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Identifying and reversing ecological barriers to successful farmland revegetation specific to tubestock planting and direct seeding in northern New South Wales

Submitted by:

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This thesis is dedicated to my husband Paul Brown with eternal love.

Abstract

Revegetation in agricultural regions across the globe has intensified over past decades in an effort to reverse widespread land degradation and conserve natural ecosystems and the biodiversity they contain. Biodiversity is essential for the physical, economic, social and cultural dimensions of human well-being, but agricultural intensification has resulted in the loss of millions of hectares of forests and natural vegetation globally. In Australia, a brief, but intense history of land clearing has resulted in the loss of 50% of forest ecosystems, with over 80% of eucalypt-dominated woodlands and forests having been altered by human endeavour. The situation on the Northern Tablelands of New South Wales reflects land clearing practices throughout the country with an estimated loss of tree cover of 50% to date. Although land clearing has eased in the past decade, tree decline continues due to recurrent episodes of rural dieback. To address this problem, substantial efforts have been made by revegetation organisations, practitioners and landholders to re-establish native trees in the region, but plantings often fail. This research was conducted to identify and reverse ecological barriers preventing the success of revegetation in temperate upland pastures. The work focused on tubestock plantings and direct seeding.

The first study was conducted to determine if existing native shelterbelts can be evaluated in terms of survival and growth to identify the environmental stresses influencing planted eucalypt establishment and growth on the Northern Tablelands. Most ‘on-ground’ revegetation is designed and implemented with no thought given to follow-up scientific monitoring. Monitoring is important not only to justify the large amounts of public funding directed into revegetation activities, but also because it demonstrates whether targets have been achieved, and provides opportunities to learn from and improve upon past failures. Six-year old shelterbelt plantings consisting of *Eucalyptus nitens, E. pauciflora* and *E. viminalis* were examined to identify potential biotic and abiotic stresses influencing tree performance. Topographic position, altitude, slope, temperature, soil type, soil moisture and weed control were measured and modelled in relation to tree survival and growth (height). The information theoretic approach was used to select the best-fitting model from a set of competing models. Poor weed control and subzero temperatures were identified as the predominant stresses affecting eucalypt survival during the monitoring period. Subzero temperatures also significantly influenced tree growth.

Given these results, the second study compared the performance of five native tree and shrub species grown in tall Corflute® tree guards and milk cartons at three landscape positions (lower slope, mid slope and upper slope) in an open temperate pasture. Seedlings in tall guards survived better than seedlings in milk cartons at mid and upper-slope landscape positions. Height was also greater for seedlings in tall guards than milk cartons at all landscape positions. Eucalypts in particular benefited from tall guards, with height growth up to three times greater than in milk cartons. Tall guards increased the temperature surrounding seedlings inside the guards, extending the growing period.

Next, the efficacy of direct seeding as a revegetation technique was investigated. Prior to conducting this investigation, three trial sites were established and monitored for 3–6 months at Bingara, Ben Lomond and Invergowrie. Recruitment and subsequent establishment was so poor that the trials were considered a failure. This study compared the effects of three sowing methods (KB seeder, modified Chatfield planter and hand sowing) and three bulking materials (rice, chicken crumble and smoked vermiculite) on the recruitment of direct-seeded acacias and eucalypts. Recruitment was highest with the KB seeder followed by the Chatfield seeder and hand-sown methods. There were no significant differences in recruitment among bulking materials. Eucalypt recruitment was low compared to the recruitment of acacias. Recruitment peaked in mid May (8 weeks post-sowing) for acacias and in early July (15 weeks post-sowing) for eucalypts, but declined markedly for both genera during the reminder of the study. Some seedling losses were incurred following the first heavy frost, but most were attributed to an invasion of rat’s tail fescue (*Vulpia myuros*) in late winter, and waterlogging in the lower areas of the site due to above-average rainfall between May and August.

Based on the results of the previous study two subsequent investigations were designed and implemented. To address the problem of weed invasion in direct-seeded revegetation, the effect of eight herbicide oversprays on the survival of 11 native tree and shrub species was examined. Seedling survival was assessed at 1, 2, 7 and 8 weeks post-spraying. Survival was greatest in seedlings treated with imazethapyr and isoxaflutole, and least in seedlings treated with diflufenican and glyphosate. There were also significant differences in survival between species, with *Dodonaea viscosa, Acacia pendula* and *Senna artemisioides* exhibiting the highest tolerance to the greatest range of herbicides, and *Atriplex nummularia, Casuarina cristata* and *Einadia nutans* exhibiting the lowest tolerance. Species varied in their tolerance of different herbicides, due to the selective nature of the different modes of action of the active ingredients and their differential uptake, translocation and metabolism between species.

To address the problem of poor eucalypt recruitment, the effects of three seed-coating treatments (coated seed, seed coated with MycoApply® and uncoated seed), four watering regimes (30 mL per day, 30 mL per 3 days, 30 mL per 5 days and no water), and two seed-sowing methods (surface-sown vs buried beneath a 5-mm vermiculite layer) on eucalypt germination and early establishment were examined. Coating the seed with microbial inoculants as well as daily watering significantly increased germination. The interaction between sowing method nested within coating treatment and watering regime was significant. Soil moisture was essential for eucalypt germination and MycoApply® appeared to enhance water capture during the critical early post-germination phase when desiccation was most likely. When soil moisture was limited, seed burial beneath vermiculite was important because it increased seed-soil contact, providing better access to soil moisture, but only in seed coated with microbial inoculants. Seed-coating treatments did not benefit seedling growth (height) because soil moisture was not limiting.

The final study examined the effects of four weed-control treatments (scalping, glyphosate, sugar and glyphosate, and sawdust and glyphosate) on the recruitment of native grasses and weed suppression. Recruitment of native grass was significantly higher in scalped plots compared to other treatments. The most effective weed-control treatments were scalping and the combinations of sugar and sawdust with glyphosate. Scalping and carbon (sugar and sawdust) addition controlled weeds by depleting weed seed banks, and alleviating soil nutrient enrichment. In combination with glyphosate application, which removed above-ground biomass, these techniques were effective strategies for combatting competitive invasive weeds.

A summary of the main findings, study limitations and recommendations for future research were presented in the final chapter.

Declaration

I certify that the substance of this thesis has not been submitted for any degree and is not currently being submitted for any other degree or qualification. I certify that, to the best of my knowledge, any assistance received in preparing this thesis, and all resources used have been acknowledged.

