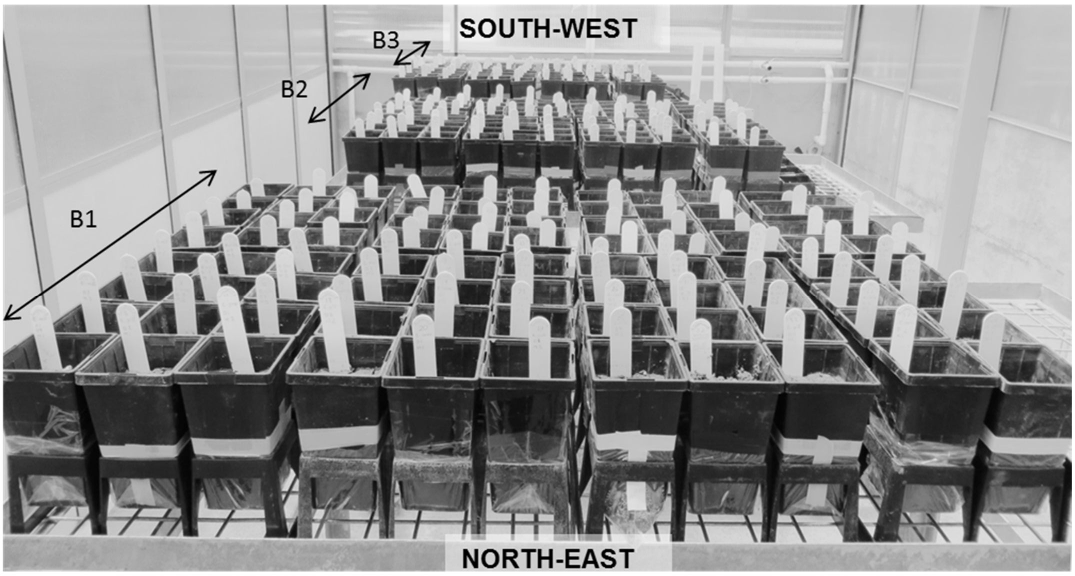


Fig. 6.1. Glasshouse bay containing three blocks (B1, B2 and B3), each block containing one pot representing one treatment combination, in addition to control pots. Each pot contained 25 seeds.

Soil watering regimes were based on 100 years of November–December rainfall records from Bellata, NSW (www.bom.gov.au, accessed March 2016). These months coincided with the most likely occurrence of sufficient rainfall to germinate seeds and permit seedling development (Carr *et al.* 2007). Rainfall means were ranked, divided into quintiles and assigned to three moisture regimes representing flood (upper quintile), moist (middle quintile) and dry (lower quintile) conditions to represent the range of likely rainfall scenarios. The daily rainfall data (12 mm, 3 mm and 0.7 mm respectively) were converted to the equivalent amount of water per pot in each moisture treatment. Pots were watered with (1) 61 mL of rain water daily for the flooded scenario to maintain saturated soil, (2) 16 mL daily in the moist treatment to keep the soil water content at about field capacity and above the permanent wilting point at the surface (Rab *et al.* 2011), and (3) 4 mL every second day for the dry scenario. Emergence was counted every second day and the number of sprouted seedlings and cumulative emergence was calculated for the entire period of the trial.

The experiment was performed in a controlled day/night temperature regime of 35°C/18°C, under natural lighting from 28 October to 27 December 2013 in two bays in a glasshouse at Armidale, NSW. Data loggers detected minor temperature variation among blocks and between bays, apparently because of uneven airflow, affecting the soil moisture content of pots.

Statistical analysis

So as to compare total emergence among treatments and their interactions, we analysed the cumulative number of emerged seedlings expressed as a proportion of the total number of seeds sown in each pot (25) using generalised linear models (GLMs). The watering regime, species and depth were treated as fixed effects and block nested within bay as a random effect. A logit function was used with a quasi-binomial error structure to account for over-dispersion in the data (O'Hara and Kotze 2010). All analyses were performed using R 3.2.3 (R Core Team 2016). A full model was fitted initially, from which non-significant terms were dropped, producing the following generalised linear model:

$$Y_{ijkl} = \mu + M_i\,(S_j + D_k) + G_l/B_m + \varepsilon_{ijklm} \quad (1)$$

where Y_{ijkl} was percentage seedling emergence; μ was the overall mean; $M_i\,(S_j + D_j)$ was the interaction of the ith watering regime, M, by the sum of the jth species, S, plus the kth depth, D; G_l/B_m was the random effect of the mth block, B, in the lth bay, G, and ε_{ijklm} was the error term. We used orthogonal contrasts to compare percentage emergence among the different combinations of the interaction between moisture level and sowing depth. However, because of the large number of contrasts, we used multiple comparisons of means (lsmeans function, Tukey method) to compare the effects of moisture and species on emergence. A second model was fitted substituting the mean seed weight per species for species.

Results

Across the whole experiment, emergence of eucalypt seedlings averaged 16% per pot, with minimum and maximum values of 0% and 72% per pot, respectively. Seedling emergence differed little between bays (14% and 17%, respectively), but varied from 14% to 21% among blocks because of uneven air flow and differential exposure to sunlight within bays. After accounting for the non-significant terms, the most parsimonious generalised linear model of seedling emergence contained two significant two-way interactions between moisture and sowing depth, and moisture and species (Table 6.2). The effect of block nested within bay was significant, but the deviance accounted for was small in relation to treatment effects.

Table 6.2. Summary of analysis of deviance of seedling emergence of six species of eucalypt in relation to the effects of watering regime and burial depth.

Source of variation	df	Deviance	Residual deviance	*F*	*p*	
Null			1971			
Moisture	2	45	1926	10.4	4.1E-05	***
Species	5	174	1753	16.2	2.6E-14	***
Depth	3	675	1078	105.2	9.0E-48	***
Bay	1	10	1068	4.6	3.2E-02	*
Moisture*Species	10	154	914	7.2	2.7E-10	***
Moisture*Depth	6	114	800	8.9	5.5E-09	***
Bay:Block	3	60	740	9.4	5.9E-06	***

d.f., degrees of freedom; $^*P \leq 0.05$, $^{***}P \leq 0.001$

Combined effect of moisture and depth

Eucalypt seedling emergence was greatest for surface-sown seeds in the moist and flood treatments (35% and 37%, respectively) and significantly greater than from seeds sown at 6-mm (20–21%) or greater depths under any moisture regime (Fig. 6.2). The significant interaction between soil moisture and sowing depth was due to the fact that emergence of surface-sown seeds in the dry treatment was significantly lower than for seeds sown at 6-mm depth under dry conditions (Fig. 6.2).

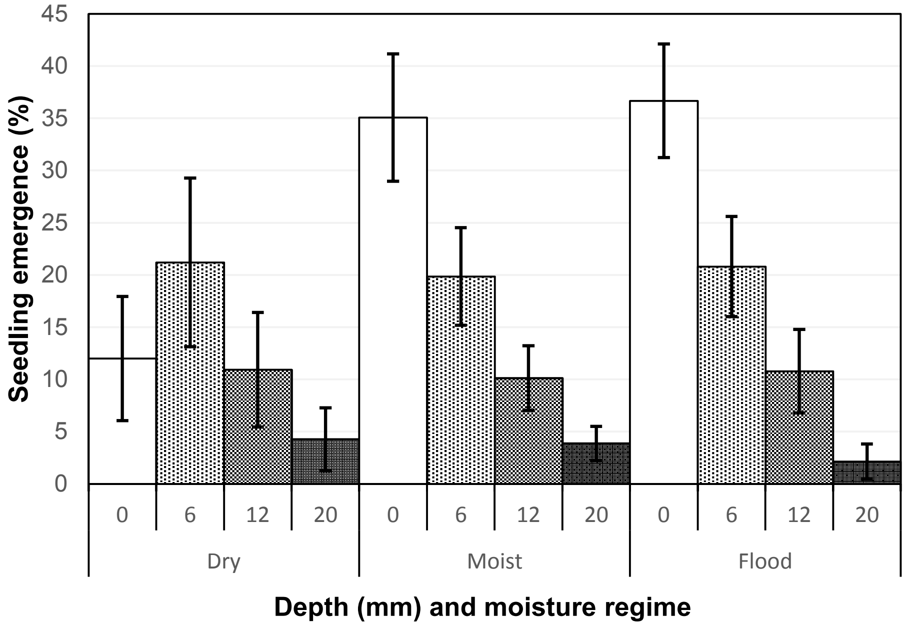


Fig. 6.2. Mean seedling emergence (%) of the six species of eucalypt at three moisture regimes and four sowing depths (0, 6, 12 and 20 mm). Error bars are ± 95% confidence intervals.

Orthogonal contrasts of the different combinations of depth and moisture indicated that seedling emergence differed among depths regardless of moisture, except that there was no difference in emergence between 0-mm and 12-mm sowing depths in the dry treatment. The difference in emergence between 12-mm and 20-mm depths was only marginal ($P = 0.06$).

Emergence also varied with species and moisture regime (Table 6.2, Fig. 6.3). *Eucalyptus melanophloia* had the highest mean emergence (25.5%) and *E.*

populnea had the lowest (9.5%) of the six species tested. Large-seeded *E. melanophloia* responded differently from the other five species, with the highest emergence (30.0%) in the dry treatment. In contrast, small-seeded *E. pilligaensis* and *E. populnea* had very low emergence in the dry treatment (2.6% and 1.6%, respectively), performing best, albeit modestly, in the flood treatment (20.0% and 14.2%, respectively). *Eucalyptus blakelyi* and *E. melliodora* responded similarly, although differently again, by exhibiting best emergence in the moist treatment (16.0% and 16.6%, respectively) and lowest emergence in the dry treatment (14.6% and 12.2%, respectively), although the emergence for both was significantly better than that for *E. pilligaensis* and *E. populnea. Eucalyptus camaldulensis* responded somewhat similarly to *E. blakelyi* and *E. melliodora*, except that emergence was similarly high under both moist and flood treatments.

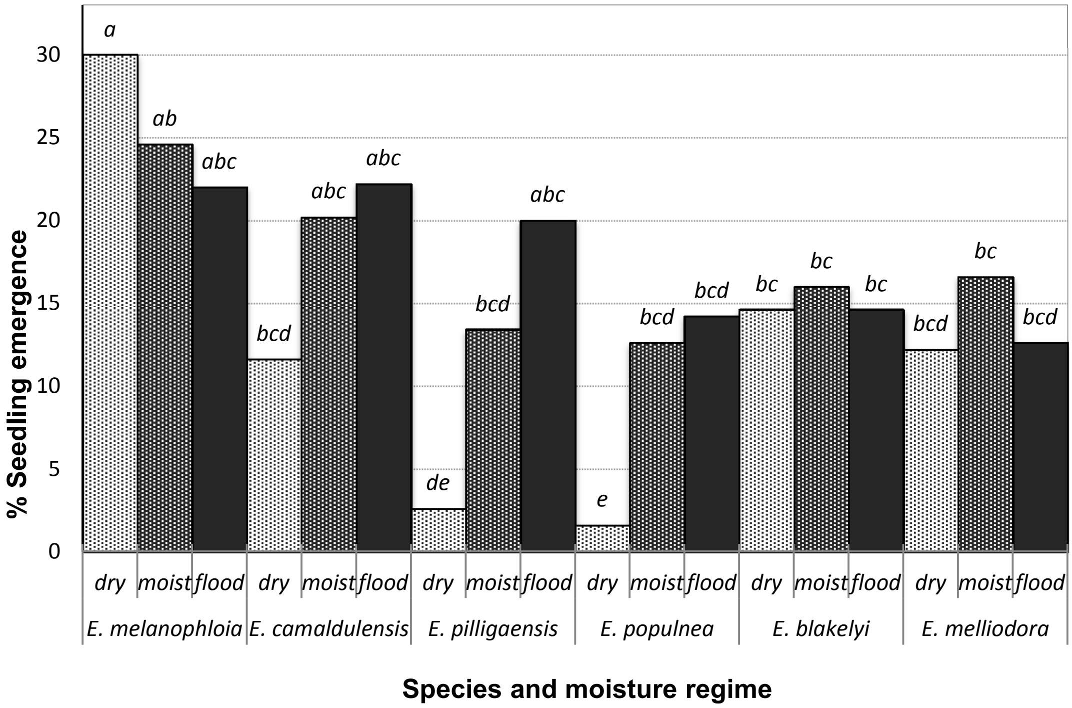


Fig. 6.3. Mean cumulative seedling-emergence percentage of six eucalypt species in relation to three moisture treatments. Means with the same letter are not significantly different (Tukey method, α = 0.05).

The results of substituting species with mean seed weight in a second GLM were comparable to those of the first model, with significant two-way interactions between moisture and sowing depth, and moisture and seed weight (Table 6.3). The interaction between moisture regime and species in the first model was readily explained as the interaction between moisture and the effect of each species' seed weight in the second model. Emergence increased with seed weight but the rate of increase was much stronger in the dry treatment than in the flood treatment, with the result being intermediate in the moist treatment (Fig. 6.4)

Table 6.3. Summary of analysis of deviance of seedling emergence after substituting the six species by their seed weight in relation to the effects of watering regime and burial depth. d.f., degrees of freedom; *$P \leq 0.05$, ***$P \leq 0.001$.

Source of variation	df	Deviance	Residual deviance	*F*	*p*	
Null			1971			
Moisture	2	45	1926	9.0	1.2E-04	***
Weight	1	174	1753	63.8	2.1E-14	***
Depth	3	675	1078	91.0	2.8E-43	***
Bay	1	10	1068	4.0	0.05	*
Moisture*Weight	2	154	914	12.9	4.0E-06	***
Moisture*Depth	6	114	800	7.7	9.4E-08	***
Bay:Block	3	60	740	8.0	3.5E-05	***

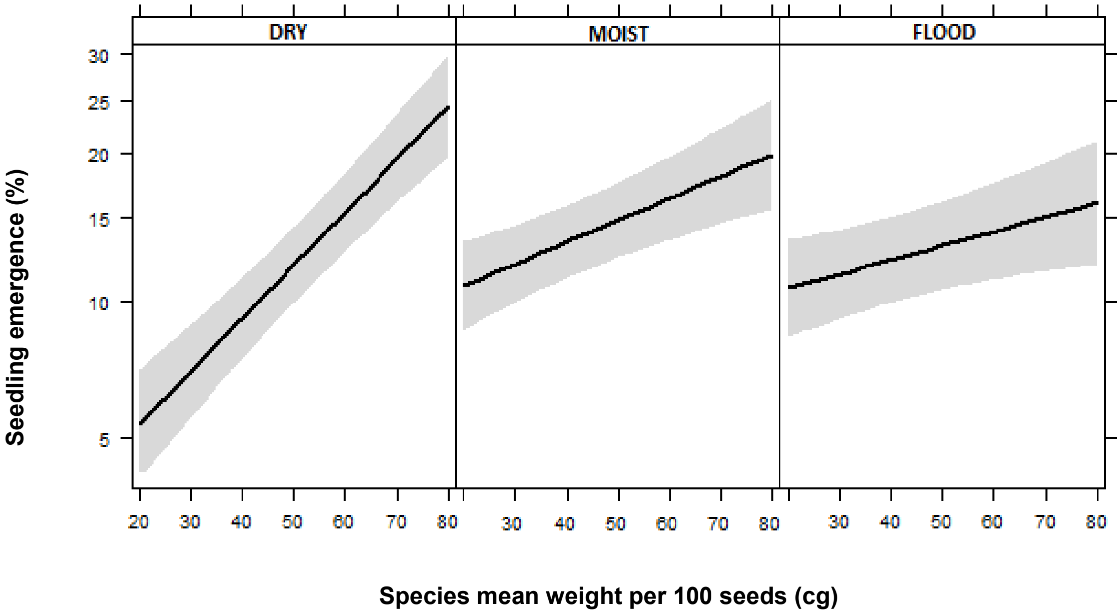


Fig 6.4 Predicted seedling emergence (%) of eucalypts in relation to mean species seed weight and moisture regime. Shading indicates the 95% confidence intervals.