[redacted]

Sharon L. Brown

9 January 2017

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Note to the examiners

The thesis has been written in the style of a thesis by publications. The formatting aligns with that used by the Australian Journal of Botany for consistency, even though each chapter will be submitted to different journals. As Chapters 2 to 7 have been prepared as manuscripts, there is some repetition for which I apologise in advance. However, it is an inevitable consequence of preparing the thesis in the format of a series of journal articles.

Chapter 2 will be submitted to Agroforestry for publication.

Chapter 3 has been submitted to Applied Vegetation Science for publication.

Chapter 4 will be submitted to Restoration Ecology for publication.

Chapter 5 will be submitted to Ecological Management and Restoration for publication.

Chapter 6 will be submitted to The Australian Journal of Botany for publication.

Chapter 7 has been accepted by the Rangeland Journal for publication.

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“Nowhere else had technological man lately occupied so fragile an ecosystem;

nowhere else had settlers utterly failed to identify with their new landscapes;

nowhere else was man’s environmental impact so patently reprehensible.”

Rolls 1999

CHAPTER 1

Literature Review and Thesis Objectives

1.1 Background

Revegetation in agricultural regions across the globe has intensified over past decades in an effort to reverse widespread land degradation and conserve natural ecosystems and the biodiversity they contain (Pimentel *et al.* 1992; Munro *et al.* 2009). Biodiversity is essential for our physical, economic, social and cultural well-being (Millennium Ecosystem Assessment 2005), yet the intensification of agriculture has resulted in the loss of millions of hectares of forests and natural vegetation globally (Sanderson *et al.* 2002; Scherr and McNeely 2008). Agriculture's ecological footprint is currently estimated to take up at least 30% of the global landscape with crops and pastures and a further 20% with intensive livestock grazing (Scherr and McNeely 2008). Human population projections of a 33% increase by the year 2030 are expected to result in a further allocation of 1 billion ha to agriculture, an area larger than the United States (Tilman *et al.* 2001; Cohen 2003). A major challenge of the future will be to create agricultural landscapes that meet joint production and conservation ideals (Scherr and McNeely 2008). This will require a shift away from traditional farming practices and ideologies towards sustainable eco-agricultural systems that focus on the implementation of ecologically sensitive management strategies (Scherr and McNeely 2008). Increasing native tree cover will play a key role in facilitating this transition (Hobbs 1993; Scherr and McNeely 2008).

In the past, revegetation efforts have often been below expectations and marred by poor survival of native germinants and planted seedlings (Close and Davidson 2003; England *et al.* 2013; Leslie *et al.* 2013; Hallett *et al.* 2014). Successful establishment of native trees is influenced by a complex of factors, including drought (Engelbrecht *et al.* 2005; Hallett *et al.* 2014; Silva *et al.* 2016), cold temperature and frost (Ball *et al.* 1991; Close *et al.* 2000), lack of pre- and post-planting management (Fagg 1988; Prober and Lunt 2008; Carr *et al.* 2009; England *et al.* 2013), and various other biotic and abiotic stresses associated with land management practices (Close and Davidson 2003). To improve revegetation efforts, more effective revegetation methods based on theory and practice need to be developed (Bartholomew 1998).

This is particularly important for future revegetation activities on the Northern Tablelands of New South Wales. The Northern Tablelands is a region of elevated undulating plateaus located atop the Great Dividing Range in northern NSW. The main land uses in the region are wool, lamb and beef production, and forestry (Carr 2009). The region experiences mild summers and cold winters (Armidale Airport, –30.53º, 151.62º; 1079 m a.s.l.; January maximum daily temperature, 25.9ºC, minimum daily temperature, 13.2ºC; July maximum daily temperature, 12.1ºC, minimum daily temperature, 1.2ºC). Mean annual rainfall ranges between 600 mm and 1100 mm, with 60% of rainfall occurring in summer (October–March) (Bureau of Meteorology 2015). The clearing of the native woody cover in the region began in the mid 1800s and reached a peak in the 1920s and 1930s (Wilson and Lonegran 2013). However, subsequent tree loss has been exacerbated by the dieback of eucalypts and less than 50% of the original tree cover remains (Nadolny 2008). Re-establishing trees on the tablelands is challenging because of the cold temperatures, frosts and waterlogging experienced in winter. While exotic species, such as pines, poplars, elms and oaks tend to survive quite well, native species often succumb under these conditions, resulting in poor revegetation outcomes (Reid *et al.* 2013; Gordon Williams and Cam Banks, pers. comm.).

This chapter provides an introduction to the research by first discussing the potential value of native trees on farms (in terms of increasing agricultural productivity and biodiversity conservation), and tree loss in agricultural landscapes. Different revegetation techniques are discussed, as well as the barriers that impede success. Finally, various conceptual frameworks for farmland restoration are identified, followed by the general aims and objectives of the thesis.

1.2 The value of native trees on farms

1.2.1 The economic benefits of native trees

Native trees confer a range of economic benefits to landholders in terms of increased productivity through the sheltering of crops, pastures and livestock (Bird *et al.* 1984; Reid and Landsberg 2000; Cleugh 2003). Shelterbelts are narrow lines of trees that often follow a linear landscape feature, such as a road or fence line, and are an effective way of merging agriculture and biodiversity without financial sacrifice (Fig. 1.1) (Dames and Moore 1999; Cleugh 2003; Eden and Cottee-Jones 2013). Despite arguments that shelterbelts compete for soil moisture and nutrients, there is strong, consistent evidence from studies worldwide demonstrating yield increases in a range of crop and pasture species (Bagley 1988; Kort 1988; Cleugh *et al.* 2002; Brandle *et al.* 2012). Shelterbelts improve growing conditions by reducing wind speed and modifying the surrounding temperature and humidity (Kort 1988; Cleugh 2003). This prevents moisture loss and erosion of topsoil, in addition to ameliorating leaf damage, head loss, lodging and flower abortion in crops (Kort 1988; Reid and Landsberg 2000; Cleugh 2003). Shelterbelts can improve yields of some crops by up to 50% (Bird *et al.* 1984; Brandle and Hintz 1988). In Australia, increased yields in sheltered crops of wheat, oats and lupins of 25%, 47% and 30%, respectively, have been reported (Bicknell 1991; Burke 1991). Shelterbelts also play an important role in providing shade and shelter for livestock (Lynch and Donnelly 1980; Reid and Landsberg 2000; Cleugh 2003; Murgueitio *et al.* 2011). The demonstrated effects of heat stress on beef and dairy cattle include decreases in live weight yield of up to 30%, reduced fertility, as well as increases in abortion rates, low birthweight calves and calf mortality (Cremer *et al.* 1990; Gregory 1995; Moons *et al.* 2014). In addition, milk production is reported to be 26% lower in unsheltered dairy cows compared to those provided with adequate shade (Fitzpatrick 1994).

Shelterbelts are also crucial in mitigating the effects of cold exposure on livestock (Reid and Landsberg 2000). Much of the research demonstrating the capacity of shelterbelts to reduce hypothermia-related mortality has been conducted on the Northern Tablelands of NSW (Lynch and Donnelly 1980; Lynch *et al.* 1980; Bird *et al.* 1984; Hinch *et al.* 2013). In this region, sheep and lamb losses during winter are high because lambing and shearing typically coincide with cold weather (Donnelly *et al.* 1974; Lynch *et al.* 1980). Shelterbelts have been shown to reduce sheep deaths by up to 50% (Reid and Landsberg 2000; Andrews and Thompson 2009). Similarly, neo-natal mortality in lambs can be reduced by half in paddocks protected by shelterbelts (Lynch *et al.* 1980).

Figure 1-1 (A) Native shelterbelt at 'Winterville', Tenderton, NSW, and (B) sheep enjoying the shade provided by planted trees at 'The Hill', Terrible Vale, NSW

Source: Photographs by Sharon Brown

In addition to providing shelter, native trees may offer benefits to landholders in term of livestock nutrition and parasite control. Recent research has demonstrated that native forage trees, including members of the *Mimosaceae, Fabaceae, Chenopodiaceae*, *Myoporaceae* and others contain condensed tannins (CT), which increase protein flow to the small intestine, thus improving nutrition and immune response to infection, as well as increasing liveweight and the rate of wool growth. In addition, CTs have anthelmintic properties by inhibiting the development of eggs and larvae of intestinal parasites (Molan *et al.* 1999; Kahiya *et al.* 2003; Kotze *et al.* 2009). When forage crops containing CTs are fed to grazing livestock (sheep, goats and deer) it is possible to minimise contamination of pasture with infective larvae and reduce dependency on anthelmintic drenches (Molan *et al.* 1999).

1.2.2 Conserving biodiversity in eco-agricultural systems

Conflict between the competing needs of agriculture and biodiversity conservation exists worldwide (Waters *et al.* 2013). Rural livelihoods need to be maintained, and demands for agricultural land to produce food, fuel and fibre will increase as predictions of a burgeoning human population become a reality (Fischer *et al.* 2008; Cary and Roberts 2011; Waters *et al.* 2013). At the same time, biodiversity is being lost at an unprecedented rate due to the destruction of natural habitats, fragmentation and loss of fine-scale landscape connectivity (Tscharntke *et al.* 2005). Productive agricultural landscapes are more reliant on plant and animal biodiversity in adjacent natural ecosystems than once thought (Altieri 1999). Natural ecosystems perform a variety of ecosystem services, essential to primary productivity (Pimentel *et al.* 1992; Fischer *et al.* 2008; Scherr and McNeely 2008; Power 2010). Ecosystem services are defined as the biological underpinnings essential to economic prosperity and other aspects of human well-being (Daily 1997). In eco-agricultural systems, the ecosystem services that flow from natural vegetation contribute substantially to the health and wealth of productive landscapes through the provision of clean water (by regulating the capture, infiltration, retention and flow of water across the landscape) and the maintenance of soil health though the input of organic matter and enhanced nitrogen fixation from native vegetation (Pimentel *et al.* 1992; Power 2010). In addition, native trees provide habitat for a range of beneficial insects that are essential for crop pollination, as well as supporting populations of birds and insect species that are the natural enemies of many common crop pests (Pimentel *et al.* 1992; Swinton *et al.* 2007; Power 2010).

Over the past century, nature reserves and national parks were created to balance land transformation and intensive land use, playing a key role in biodiversity conservation. With the encroachment of agricultural landscapes, less than 6.4% of global land area is currently allocated to conservation (Bengtsson *et al.* 2003). There is a need to reconcile production and production-dependent rural livelihoods with healthy ecosystems by developing eco-agricultural systems that allow the coexistence of nature and agriculture (Scherr and McNeely 2008). Such integrated systems have the potential to generate co-benefits for production, biodiversity and local people (Scherr and McNeely 2008).

1.3 Tree loss in agricultural landscapes

Global tree loss in agricultural landscapes is primarily attributed to agricultural intensification and its consequences (Lindenmayer and Burgman 2005). Land clearing is the most significant and widely reported cause of natural ecosystem destruction and degradation (Reid and Landsberg 2000; Lindenmayer and Burgman 2005). Estimates of land clearing suggest that more than 50% of global terrestrial ecosystems have been totally cleared and a further 30% are degraded or modified (Lindenmayer and Burgman 2005). In Australia, most land clearing has occurred in the last 100 years due to the invention of mechanised farm equipment (Bullen *et al.* 1990). By the 1980s, 38% of Australia's forests had been severely modified by clearing (Wells *et al.* 1984). The most recent estimate shows that Australia's native forests constitute only 19% of total land mass (Bureau of Rural Sciences 2010).

On the Northern Tablelands of NSW, eucalypt woodlands have been extensively cleared to make way for improved pasture and cultivation (Curtis 1990a). The region remains one of the most highly cleared parts of Australia (Bradshaw 2012). Land management proposals for the Namoi, Gwydir and Macintyre River catchments have estimated that to sustain land management on the Northern Tablelands, at least 887 000 ha need to be revegetated (Peasley 1995; Donaldson 1996; Donaldson and Heath 1997).