Discussion

Emergence was low (average of 16%) from vertosol soils despite high seed viability (88–98% germination) of the six eucalypt species used in the experiment and the provision of ample moisture in two watering treatments as well as optimum temperatures. Emergence was greatest under moist and flood conditions from surface-sown seeds, declining with increasing depth. However, under dry conditions, emergence was greater from 6-mm depth (21% emergence) than from surface-sown seeds (12%). Emergence was positively related to seed size, most markedly under dry conditions and least so under flood conditions. This is probably because large seeds can emerge from a greater depth than can small seeds, coupled with the fact that small seeds dry out faster than large seeds under dry conditions (Leishman and Westoby 1994).

It is clear from this work that the direct seeding of eucalypts in vertosols requires precision placement in relation to depth and optimum moisture conditions. Season of sowing may be less important in north-western NSW than elsewhere, because the region has a more variable, drought-prone climate than do other parts of the Australian wheatbelt (Chenu *et al.* 2013), meaning that optimal sowing conditions could occur anytime. Preparation of a seedbed with fine tilth to facilitate precision placement of small seed just below the soil surface and artificial watering after sowing or sufficient rainfall will be beneficial to create a suitable micro-environment for germination and emergence (Facelli *et al.* 1999).

With the cracking clay vertosol soils used in the present experiment, many surface-sown seedlings in the dry treatment emerged from cracks that developed in the soil surface, where seeds were washed on watering, and where conditions for seedling emergence were probably more suitable than at the surface (Fig. 6.5). These results are consistent with those of studies of non-eucalypt species in similar soils, where surface-sown seeds did not emerge during intermittent rain and hot periods, but emerged when sown below the soil surface (Watt and Whalley 1982a; Watt and Whalley 1982b; Younger and Gilmore 1978). Small seeds with limited nutrient reserves are disadvantaged by sowing at depth because the seedlings do not have the resources to reach the surface (Long *et al.* 2015). Small seeds sown on the surface of vertosol soils have previously been observed lodging in cracks in crusted soils, where they find suitable micro-environmental conditions for germination and seedling survival (Chambers and MacMahon 1994).

Fig. 6.5 *Eucalyptus blakelyi* seedlings emerging from surface-sown seed under (a) dry, (b) moist and (c) flood watering treatments.

The largest-seeded species, *E. melanophloia*, exhibited the highest emergence overall (26%) of the six species, and was particularly successful when seeds were sown at the surface, by emerging from soil cracks in the dry treatment (30%). Emergence of *E. melanophloia* was somewhat lower under moist (25%) and flood (22%) conditions, but these values were still high compared with the other five species. Maximal emergence of *E. melanophloia* under dry conditions could have been anticipated, because seedlings of the species are adapted to tolerate moisture stress and optimise water intake under dry and irregular rainfall conditions (Prebble and Stirk 1980). *Eucalyptus melanophloia* establishes readily after rainfall, despite the inability of the soils where it occurs to retain moisture for long (Fensham and Fairfax 2007).

Eucalyptus camaldulensis with moderately large seeds exhibited best emergence under flood (22%) and moist (20%) conditions, but was less successful in the dry treatment (12%). These responses accord with the natural occurrence of this species on heavy clay soils with access to a watertable and along river banks in inland Australia (Costermans 2009), on floodplains and along watercourses and river banks, and its known tolerance of frequent or periodic flooding (Stefano 2002; Thorburn and Walker

1994). The seed of *E. camaldulensis* can float for up to 48 h in flood waters, and germination and establishment occur frequently after heavy rainfall or major flooding, particularly in drift lines (Stefano 2002). In previous trials with this species, full seed imbibition has been more critical in achieving high germination than other factors such as optimum temperature (Grose and Zimmer 1958). *Eucalyptus camaldulensis* populations are also differentially sensitive to drought, with the germination of larger-seeded populations being more constrained by low soil hydraulic conductivity than that of smaller-seeded populations (Stoneman 1994).

Eucalyptus pilligaensis and *E. populnea* responded similarly to *E. camaldulensis* in relation to the three watering treatments, but exhibited lower mean emergence (12% and 10%, respectively, compared with 18% in *E. camaldulensis*; Fig. 6.3. The similarity in response of *E. pilligaensis* and *E. populnea* can be explained by their co-occurrence on floodplains in the Namoi catchment in north-western NSW (ALA 2016), and their adaptation to the same environmental conditions for establishment (Webb *et al.* 2002).

The seed of all six species used in the present experiment should germinate readily and rapidly without pre-treatment under optimal conditions (Boland *et al.* 1980). However, the vertosol soils used in the present experiment were disadvantageous for eucalypt germination and seedling development because mean emergence averaged only 16% per pot and maximum emergence was only 72% (one pot of *E. melanophloia* sown at 6-mm depth under dry conditions). The specific limitations of vertosol soils for germination of eucalypt seeds and seedling emergence are as follows (Coughlan and Loch 1984; Spaargaren 2008; Watt and Whalley 1982b): (1) the brief temporal window over which physical conditions are suitable for germination at the surface in vertosols, because of the rapid drying of the surface soil; (2) soil swelling on hydration, restricting aeration, infiltration, permeability and drainage; (3) soil shrinkage on drying, producing deep surface and subsurface cracking; (4) crusting of the surface soil layer following cessation of rainfall; (5) agricultural use making the topsoil cloddy or dispersive, and prone to waterlogging, and (6) soil churn, when the soil dries out and deep wide cracks open up from the surface downward, clods of surface soil fall into the cracks, pushing up the subsurface soil when the soil rehydrates and swells and mixing surface and subsurface materials. All but the last of these problems were evident in at least some of the treatments in the present experiment.

Conclusions

Vertosol soil conditions are harsh for eucalypt-seed germination, particularly after intensive farming, and are heavily influenced by moisture. Seedling emergence was greatest from surface-sown seeds in moist and flood-watering treatments and declined with depth to almost nil at 20-mm depth. However, surface-sown seeds under dry conditions showed reduced emergence, being similar to the seedling response from 12-mm depth. All species exhibited greatest emergence at 0–6-mm depth, with seed size being less important than moisture and proximity to the surface. Eucalypt species responded differently to the three watering treatments. Opportunities for the successful direct-seeding of eucalypts in vertosol soils in north-western NSW may be infrequent because of the need for an adequate soil-moisture store, a dry period to enable heavy machinery to be used for seedbed preparation and sowing with precision seed placement just below the soil surface, followed by a rainfall event of a sufficient magnitude to permit germination and seedling establishment. Alternatives to direct seeding, such as establishment of eucalypts from tubestock in vertosols in north-western NSW, ought to be considered when the conditions for successful direct seeding are not ideal.