Tree loss is also associated with agricultural practices that lead to land degradation by altering the physical, biological and chemical properties that maintain heathy ecosystems (Lindenmayer and Burgman 2005). Degradation that threatens the persistence of native trees in agricultural landscapes takes many forms:

- The replacement of deep-rooted native woody species with shallow-rooted crop and pasture species alters hydrological processes resulting in salinisation, and localised waterlogging (Mitchell 1991; Hobbs 1993; Prober and Smith 2009);
- Overuse and mismanagement of herbicides and pesticides lead to an accumulation of toxic pollutants in wetlands, aquifers and soil profiles (Scherr and McNeely 2008);
- Livestock grazing negatively affects soil structure, reduces water infiltration, prevents recruitment of native trees and enriches topsoil nutrients,

particularly around paddock trees and in stock camps (Fischer *et al.* 2009; Prober and Smith 2009), and

- Superphosphate application elevates soil nitrogen and phosphorus levels favouring the invasion of exotic nitrophilic weeds, and may be detrimental to soil microbial communities and fungal symbionts that form important associations with the roots of native trees (Prober and Smith 2009; Perry *et al.* 2010).

The detrimental impact of degradation processes on native trees has been observed worldwide (Curtis 1990a). In Europe, North America and Japan, tree loss is primarily associated with unnatural chemical imbalances in the atmosphere, soil and water (Jurskis 2005). Similarly, chemical changes in soils caused by agriculture are responsible for tree loss in Australian rural landscapes (Landsberg *et al.* 1990; Granger *et al.* 1994; Marsh and Adams 1995; Lindenmayer and Burgman 2005). Known as rural dieback, significant increases in the premature decline and death of eucalypt trees have been widely reported since the 1960s in all states (Reid and Landsberg 2000; Lindenmayer and Burgman 2005). However, it appears to be more pronounced in temperate landscapes, where climatic extremes accelerate rural dieback in association with particular land management practices (Jurskis 2005). The New England Tablelands bioregion in northern NSW is one region where rural dieback has been a prevalent and recurring problem. Some 50% of tree cover has been lost since the late 1970s (Nadolny 2008). The problem is exacerbated by a lack of recruitment to replace dieback-affected tree populations, which is often characteristic of grazed landscapes (Reid and Landsberg 2000; Fischer *et al.* 2009). Revegetation is, therefore, essential to keep pace with tree loss and reverse degradation processes.

1.4 Revegetation techniques

Revegetation techniques fall into three categories: (1) natural regeneration, (2) direct seeding, and (3) tubestock planting.

1.4.1 Natural regeneration

The goal of natural regeneration is to recreate natural communities that once existed by providing the time and space for native species to recolonise through recruitment from the soil seed (or propagule) banks, seed dispersal or vegetative means in the absence of human intervention. Natural regeneration is often the preferred method of revegetation because it is cost-effective, doesn't require planting or management input, and has the added advantage of retaining the character and native species of an area (Curtis 1990b; Whisenant 1999), usually resembling the vegetation that previously existed (Cramer *et al.* 2008). The growing trend in the abandonment of agricultural land due to socio-economic changes has provided the opportunity for succession theory to be studied in depth (Cramer *et al.* 2008). In Australia, these studies often report a lack of trajectory towards previously existing natural communities, where degraded weed-dominated vegetation can persist in abandoned fields for decades (Cummings *et al.* 2007; Standish *et al.* 2007; Cramer *et al.* 2008). This is one of the main reasons why natural regeneration as a revegetation strategy often fails (Whisenant 1999). The potential for degraded agricultural lands to transition to a pre-existing state is determined by past and present biotic and abiotic disturbance regimes, such as weed invasions and elevated soil-nutrient levels (Cramer *et al.* 2008; Greipsson 2011; Lake 2013). When the intensity, duration and frequency of disturbance are too great, succession towards the pre-existing native, self-regulating community cannot progress without active restoration interventions to ameliorate disturbance impacts and establish trajectories of recovery (Lake 2013; Miller *et al.* 2013). Other factors impeding successful natural regeneration include the ability of native species to disperse to a new site (propagule limitation), and their ability to establish and survive once there (microsite limitation) (Hobbs and Walker 2007; Standish *et al.* 2007; Scott and Morgan 2012). Propagule limitation may result from several processes, including low reproductive output, lack of persistent seed banks, a decline in natural pollinators, seed predation, as well as restricted seed dispersal. Microsite limitation is influenced by the number and quality of microsites available, poor quality seedbeds, competition from exotic weeds and grasses, unfavourable microclimatic and disturbance conditions, and limited resources (Whisenant 1999; Greipsson 2011).

1.4.2 Direct seeding

Direct seeding is a generic term for sowing native tree and shrub seeds into a ripped, ploughed or scarified seed line or seed bed. The technique has practical, cost-effective applications to broad-scale revegetation in agricultural settings (Piggott *et al.* 1987; Schneemann and McElhinny 2012). The advantages of direct seeding are that it is rapid (e.g. a single operator is able to seed 20–30 km of trees in a day), the resulting vegetation is structurally diverse if multiple species are sown and naturally spaced seed broadcasting is used, and the germinants are better adapted to local conditions (Knight *et al.* 1997; Rawlings *et al.* 2010). Various methods are used to introduce seeds into new sites, including broadcast seeding, aerial seeding, hydro-seeding and commercial agricultural seeders and drill seeders (Whisenant 1999; Greipsson 2011). In Australia, direct seeding has had varying degrees of success (Carr *et al.* 2009; van Andel and Aronson 2012). For example, in South Australia and Victoria where the technique was first used in Australia, direct seeding is the predominant method of revegetation and successful with a broad range of species. In other states, direct seeding has not been as successful and there is considerable scope for improvement (Carr *et al.* 2009). Failures with direct seeding are attributed to poor germination of native seed, sowing outside the optimal growing season, lack of soil moisture availability, inadequate ground preparation, poor species selection, and losses due to seed predation and competition with exotic weeds and pasture grasses (Carr *et al.* 2009; van Andel and Aronson 2012; Palma and Laurance 2015). Access to high-quality and appropriately sourced germplasm is also important for improving the success of direct seeding and ensuring new populations are functional and resilient (Broadhurst *et al.* 2008). Traditionally, the use of local-provenance seed has been considered best practice, given the potential for maximising local adaption and minimising outbreeding depression (Broadhurst *et al.* 2008). However, there is a growing body of research that suggests that sourcing seed from a range of locations with matching habitat profiles is a better approach. Known as composite provenancing, this technique creates genetically diverse populations with an increased capacity to resist future environmental challenges, particularly in relation to climate change projections (Mortlock 2000; Williams 2007; Broadhurst *et al.* 2008).

1.4.3 Tubestock planting

In unpredictable and inhospitable environments where direct seeding seldom succeeds, transplanting whole plants is the most viable alternative (Whisenant 1999). Tubestock planting involves the introduction and establishment of nursery-grown seedlings into areas set aside for revegetation. Three planting techniques are used: (1) manual planting using a shovel or a specially designed hand planter, such as a Hamilton planter or a pottiputki; (2) partially mechanised planting using an auger or post-hole digger, or (3) fully mechanised planting using a tractor-drawn planter, such as a Chatfield planter (Schirmer and Field 2002). Although the cost of tubestock plantings is higher than natural regeneration and direct seeding, the technique is often preferred because trees can be established in the landscape in a relatively short timeframe and the results are immediate (Rawlings *et al.* 2010). An additional benefit of tubestock planting is that it requires smaller amounts of seed than direct seeding, thus reducing seed exploitation pressure on remnant populations of native plants that are prone to overharvesting as the demand for native seed increases (Mortlock 2000; Broadhurst *et al.* 2008). Successful tubestock planting is dependent on the procurement of high-quality seedlings. Seedlings selected for revegetation should have healthy, disease-free leaves and a well-developed system that does not protrude from the pot or tube (Lindenmayer *et al.* 2010). Underdeveloped root systems and root deformations, such as J-rooting, may be related to seed source and poor nursery propagation techniques, both of which can result in early death, retarded growth and instability in windy conditions (Thomas *et al.* 2008). J-rooting is a particularly common problem in eucalypts and occurs when the taproot grows upwards or sideways, sometimes forming a complete circle before turning downwards (Thomas *et al.* 2008). In addition, seedlings should be hardened off in the nursery to suit the conditions they will be planted into (Lindenmayer *et al.* 2010).

The advantages and disadvantages of natural regeneration, tubestock planting and direct seeding are outlined in Table 1.1.

Table 1-1 The advantages (+) and disadvantages (−) of three revegetation techniques (Rawlings *et al.* 2010)

Natural regeneration	Direct seeding	Tubestock planting
(+) Lowest establishment costs	(+) Lower establishment costs	(−) Highest establishment costs
(−) May have to wait a long time for results	(−) May have to wait 3–5 years for results	(+) Revegetation is quick and visible to passers by
(+) Natural spacing	(+) Natural spacing and more diverse structure	(−) Often results in unnatural spacing
(+) Establishes healthiest plants	(+) Establishes healthy plants	(−) Poor root development due to poor nursery practice
(+) Plants are well adapted to site	(+) Germinants are well adapted to site	(−) Adaptation to site depends on provenance
(+) Uses naturally available seed	(−) Uses large quantities of seed	(+) Small quantities of seed
(+) Requires little labour	(±) Requires moderate labour	(−) Labour-intensive
(−) Long establishment times lead to more maintenance such as weed control	(−) Long establishment times lead to high maintenance such as weed control	(+) Distinct rows can facilitate maintenance
(−) Fertiliser and other soil additives cannot be added	(+) Fertiliser and other soil additives can be added	(+) Fertiliser and other soil additives can be added
(−) Tree guards cannot be used	(−) Tree guards cannot be used	(+) Tree guards can be used to protect seedlings
Other considerations		
(+) Missing species may re-establish from seed bank	(−) Most vulnerable of three approaches to climate variability	(+) Immediate results
(−) Needs a nearby seed source	(−) Ants can predate seed	(±) Uniform establishment and growth

1.5 Barriers preventing successful revegetation

The success of revegetation is often difficult to measure because of inadequate monitoring following planting or sowing, and publication bias towards successful experiments, given that many failed attempts are not reported (Field *et al.* 2007; Atyeo and Thackway 2009; Florentine *et al.* 2011; Godefroid *et al.* 2011). However, there is evidence in the literature to suggest that revegetation activities, both in Australia and overseas, are often unsuccessful (Close and Davidson 2003; Fahselt 2007; Godefroid *et al.* 2011; Leslie *et al.* 2013). Ultimately, the success of revegetation is dependent on the long-term survival of direct-seeded germinants and planted tubestock (Andrews 2000; Close and Davidson 2003; Graham *et al.* 2009).

On the Northern Tablelands of NSW, there are many barriers to successful revegetation (Fig. 1.2). This research identified and systematically targeted the following barriers: (1) cold temperature and frosts during seedling establishment and growth; (2) competition with weeds at various stages in the life cycle of native trees and shrubs; (3) defective direct-seeding techniques; (4) inadequate soil moisture during seed germination and seedling establishment; (5) lack of microbial symbionts, which enhance water capture during germination; (6) poor germination and recruitment of direct-seeded eucalypts, and (7) prior land use and disturbance (e.g. N and P soil enrichment from fertiliser application, discussed above).

Subzero temperatures reduce the survival of planted seedlings and mature trees through mechanical injury to leaves and cold-induced photoinhibition (Murata *et al.*