Chapter 7: Synthesis and general conclusions

Introduction

This thesis investigated the seed traits for successful germination of 73 plant species that occur in endangered communities native to north-western NSW. The aim was to generate knowledge to assist the ecological restoration of retired farmland, particularly the cracking-clay vertosol soils of the North-West Plains. Previous revegetation efforts in the region on these soils and utilising some of these species in direct seeding or as planted (tubestock) seedlings have had mixed results, with success varying among species and sites (Reid *et al.* 2009). The constraints for successful revegetation that have been identified with many of these species are issues of seed management and seed ecology including seed quality, viability, persistence, storage, germination requirements and dormancy. The first step in assuring successful revegetation through propagation of seed in the nursery or directly in the soil at a revegetation site is understanding the seed, its ecology and management requirements. However, there is insufficient scientific information about the seed ecology of many species (Baskin and Baskin 2004a; Budelsky and Galatowitsch 1999; Hossain *et al.* 2014; Long *et al.* 2015). For broad-scale restoration, direct seeding is generally considered more convenient than other methods such as tubestock plantings, due to reduced labour and material costs and the ability to sow a diverse seed mix (Gibson-Roy *et al.* 2007; Hallett *et al.* 2014). However, in addition to understanding the germination requirements of species, direct seeding requires additional knowledge about site suitability, soil moisture conditions and other environmental factors required to create a suitable microenvironment for germination and early seedling growth (Moles and Westoby 2006).

The objectives of this chapter are to: (1) summarise the major findings about seed ecology from this research; (2) recount the limitations of the work; (3) explain the significance of the research in relation to our conceptual understanding of plant germination and establishment biology; (4) highlight the main practical contributions and recommendations for management and public policy, and (5) propose future research directions.

Summary of main findings

Chapter 3 identified the most favourable conditions of light and temperature for germination of 14 seedlots of ten species of *Eucalyptus* (*E. albens*, *E. blakelyi, E. chloroclada, E. dealbata, E. camaldulensis, E. melanophloia, E. melliodora, E. pilligaensis*, *E. populnea* and *E. sideroxylon*) from the study region, and of varying seed size. The germination response of the 14 seedlots to three seasonal regimes of alternating day/night temperature – 15/5°C (winter), 25/15°C (spring) and 35/25°C (summer) – and two light conditions (light/dark and dark) was assessed in germination cabinets. Germination response varied between species and between seedlots within species. Winter and spring temperatures and light yielded higher overall average germination (96%) than summer temperatures in continuous dark (93%). Seedlots of *E. chloroclada*, *E. blakelyi E. sideroxylon, E. melliodora* and *E. melanophloia* germinated consistently well under all conditions, whereas seedlots of *E. albens, E. dealbata*, *E. melliodora*, *E. pilligaensis* and *E. populnea* germination were affected by treatment. Germination of small seeds was greater in the presence of light while large seeds germinated better in continuous darkness. Time to germination was about threefold faster under summer and spring temperatures than winter temperatures. The results were a useful guide to the temperature and light conditions required to achieve maximum germination of these seedlots.

In Chapter 4 practical and cost-effective methods to break physical seed dormancy in ten acacia species from native communities in north-western NSW were identified. Methods were evaluated that could be applied to bulk lots of seed in order to achieve rapid and uniformly high germination. Germination tests were conducted after seed pre-treatment by mechanical scarification (two intensities) or hot water in ten *Acacia* species (*A. deanei, A. decora, A. excelsa, A. leucoclada, A. ligulata, A. melanoxylon, A. oswaldii, A. pendula, A. salicina* and *A. stenophylla*). Tests were conducted under two temperatures (25/15°C and 15/25°C) in alternating 12-h light/dark conditions in germination cabinets. Time to germination was also measured. The results varied with species, but the three dormancy-breaking treatments all significantly increased germination percentage or time to germination in all but *A. melanoxylon* compared to untreated controls. Temperature had an effect only with *A. decora*. Germination was greater and/or more rapid after mechanical scarification than the hot-water treatment, and three passes with the scarifier resulted in greater and more rapid germination than one pass. Scarifying seeds was the most useful and practical method to improve germination in nine out of ten species. Triple scarification can be recommended to treat at least some seed in each batch of eight of the *Acacia* species before direct seeding, to increase germination and improve the efficiency of seed use.

Chapter 5 identified constraints to the germination of 47 of the 49 plant species tested, investigated pre-treatments to overcome germination limitations, and estimated the suitability for direct seeding or propagation of these species from seed. The test consisted of an evaluation of seed viability percentage, followed by germination under three different seasonal temperatures (alternating day/night, 15/5°C, winter; 25/15°C, spring or autumn; 35/25°C, summer), with and without seed pre-treatments to promote germination. Tests were conducted under controlled conditions in germination cabinets. Seed viability varied widely among species, being >75% in 38 species and <50% in seedlots of only eight species. Seed age was the main factor affecting viability. Temperature affected germination in 27 species, most germinating better under spring/autumn temperatures. Seeds of 22 species had higher germination after pre-treatment, physiological dormancy being the most frequent type of germination constraint in species with seeds requiring pre-treatment. However, physical and morphological dormancy, combined dormancy and treatments that increased germination of non-dormant seeds were also identified. Seeds of species that obtained high germination in this experiment (>75%), with or without pre-germination treatment, can be used in direct seeding, providing all other environmental requirements for germination are met.

Chapter 6 determined the effect of sowing depth, moisture regime and seed mass on the germination of six eucalypts in vertosol (cracking-clay) soils from north-western NSW, identifying the factors that regulate seedling emergence in these soils in a glasshouse environment. The experiment consisted of sowing seed of *E. blakelyi, E. camaldulensis, E. melanophloia, E. melliodora, E. pilligaensis* and *E. populnea* at four soil depths (0, 6, 12 and 20 mm) under three soil-moisture scenarios (dry, moist and flooded) and assessing seedling emergence. Emergence was low (overall average, 16%) despite high seed viability in all species and provision of optimum temperatures and ample moisture in two of the three watering treatments. All six species exhibited greatest emergence from 0–6-mm depth. The effect of seed size was important under dry conditions close to the surface. Species responded differently to the three watering treatments: *Eucalyptus melanophloia* was the only species to exhibit maximum emergence in the 'dry' watering treatment. The floodplain species, *E. camaldulensis*, *E pilliganesis* and *E. populnea*, had highest emergence under flood conditions, and *E. blakelyi* and *E. melliodora* exhibited intermediate emergence in relation to all three soil-moisture treatments. Vertosol soil conditions are harsh for eucalypt germination and seedling emergence. Although direct seeding of these species in vertosol soils may be successful on occasions, suitable conditions for seedling emergence will probably be infrequent and alternatives to direct seeding may need to be considered for these species.

Significance of the research and practical contributions

This research contributed to theory and practice by: (1) providing new knowledge on the seasonal temperature conditions most suitable for the germination of seeds of 73 plant species that are important structural components of six endangered ecological communities in north-western NSW; (2) proposing effective pre-treatments for large quantities of seed of *Acacia* species prior to direct seeding; (3) producing new information on germination constraints in these species and methods to overcome them in several species that had not been documented previously, permitting their inclusion in existing classifications of germination constraints; (4) confirming techniques to overcome dormancy or germination constraints in species for which the information was known for related congeneric species, allowing the methods to be applied for plant propagation and revegetation in both nursery and field; (5) generating information that allows species' requirements for seed germination to be projected to the environmental conditions at sites where direct seeding is planned, including sowing season, to increase the chances of restoration success; (6) rejecting popular ideas about seed dormancy in some species when the seed constraints are actually related to seed viability and vice versa, and (7) expanding our knowledge of the ranges in temperature, moisture and sowing depth and the combinations of these interacting conditions that are favourable for germination. These points are expanded on below.