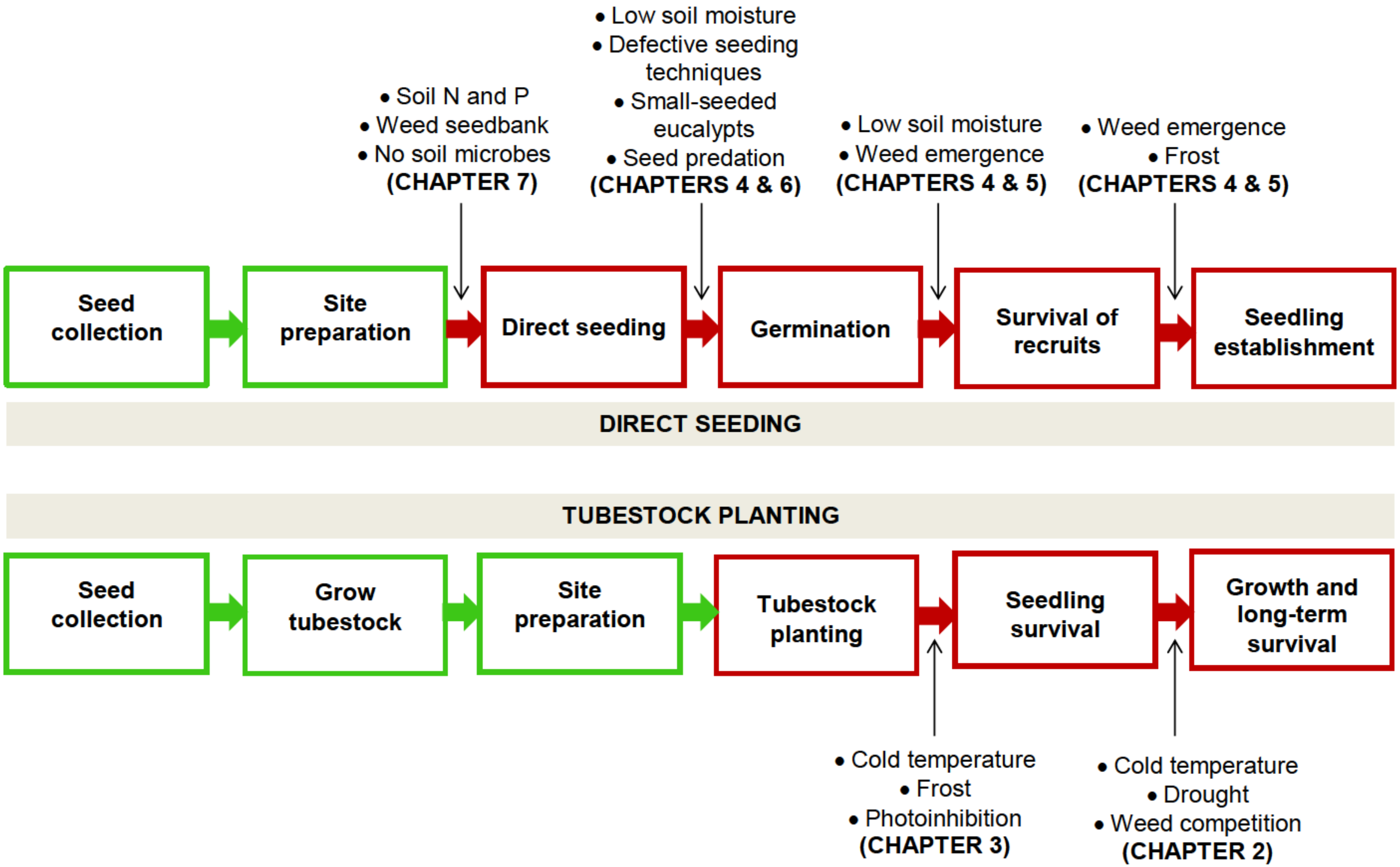

Figure 1-2 Flow chart identifying barriers that are potential thresholds (arrows) preventing the success of direct seeding and tubestock planting in northern NSW.

2007; Close 2012). The effects of winter temperature and frost are exacerbated in exposed, over-cleared and undulating landscapes, where cold-air drainage is a natural phenomenon (Reid *et al.* 2013). Without adequate protection, substantial losses of planted seedlings occur. In addition, mature trees often fail to thrive, exhibiting a stunted juvenile form typical of cold-climate vegetation (Farrell and Ashton 1973; Gilfedder 1988).

Poor weed control often accounts for revegetation failures. Fast-growing exotic annuals are invasive in habit and compete with native plants for nutrients, light, space and water (Carr *et al.* 2009; Willoughby and Jinks 2009; Gibson-Roy *et al.* 2010; Cole *et al.* 2016). Heavy weed burdens may decrease the survival of native seedlings and germinants by up to 80% (Landcare 2003; Willoughby and Jinks 2009). Timely and effective weed management frees up important resources and allows moisture to accumulate in the soil profile, both of which are critical for the germination of sown seed and the survival of seedlings and tubestock during establishment (Hagon and Chan 1977; Lloret *et al.* 1999; Windsor *et al.* 2000; Close and Davidson 2003; Engelbrecht *et al.* 2005; Mercuri *et al.* 2005; Padilla and Pugnaire 2007; Carr *et al.* 2009).

The success of direct seeding is potentially impeded by defective seeding techniques. As part of this research, three additional direct-seeding experiments were undertaken, but were not written up in this thesis because recruitment and subsequent survival of recruits was either low or absent. These failures exemplify the notoriously challenging field in which restoration ecologists work. The first of these experiments was undertaken at the Living Classroom in Bingara, where 24 × 15-m lines were direct-seeded using the purpose-built KB mechanical precision seeder to test the effects of three soil additives (smoked vermiculite, water-holding crystals and microbial inoculants) on the germination of twelve plant species native to the North West Slopes and Plains of NSW. Of the nine genera represented in the seed mix (*Acacia, Eucalyptus, Senna, Geijera, Callitris, Dodonaea, Notelaea, Beyeria* and *Maireana*), only three *Acacia* species and one *Dodonaea* species germinated (a total of 45 recruits from 3.0 kg of seed). Recruitment was unevenly distributed across the site with most of the recruits occurring in the first four rows and no recruits present in the last four rows. Subsequent survival of seedlings over the next 12 months was low.

The second experiment was conducted at Glen Morgan, Ben Lomond, NSW, and involved monitoring the recruitment of Northern Tablelands native trees and shrubs in nine genera (*Acacia, Eucalyptus, Callistemon, Olearia, Melaleuca, Leptospermum, Cassinia, Casuarina* and *Angophora*) along 9 km of direct seeding (in triple rows spaced 2 m apart) sown by the KB seeder. Recruitment was poor, consisting only of *Acacia* and *Angophora* recruits. The same pattern of recruitment observed at the Bingara site was evident at this site with a noticeable absence of recruitment in areas that were direct-seeded last. While it was confirmed that seed was present in the hopper for the duration of the direct seeding, equipment failure or vibration of the seed hopper may have caused the seed to separate from the bulking material, resulting in the uneven distribution of seed.

The third experiment took place on a property at Invergowrie, NSW, with the aim of comparing the effects of three seed coating treatments (coated seed, seed coated with MycoApply® and uncoated seed), three ground-preparation treatments (cultivated, removal of ground cover with glyphosate, and control), two watering treatments (10

L water per plot every 3 days and a nil watering treatment) on the recruitment of twelve *Eucalyptus* species hand sown into 1 × 1-m plots) *in situ*. With no new recruits observed in the month following seeding, the decision was made to re-seed the site. An additional treatment of permethrin insecticide was added to one half of each plot following field observations that a large proportion of the seed, despite the brightly coloured coating, was harvested by ants. After failing the second time, this experiment was repeated under controlled conditions in a glasshouse.