1. The lack of knowledge of seed characteristics and germination traits in native species constitutes one of the main obstacles to the use of native seed for revegetation (Hay and Probert 2013). Numerous Australian species have not yet been examined in relation to their germination traits (Bell 1999; Clarke *et al.* 2000; Jurado and Westoby 1992; Merritt *et al.* 2007). The results obtained in this research contribute to knowledge by providing useful insights into 73 native plant species from north-western NSW, although many information gaps still need to be filled in order to build a solid knowledge base about the seed ecology of these species.

2. Restoring biodiverse communities by large-scale revegetation requires the use of large quantities of seed ready for propagation, as seeds are the principal means of revegetation (Merritt and Dixon 2011). This research proposes effective pre-treatments for preparing large quantities of seed of *Acaci*a species for direct seeding. However, broad-acre restoration of ecological communities requires scaling up the variety of species and ensuring that dormancy and other constraints

have been overcome in at least a proportion of the seed of each species so that it is ready to geminate, not only bulking up the quantity of seed.

3. The results of treatments and environmental conditions for increasing germination percentage in these 73 species will be useful for seed management. However, the results and conclusions drawn from this research were based on experiments with few or mostly only one seedlot per species, and therefore cannot be assumed to apply to the diversity of populations of each species, even in north-western NSW. Germination response to environmental conditions and seed pre-treatments varies not only among seeds in the same population or from the same maternal plant, but also among seedlots of different ages and provenances (Bischoff *et al.* 2006; Burrows 2000; Close and Davidson 2003; Pohlman *et al.* 2005). Consequently, differences in germination response to a given treatment can be expected among seedlots of the same species.

4. Seed users need to be able to integrate knowledge and technology for effective seed use, to cope with the demands of large-scale restoration (Mangold 2012). This research provided practical recommendations for effective seed use and seed management for revegetation, emphasising ‘low-tech.’ seed pre-treatments that can be applied directly and easily to seed to increase germination. However, several of these treatments (e.g. extracting endosperms and embryo of *Eremophila* spp. and *Geijera parviflora* from the hard fruit and seed coat) cannot be used to pre-treat large quantities of seed for large-scale revegetation due to labor intensity and cost.

5. Other investigations of the germination response of the seed of native species for revegetating cracking-clay vertosol soils in north-western NSW – the focus of the research reported in this thesis – have been conducted recently by Arnold *et al.* (2014b). The research reported in this thesis includes the germination response of six eucalypt species, which had not been tested previously under varying environmental conditions in vertosols. The information includes guidelines for direct seeding of eucalypts in vertosols. In general, the experiments conducted as part of this thesis attempted to match species' requirements for seed germination with the environmental conditions occurring at sites where direct seeding is projected (i.e. seasonal temperatures, rainfall, light).

6. At present, management of seeds for restoration is undertaken largely by end users (seed suppliers, non-governmental organisations, and individuals or community-based groups supported by government programs) who frequently have restricted access to scientific information and training (Merritt and Dixon 2011). As a result, mistaken beliefs are commonly encountered about germination constraints, based on experience but lacking the scientific rigour to interpret observations correctly. This research was able to reject popular notions about seed constraints attributed to dormancy in some species, which are actually related to seed viability and vice versa.

7. Because seeds in nature respond to numerous interacting environmental factors rather than individual factors for germination, it is important to assess germination response in relation to interacting stimuli simultaneously (Pywell *et al.* 2003). This thesis contributed by expanding knowledge of the environmental ranges and

combination of treatments and environmental factors that stimulate germination in various species.

As the seed of closely related taxa (other sub-species, congeners or members of the same family) frequently share seed characteristics and germination traits (Baskin and Baskin 2014), techniques useful for relieving dormancy or germination constraints in related species might be incorporated where information at the species level is not available. However, tests should be conducted to confirm the utility of such treatments prior to use in restoration in the nursery or field.

Management recommendations

As demonstrated in Chapters 3 and 5, the germination response of a species can vary with seedlot. Therefore, it is important to keep records of seedlot collections, comprising the date, collection site, storage and any other handling issues. These data provide useful information about the idiosyncrasies of seed ecology and germination traits specific to seedlots, and help expand information around intraspecific genetic and phenotypic variability (Violle *et al.* 2012).

Seed management practices would benefit by including strategies for handling, cleaning and storing seed between the point of collection and eventual use, to minimise negative influences on seed viability that may be important when seed is stored, as seen in Chapter 5. In particular, rigorous testing of the viability and quality of each seedlot must be conducted each time prior to using seed, to avoid using seed that has been rendered unfit through inadequate handling and management practices.

Stored seed should also be tested periodically to avoid storing unviable seed, corroborate viability and detect issues that arise during storage.

Pre-treatments and environmental conditions that resulted in maximum germination percentages in Chapters 3–6 are recommended for use with other seed of the same species. However, variations in seed attributes due to seedlot can result in different outcomes. Nevertheless, in the case of seed thought to require specific pre-treatments to increase germination (species in Chapter 5), overcoming dormancy or other germination constraints must be assured or confirmed prior to using the seed in restoration programs.

Site monitoring of environmental conditions and weather forecasts prior to direct seeding in the field is vital to predicting sowing conditions associated with a high likelihood of seed germination and seedling establishment. Ideally a match between seed requirements for germination and the sowing environment should be sought, and the likelihood of success estimated (as in Chapter 6). Additionally, other factors such as the timing of germination and seedling growth rate in relation to soil conditions, seedling hardiness, competition, and other factors can determine the success of seedling emergence and need to be considered prior to sowing. This situation was clearly illustrated comparing the results of Chapter 3 where eucalypt germination was mostly high and Chapter 6 where several of the same species exhibited low emergence.

Limitations of the work

Although the seed treatments used in this research met the objective of increasing germination percentage in most species (Chapters 3–6), not all of the practices may be feasible for large-scale, commercial propagation due to high labour costs (e.g. some species in Chapter 5). In such cases, the trade-off between benefits and costs of particular treatments per seedlot will need to be established prior to use of the seed.

All pre-treated seed in this research was germinated under controlled or semi-controlled conditions, with the intention of being able to extrapolate the results to nurseries for the production of seedlings as well as to direct seeding in the field. However, it is possible that some seedlots may respond differently under field conditions to those experienced in the glasshouse or germination cabinet. Variable responses should be expected from time to time.

As shown by all the experiments in this thesis, the provision of suitable conditions for the time required until germination is essential. This can prevent other germination constraints such as secondary dormancy, which occurs when conditions to which seed are exposed are unsuitable for germination (Baskin and Baskin 2004b; Karssen 1980).

Direction of future research

Seed availably for this project was dependent on the seasonal conditions for seed production prior to and during the research, as well as the accessibility of collection sites, collectors' time and technical resources. This allowed the testing of the seed of 73 priority species from the six endangered communities of interest in north-western

NSW. However, these communities comprise a much wider diversity of plant species across all vegetation strata (tree, shrub, vine, herb, etc.). Further experiments should be undertaken with the remaining species to increase the fidelity of the future restoration of these plant communities and promote revegetation of plants in all of the available life-form niches to reduce weed invasion.

In several of the species tested, the percent germination obtained did not match the percent seed viability, despite the increase in germination percentage of treated seeds over untreated control seed. Therefore, more suitable seed pre-treatments that increase germination to match the seed viability percentage should be sought. Similarly, more research is needed for most species to find treatments that are commercially viable for treating the quantity of seed required for broad-acre restoration.

More research that compares the factors that affect germination under controlled, semi-controlled and field conditions would be useful to better understand the interactions between environmental factors and their synergistic impact on germination in the different situations. This would help researchers better understand, manage, target or avoid the factors that promote or constrain germination.

Successful germination and emergence do not guarantee seedling establishment (Ceccon *et al.* 2015; Moles and Westoby 2006). Research on the plant life-stages subsequent to germination would also be useful to better understand the factors leading to the success or otherwise of different restoration practices.

Conclusions

Low or null seed viability and the need for seed pre-treatment were responsible for the poor germination of some plant species common to endangered communities in north-western NSW, species which had been reported as problematic or 'recalcitrant' by seed users. The information provided about seed viability, environmental requirements and pre-treatments required for maximum germination should be useful for seed users and revegetation programs, either through production of seedlings in the nursery or direct seeding in the field. The seed of several of the species tested does not require pre-treatment for germination do respond to ambient environmental conditions, which can either have a positive effect, stimulating germination, or a limiting effect. The germination of seeds with a germination constraint was generally greater after pre-treatment than under control conditions. Therefore, it is worthwhile investing in research to find suitable conditions and treatments before embarking on plant propagation with seed. More than one method can be used to pre-treat the seed of species to improve germination. It is important to select the most suitable option in terms of germination outcomes, costs, feasibility and time.

Field conditions such as those encountered with vertosol soils can be harsh for germination and seedling emergence. However, windows of opportunity do occur and planning and forecasting will maximise the likelihood that direct seeding on these soils is timed to occur at a time when most or all species are most likely to germinate and emerge. Given the difficulties associated with floodplain vertosols, however, aerial broadcasting of seed after floods may be more successful than using conventional seeders on the ground and trying to time sowing for between antecedent rain that fills the soil moisture store and sufficient and immediate follow-up rain to maintain sufficient moisture in the topsoil.

Glossary

Biodiversity refers to biota in terms of taxonomic and genetic diversity, the variety of life forms present and the community structure thereby created, and the ecological roles performed.

Dieback is the death of trees, usually produced by weakening environmental effects or diseases working against trees for long periods, even years until the tree cannot cope and dies.

Direct seeding is a revegetation technique that involves sowing seed directly into prepared ground.

Dormancy is a property of some seeds, inhibits germination of viable seed under environmental factors that would be otherwise favorable for germination.

Ecological restoration is the process of assisting the recovery of an ecosystem that has been degraded, damaged, or destroyed. A legitimate and indeed important object of much ecological restoration is the reintegration of fragmented ecosystems and landscapes, rather than focusing on just a single ecosystem.

Ecosystem consists of the biota (plants, animals, microorganisms) within a given area, the environment that sustains it, and their interactions. A **natural landscape** or **ecosystem** is one that developed by natural processes and that is self-organizing and self-maintaining.

Ecosystem functions are the dynamic attributes of ecosystems, including interactions among organisms and interactions between organisms and their environment. They are the basis for self-maintenance in an ecosystem.

Ecosystem services: refers to the benefits humankind obtains from ecosystems. These include provisioning services such as food, fibre, medicines and water; regulating services such as flood and disease control; soil formation; pollination; cultural services such as spiritual, recreational, educational and aesthetics; and supporting services such as nutrient cycling that maintain the conditions for life.

Environment what sustains the biota of an ecosystem includes the soil or substrate, the atmospheric or aqueous medium, hydrology, weather and climate, topographic relief and aspect, the nutrient regime, and the salinity regime.

Functional groups Assemblages of organisms can also be recognized by their functional roles in the ecosystem (e.g. primary producers, herbivores, carnivores, decomposers, nitrogen fixers, pollinators). All functional species-groups must be represented if a restored ecosystem is to maintain itself

Habitat refers to the dwelling place of an organism or community that provides the requisite conditions for its life processes.

Landscape consists of a mosaic of two or more ecosystems that exchange organisms, energy, water and nutrients.

Land degradation is a process in which the quality of the biophysical environment (land, soil, vegetation, water) is deteriorated by excessive or inappropriate explotaition caused by human-induced processes acting upon the land. It is viewed as to be harmful or undesirable.

Land reclamation is the gain of land from the sea, or wetlands, or other water bodies, but it is also the restoration of productivity or use to lands that have been degraded by human activities or impaired by natural phenomena. Land reclamation frequently requires inputs which are costly, labour-demanding or both.

Land rehabilitation shares with restoration a fundamental focus on historical or pre-existing ecosystems as models or references, but rehabilitation emphasizes the reparation of ecosystem processes, productivity and services.

Land use comprises the management and modification of the environment though uses such as settlements, agricultural land, pastures or managed forests.

Native biodiversity refers to species that evolved in that geographic area, and occur naturally there, as opposed to having been transported by humans in recent times.

Natural regeneration is the term used to describe the growth of plants from seed naturally distributed to the site. Natural regeneration relies on existing seed sources, such as soil or canopy stored seed, or seed transported to the site by water, wind or animals in the area to be revegetated.

Pantropical is the distribution or occurrence throughout the tropical regions of the earth.

Reference ecosystem is an actual site, its written description, or both, that serves as a model for planning a restoration project and its later evaluation.

Reforestation is the re-establishment of a depleted forest and woodlands

Revegetation is the process of reconstructing vegetation and habitat through planting, direct seeding, brush matting and transplanting as well as rebuilding the soil of disturbed land.

Scarification of seed is the weakening, opening, or disruption of the seedcoat used to encourage germination.

Self-mulching is the capability of various heavy clay soils, usually vertosols to re-aggregate its clay forming a loose granular mulch of fine aggregates at the surface after wetting and drying, or freezing and thawing

Species composition is the taxonomic array of species present, and species richness, i.e. the number of different species present.

Stratification is a method used to relieve seed physiological dormancy that consists in the treating stored or collected seed prior to sowing to simulate natural winter or summer conditions that a seed must endure before germination.

Tubestock Planting or **'Tubestock'** is the term for seedlings that have been raised in small nursery tubes, for transport to the planting site

Wildlife is the native fauna and flora of a region that grow in natural conditions usually independently of people.

Appendix 5.1 List of all seedlots used in this research, their viability (percentage), seed age at the time of testing, and optimum temperature for germination based on statistical analyses of deviance. Taxonomic nomenclature as in Australia's Virtual Herbarium (AVH 2015)

Species	Family	Viability		Seed Age	Germination season (optimum)	*P*	Collection site	
		(%)	Test	(years)			Latitude	Longitude
Acacia harpophylla	Fabaceae	99.8	TTZ	0	Any	ns	−29.4149	149.7063
Acacia harpophylla SL 2		0		3			−30.4149	148.7700
Alectryon oleifolius	Sapindaceae	83.5	TTZ	0	Spring/autumn		−29.8694	149.7971
Alectryon oleifolius SL 2		4.2		3			−29.0481	149.6550
Allocasuarina littoralis	Casuarinaceae	65.3	TTZ	U	Spring/ autumn	***	−30.2823	152.0185
Alstonia constricta	Apocynaceae	88.1	TTZ	0	Summer	***	−29.5565	149.6573
Alstonia constricta SL 2		3.8		5			−29.0541	150.4778
Angophora floribunda	Myrtaceae	55.7	CUT	1	Summer, spring/ autumn †	ns	−30.7586	151.3624
Apophyllum anomalum	Capparaceae	85.6	TTZ	0	Summer	***	−28.8917	150.2847
Atalaya hemiglauca	Sapindaceae	89	TTZ	2	Summer, spring/ autumn †	ns	−28.7846	149.3697
Atriplex nummularia	Chenopodiaceae	84.3	SQ	3	Spring/ autumn, winter	***	−29.0173	149.2555
Atriplex semibaccata		76.9	SQ	0	Summer	***	−29.8132	148.8991
Brachychiton populneus	Malvaceae	80.3	TTZ	4	Spring/autumn	**	−28.6053	150.3596
Cadellia pentastylis	Surianaceae	87.4	TTZ	0	Summer, spring/ autumn †	ns	−29.8482	150.1567
Capparis lasiantha	Capparaceae	78.7	TTZ	0	Spring/ autumn	ns	−29.8874	148.9745
Capparis lasiantha SL 2		4.6		3			−28.6848	149.9701
Capparis mitchellii		81.5	TTZ	0	Spring/ autumn, summer	ns	−30.3464	149.6828
Capparis mitchellii SL 2		27.4		4			−31.1886	150.3048
Casuarina cristata	Casuarinaceae	77.7	TTZ	4	Summer, spring/ autumn †	ns	−29.2698	150.121
Casuarina cunninghamiana		53.8	TTZ	4	Summer	*	−30.0327	150.3153
Clematis microphylla	Ranunculaceae	85.2	CUT	0	Spring/autumn	***	-30.5236	151.6718