Water stress is an important cause of plant mortality in planted seedlings and direct-seeded recruits (Palma and Laurance 2015). Mortality can be alleviated by deep ripping 6 months prior to planting or seeding to ensure a full soil moisture profile. However, in the absence of adequate soil moisture, post-planting irrigation is essential, particularly for planted tubestock. Past research has consistently reported high survival rates of watered compared to non-watered plantings (Close and Davidson 2003; Mercuri *et al.* 2005; Geeves *et al.* 2008; Graham *et al.* 2009), but irrigation is often regarded as an unnecessary expense and is rarely practised. Management protocols incorporating either deep watering (15–25 L) at the time of planting and/or fortnightly follow-up watering if post-planting conditions are dry have the potential to significantly improve the success of revegetation (Dalton 1992; Engelbrecht *et al.* 2005; Mercuri *et al.* 2005).

Water stress is exacerbated in revegetation that is conducted outside optimal planting times (Carr *et al.* 2007). On the Northern Tablelands, most revegetation activities occur in spring, coinciding with warmer temperatures and higher seasonal rainfall (Andrews 2000). However, planting in autumn may be an alternative, provided seedlings are protected from frost (Sharon Brown, 2016, pers. obs.). The main advantage of planting in autumn is the contribution of heavy dew, fog and overcast winter days to maintaining soil moisture levels. Root systems may have more time to establish during winter before high summer temperatures reduce moisture levels in the upper soil profile. Poor survival is frequently observed in summer plantings (Carr *et al.* 2007). Ad-hoc, out-of-season plantings can be a consequence of bureaucratic decisions in government-funded projects to meet end-of-year revegetation targets, in addition to obligations to nurseries to purchase pre-ordered seedlings for projects that may have stalled or been delayed.

Adequate soil moisture is universally important for seed germination and the establishment of new recruits, particularly in smaller-seeded species that lack endosperm and need to access external resources quickly in order to survive (Weinberg *et al.* 2011). One way to overcome this is by adding microbial inoculants (containing mycorrhizae) to a coating surrounding the seed so that when the radicle emerges, symbiotic associations are formed immediately. Mycorrhizae play an important role in water acquisition and drought resistance, as well as nutrient capture and recycling in many cultivated species (Gardner and Malajczuk 1988; Linderman 1988; Ajeesh *et al.* 2015). They increase root surface area and can infiltrate the soil further than host roots, greatly enhancing host access to important soil resources (Allen 1982; Linderman 1988). On the Northern Tablelands, past European land management practices, such as widespread clearing and the application of superphosphate to fertilise pasture, have had a detrimental effect on the soil microbiology (Jurskis and Turner 2002). Sensitivity to these disturbances can reduce inoculum levels in the soil and affect the health of extant plant communities and prevent the recruitment of subsequent generations (Tommerup and Bougher 2000).

A final barrier preventing successful revegetation on the Northern Tablelands is the poor establishment of eucalypts (Curtis 1990a; Reid and Landsberg 2000). Re-establishing eucalypts in the landscape is a high priority, given the substantial tree losses to eucalypt dieback (Nadolny 2008). A common observation in direct-seeded sites is that large-seeded species, such as acacias, have substantially higher rates of recruitment and survival than small-seeded species, such as eucalypts. This trait has been linked to drought tolerance, as large seeds have an increased store of resources and produce roots greater in length and biomass compared to small seeds, both of which increase the likelihood of survival under suboptimal moisture conditions and in the presence of a competitive weed burden (Moles and Westoby 2002; Hallett *et al.* 2011; Palma and Laurance 2015). Water limitation is an inhibitor of eucalypt germination and survival (Whalley and Curtis 1991; Yates *et al.* 1996; Close *et al.* 2009). Other factors impeding eucalypt germination in direct seeding include predation of seeds by ants, rapid seed desiccation in the absence of suitable microsites, livestock grazing and seed burial at depths greater than 10 mm (Ashton 1979; O'Dowd and Gill 1984; Curtis 1990a; Bashford 1993; Battaglia and Reid 1993; Dorrough and Moxham 2005; Weinberg *et al.* 2011; Palma and Laurance 2015; Ruiz Talonia *et al.* 2016).

1.6 Conceptual models of restoration

Revegetation and restoration activities should be guided by a sound conceptual model incorporating both theoretical and applied ecology (Hobbs and Harris 2001). Such a model aims to provide a general understanding of how ecosystems work and the factors involved in system restoration, as well as an understanding of on-ground methodologies that can be applied in specific situations (Hobbs and Harris 2001;

Perring *et al.* 2015). Conceptual models of restoration have received a great deal of attention in recent decades. Three types of models have emerged. The linear succession (or climax) model (Fig. 1.3A) is the traditional model upon which restoration has been based, and incorporates the concept of plant community development and response to disturbance (Taylor *et al.* 2013). This framework encompasses models of plant succession that result in continual, proportional and predictable change along a single trajectory (culminating in a climax or final self-propagating community) through changes in biotic and abiotic stresses (James *et al.* 2013). This model posits that increasing or decreasing management intervention over time will lead to varying successional plant communities up or down the trajectory (Suding and Hobbs 2009; James *et al.* 2013), but secondary succession will always lead back to the original stable climax community (Spooner and Allcock 2006).

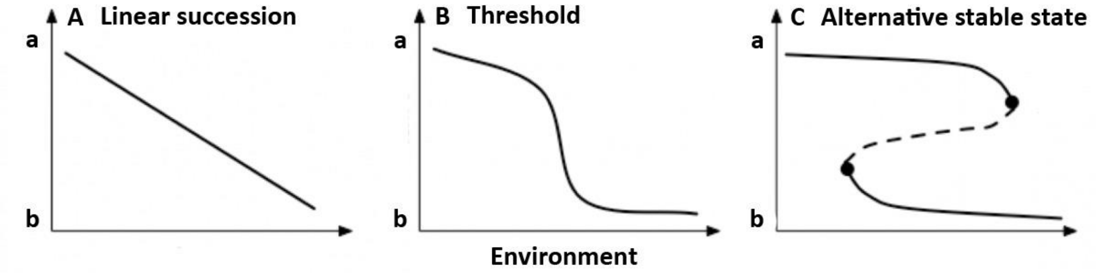

Figure 1-3 Conceptual framework models for restoration: (A) the linear succession model predicts continual and proportional change occurs along a trajectory culminating in a single climax community, (B) the threshold model predicts little change over time until a trigger induces abrupt and rapid change, and (C) the alternative stable state model predicts threshold dynamics as in (B) but also predicts that multiple alternative stable states can persist under similar environmental conditions (James *et al.* 2013)

However, linear succession models have proven to be inadequate for some ecosystem types because they fail to consider that ecosystems are highly complex and dynamic, often encapsulating multiple pathways of vegetation change rather than a single pathway (Stringham *et al.* 2003). Furthermore, biotic and abiotic stressors fluctuate over time, resulting in discontinuous and irreversible vegetation changes

(James *et al.* 2013). This has led to the development of alternative models (Stringham *et al.* 2003).