Corymbia tessellaris	Myrtaceae	86.9	TTZ	4	Summer†	*	-29.462	149.7362
Cullen tenax	Fabaceae	76.4	TTZ	1	Spring/autumn	ns	-29.6571	149.2982
Desmodium brachypodum		71.5	TTZ	4	Spring/autumn	***	-29.8756	150.5589
Dodonaea viscosa	Sapindaceae	85.1	TTZ	5	Summer	***	-31.0093	150.199
Ehretia membranifolia	Boraginaceae	79.3	CUT	U	Winter	***	-29.8694	149.7971
Einadia nutans	Chenopodiaceae	83.9	SQ	1	Summer	***	-31.0975	150.2705
Elaeodendron australe	Celastraceae	73.2	TTZ	4	Spring/autumn		-30.1545	149.9315
Enchylaena tomentosa	Chenopodiaceae	94.6	TTZ	1	Any	ns	-29.5577	149.6216
Eremophila debilis	Scrophulariaceae	85.4	TTZ	4	Spring/autumn	***	-30.1839	149.5651
Eremophila maculata		79.3	TTZ	4	Spring/autumn	***	-29.4737	149.7857
Eremophila mitchellii		68.9	TTZ	3	Spring/autumn	**	-30.9575	150.0262
Flindersia maculosa	Rutaceae	99.7	TTZ	1	Spring/autumn		-29.0220	149.0770
Geijera parviflora		95.8	TTZ	1	Spring/autumn	***	-30.3731	148.8300
Geijera parviflora SL2		53.1		3			-29.7746	144.6466
Glycine clandestina	Fabaceae	94.5	TTZ	4	Summer	*	-29.8760	150.5589
Grevillea striata	Proteaceae	86.6	TTZ	4	Spring/autumn		-30.168	149.9404
Jasminum lineare	Oleaceae	76.8	TTZ	4	Spring/autumn		-29.9341	149.8473
Jasminum suavissimum	Oleaceae	66.7	TTZ	4	Spring/autumn		-30.9886	150.5655
Lomandra longifolia	Asparagaceae	95.2	TTZ	0	Spring/autumn	***	-30.0071	152.0244
Maireana brevifolia	Chenopodiaceae	89.2	CUT	3	Spring/autumn	**	-29.4674	149.7713
Maireana brevifolia SL2		0		5			-29.4737	149.7857
Melaleuca bracteata	Myrtaceae	95.7	SQ	3	Any	ns	-30.1602	149.9669
Melaleuca trichostachya		78.9	SQ	3	Any	ns	-29.4630	148.7142
Myoporum montanum	Scrophulariaceae	89.4	TTZ	1	Summer	**	-31.1915	150.3522
Notelaea microcarpa	Oleaceae	52.7	TTZ	4	Spring/autumn	***	-32.0000	150.0000
Owenia acidula	Meliaceae	80	CUT	4	Summer	***	−29.2738	150.3522

Pandorea pandorana	Bignoniaceae	88.6	TTZ	3	Any	ns	−29.8646	150.5892
Pittosporum angustifolium	Pittosporaceae	90.5	TTZ	3	Spring/autumn, summer	ns	−29.7564	150.0173
Rhagodia spinescens	Chenopodiaceae	90.7	TTZ	2	Winter	**	−29.8130	154.9177
Santalum lanceolatum	Santalaceae	68.3	TTZ	4	Spring/autumn		−30.6272	150.1705
Senna artemisioides	Fabaceae	88.2	TTZ	3	Spring/autumn	***	−31.1886	150.3048
Solanum cinereum	Solanaceae	76.8	TTZ	4	Winter	***	−30.5857	150.1666
Sporobolus elongatus	Poaceae	93.1	SQ	3	Spring/autumn	***	−30.4019	151.3200
Ventilago viminalis	Rhamnaceae	0	TTZ	5	Summer, spring/autumn	***	−29.8694	149.7971
Ventilago viminalis SL 2		88.3		3			−29.9034	149.7514
Xerochrysum bracteatum	Asteraceae	67.4	SQ	5	Spring/autumn	***	−31.2545	150.2583

Viability test: TTZ = Tetrazolium, CUT = Cutting test, SQ = Squash. Age: 0 = fresh seed tested shortly after collection; U = unknown age; **P* ≤ 0.05; ***P* ≤ 0.01; ****P* ≤ 0.001; ns, *P* > 0.05

Appendix 5.2 List of species used, author names and recommended, seed pre-treatments for germination found in scientific and grey literature.

Species	Seed Pre-treatments
Acacia harpophylla F.Muell. ex Benth.	No seed pre-treatment required; this species is not hard-seeded (Reichman *et al.* 2006)
Alectryon oleifolius (Desf.) S.T.Reynolds	Wash seed for a few days before sowing; this can help with the germination (Bonney and Miles 2003).
Allocasuarina littoralis (Salisb.) L.A.S.Johnson	No pre-treatment required; seed germinates between 15 and 35°C, but optimum between 25 and 35°C (Crowley and Jackes 1990)
Alstonia constricta F.Muell.	No seed pre-treatment required (David Carr, personal communication)
Angophora floribunda (Sm.) Sweet	No seed pre-treatment required (Gunn 2001)
Apophyllum anomalum F.Muell.	No seed pre-treatment required (Murray 2003)
Atalaya hemiglauca (F.Muell.) F.Muell. ex Benth.	Remove seed samaras and husk, and treat with hot water to increase germination (Langkamp 1987). 100% germination obtained when seed scarified (with a scalpel) and sown in agar (Boland *et al.* 1980; Murray 2003)
Atriplex nummularia Lindl.	Germination facilitated by washing salt from bracteoles before sowing by placing seeds under running water and rubbing for several minutes (Langkamp 1987). Alternatively, rinse seeds in flowing water for approximately an hour (Florabank 2010). Seeds germinate when fruit bracts are removed. If seed removed from fruit, washing not necessary (Bonney and Miles 2003)
Atriplex semibaccata R.Br.	No seed pre-treatment required (Florabank 2010)
Brachychiton populneus (Schott & Endl.) R.Br.	Treat with hot water and allow seed to soak overnight although may not be always necessary (Florabank 2010; Langkamp 1987)
Cadellia pentastylis F.Muell.	Apply heat (http://www.environment.nsw.gov.au)
Capparis lasiantha R.Br. ex DC.	Removing the black seed from the berries and washing seed before sowing may improve germination. Seed may be prone to damping off (Murray 2003)
Capparis mitchellii Lindl.	Pour boiling water on to seeds for pre-treatment and alternate 15/25°C with 12/12-hour light/dark (71% germination)(Waratah Database 2001). Remove dispersal units and scarify with sandpaper (ANBG 2016)
Casuarina cristata Miq.	Inoculate with *Frankia* for best results (Waratah Database 2001). Light required (Gunn 2001; Langkamp 1987)
Casuarina cunninghamiana Miq.	No seed pre-treatment required (Florabank 2010), but light a prerequisite for germination (Langkamp 1987)
Clematis microphylla DC.	Pre-treat with gibberellic acid to increase rate of germination (Lush *et al.* 1984). Remove awns and provide sufficient moisture (Langkamp 1987)
Corymbia tessellaris (F.Muell.) K.D.Hill & L.A.S.Johnson	No seed pre-treatment required (Florabank 2010)
Cullen tenax (Lindl.) J.W.Grimes	Apply high temperatures or other mechanism to crack seed coat (e.g. scarification) and allow water imbibition (Morgan 1998)
Desmodium brachypodum A.Gray	No references found for this species but seeds of other *Desmodium* spp. benefit from light, smoke and 1 year storage; heat and chilling (Clarke *et al.* 2000)

Species	Seed Pre-treatments
Dodonaea viscosa Jacq.	Untreated seed can germinate over a long period due to thick seed coat that restricts water entry. Nicking seed coat or hot water treatment (just off the boil) followed by water cooling in cold water hastens germination (Murray 2003). Dunk seed in very hot water for 30 seconds, and cool down rapidly, to shorten germination time (Bonney and Miles 2003; Florabank 2010; Sweedman and Merritt 2006). Boil in water for 5 seconds (Hodgkinson and Oxley 1990). Soak in very hot water prior to sowing to assist germination (ANBG 2016)
Ehretia saligna var. **membranifolia** (R.Br.) Randell	Soak in very hot water (95°C) for about 2 minutes (Waratah Database 2001)
Einadia nutans (R.Br.) A.J.Scott	No seed pre-treatment required (Leishman and Westoby 1998)
Elaeodendron australe Vent.	Remove tough seed coat to permit germination (Curran *et al.* 2013). Seed needs to be breached by filing, chipping, cracking or accelerated composting (Erskine *et al.* 2005)
Enchylaena tomentosa R.Br.	Apply smoke water treatment for 1 hour to significantly improve germination (Murray 2003). Keep moist, add small amounts of salt mixed into potting soil for germination, or soil from where plants are growing (Bonney and Miles 2003). At 20°C, 84% germination can be obtained by removing succulent tissue and seed cleaning (ANBG 2016)
Eremophila debilis (Andrews) Chinnock	Sow from ripe, washed fruits; split fruits in halves and quarters to extract seeds prior to sowing; some seeds are damaged during process (www.greeningaustralia.org.au). Scarify fruit to promote uptake of water and oxygen (Richmond and Chinnock 1994)
Eremophila maculata (Ker Gawl.) F.Muell.	Remove seed coat for good germination (Murray 2003; Richmond and Ghisalberti 1994). Soak seed in warm water for up to 16 hours, then split hard woody fruit (Bonney and Miles 2003)
Eremophila mitchellii Benth.	Scarify fruit to promote uptake of water and oxygen (Richmond and Chinnock 1994)
Flindersia maculosa (Lindl.) Benth.	No seed pre-treatment required (Gunn 2001)
Geijera parviflora Lindl.	Fracture seed coat under pressure, then totally remove seed coat (Mole 1996; Murray 2003). Soak in warm water (20-25°C) for 2–4 hours as a general treatment (Waratah Database 2001). Soak overnight (Gillespie *et al.* 1998),or soak for 72 hours (Jurado and Westoby 1992)
Glycine clandestina J.C.Wendl.	Place seeds on preheated soil at 80°C for 60 minutes to obtain approximately 90% germination (Auld and O'Connell 1991)
Grevillea striata R.Br.	No records were found for this species. However, germination of other *Grevillea* species benefit from peeling waxy seed coat, pouring warm to hot water over seed, and then soaking for 24–48 hours. Smoke treatment combined with soaking may provide good results (Murray 2003)
Jasminum didymum subsp. ***lineare*** (R.Br.) P.S.Green	Cover freshly collected and cleaned seeds with 8-mm of seed raising media or sterilised sand (Northern Territory Herbarium 2013)
Jasminum suavissimum Lindl.	No seed pre-treatment required (Australian Native Plants Society http://anpsa.org.au)
Lomandra longifolia Labill.	Sow seed in moist soil for 6 weeks (ANBG 2016). Soaking can improve germination of other *Lomandra* species (Murray 2003). Removal of inner pericarp by leaching overcomes inhibition in other *Lomandra* species. Soak in GA_3 (50 mg L) for 24 hours to remove remaining inhibitors further improve germination; incubation at autumn temperatures for 3 months promotes germination in other *Lomandra* species (Plummer *et al.* 1995)

Species	**Seed Pre-treatments**
Maireana brevifolia (R.Br.) Paul G.Wilson	Sow in moist substrate to promote germination (Langkamp 1987). Seed germinates easily if covering structure is removed prior to sowing (Florabank 2010)
Melaleuca bracteata F.Muell.	No seed pre-treatment required (Gunn 2001)
Melaleuca trichostachya Lindl.	No seed pre-treatment is required (Gunn 2001)
Myoporum montanum R.Br.	Remove flesh from fruit and soak overnight in warm water before sowing (Murray 2003). Soak seeds in warm water overnight before sowing (Bonney and Miles 2003)
Notelaea microcarpa R.Br.	Remove flesh from seed before sowing (Murray 2003)
Owenia acidula F.Muell.	Needs light and constant moisture, not wet (http://www.sunshine-seeds.de/).
Pandorea pandorana (Andrews) Steenis	No seed pre-treatment required (ANBG 2016).
Pittosporum angustifolium Lodd.	Mix seed with small quantity of coarse sand, then wash in detergent or washing powder mixed with water, and rub to help remove inhibitor; drain water and repeat once or twice (Murray 2003). No pre-treatment required (Florabank 2010)
Rhagodia spinescens R.Br.	Soak in smoke water 1:10, 1:100 or 1:1000 dilution for 24 hours (Sweedman and Merritt 2006). Seed of most species germinates easily without treatment (Florabank 2010; Murray 2003)
Santalum lanceolatum R.Br.	Remove outer shell to improve germination and reduce the time for germination. Results can be improved by soaking in Gibberellic acid (GA_3) solution and shell does not have to be removed in this case. Addition of iron chelate, composted leaf litter and fine gravel in potting media may improve results (Murray 2003). Soak in GA_3 0.1% solution for 24 hours (Sweedman and Merritt 2006)
Senna artemisioides (Gaudich. ex DC.)	Soak in very hot (95°C) or boiling water for about 2 minutes (Sweedman and Merritt 2006). Scarification or nicking seed may give good results, but apparently seed coat hardens as the seed ages, and more aggressive treatment is required with older seeds (Murray 2003). Pour hot water over seed, allow to cool, skim off floating seed, drain off excess water and dry (Bonney and Miles 2003)
Solanum cinereum R.Br.	Wash seed before sowing. Treatment with smoke water may improve results (Murray 2003)
Sporobolus elongatus R.Br.	Sow after 9 months for physiological breakdown in dormancy to occur. Has an obligate light requirement for germination (Lodge and Whalley 1981a)
Ventilago viminalis Hook.	Enough moisture to promote germination required (Langkamp 1987). If germination less than 20%, scarification with sandpaper required (ANBG 2016)
Xerochrysum bracteatum (Vent.) Tzvelev	No seed pre-treatment required but GA_3 can improve seed performance and seedling emergence (Grzesik and Nowak 1998)

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