The threshold model (Fig. 1.3B) predicts that ecosystem structure and function remain stable over a range of environmental conditions until a trigger (e.g. fire or drought) induces an abrupt rapid change (James *et al.* 2013). Thresholds are barriers in space and time, which once crossed, can disrupt equilibrium causing an ecosystem to degrade beyond the point of self-repair (Stringham *et al.* 2003). Thresholds are identified when a change between vegetation states cannot be reversed by simply removing the disturbance that caused the initial shift (Spooner and Allcock 2006). Overcoming thresholds to return an ecosystem to a less degraded state usually requires a major restoration effort. Without intervention, a new state that supports a different suite of plant communities and new thresholds will form (Stringham *et al.* 2003).

The alternative stable-state model also incorporates the concept of thresholds, but unlike the threshold model, the alternative stable-state model predicts that multiple alternative states can persist under similar environmental conditions (Fig. 1.3C) (James *et al.* 2013). First developed by Westoby *et al.* (1989), this model accounts for abrupt shifts between different metastable states. Sometimes changes in state can be reversed if the stressor(s) causing the change are removed, but often the changes are irreversible (Hobbs and Harris 1996).

The state-and-transition model (STM) (Fig. 1.4) has emerged as the leading conceptual framework upon which to base restoration in terrestrial ecosystems (James *et al.* 2013). A primary advantage of STMs is their potential to account for vegetation change along several axes, including both continuous and reversible and discontinuous and irreversible vegetation dynamics (Briske *et al.* 2005). STMs are represented as flowcharts comprised of alternative stable vegetation states supported by a particular combination of abiotic variables, and the possible transitions between states (James *et al.* 2013). Transitions represent thresholds and restoration pathways that move a plant community from one stable state to another. They are generally viewed as irreversible without intensive management inputs (James *et al.* 2013).

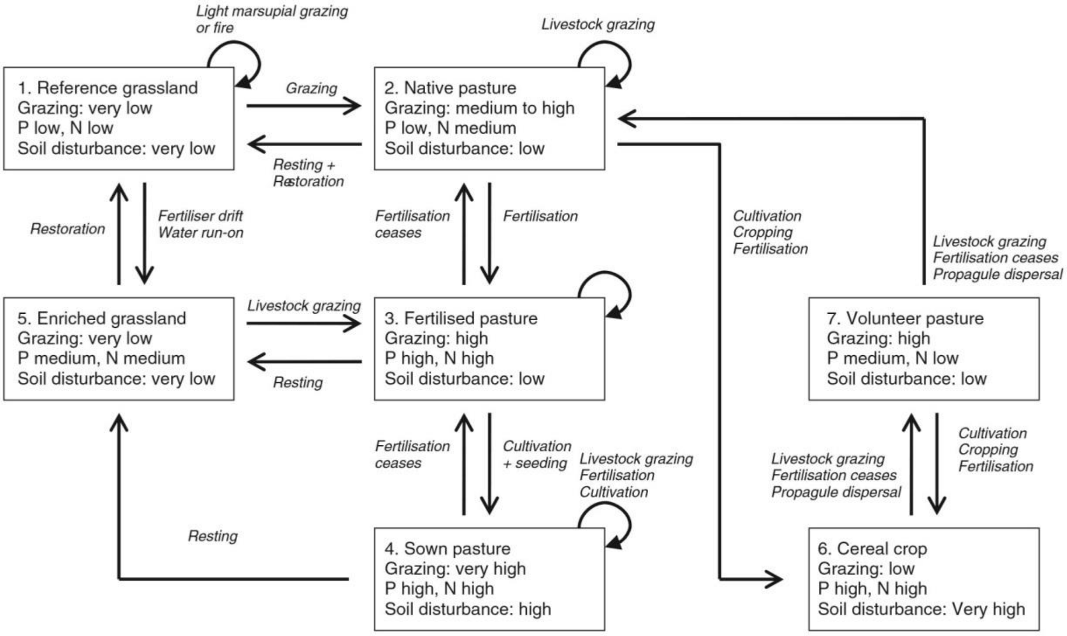

Figure 1-4 State-and-transition model for cultivated and grazed grasslands in southern Australia (Wong *et al.* 2010)

1.7 Thesis objectives

This research constitutes different studies that were designed and undertaken for the purpose of improving revegetation practices on the Northern Tablelands of NSW. The primary aim of this research was to identify and reverse ecological barriers preventing the success of direct seeding and tubestock planting in temperate upland pastures. A secondary aim was to develop a sound conceptual framework to guide and inform future restoration activities. The conceptual framework underlying the various studies undertaken in this thesis is the threshold model. In the following chapters, revegetation strategies that have the potential for overcoming biotic and abiotic barriers (thresholds) are developed.

The main objectives of this research were to:

1) Determine if evaluation of established shelterbelts of eucalypts can reveal information about the environmental stresses affecting the survival and growth of on-farm tree plantings on the Northern Tablelands;
2) Investigate the potential benefits of tree guards for providing protection against seasonal temperatures in mixed-species plantings;
3) Assess the reliability of direct seeding as a revegetation technique, with a particular focus on sowing methods and bulking materials;
4) Investigate the potential of herbicide oversprays for weed control in direct seeding by determining tolerances in a range of native species to a range of selective herbicides and glyphosate;
5) Assess the potential of seed coating treatments and water regimes to improve the recruitment of direct-seeded eucalypts, and
6) Identify suitable methods of weed control for native grass establishment.

1.8 Knowledge gaps

There is an urgent need to restore native vegetation to farmland, but there is still a poor understanding of the theoretical and practical requirements of doing so. Revegetation activities should be based upon a sound conceptual framework to inform current and future management. However, often revegetation is implemented in an *ad hoc* manner, with little forethought to the biotic and abiotic processes that underpin the structural and functional dynamics of the resulting vegetation, or the types of disturbances (past and present) that are likely to act as ecological thresholds. In this thesis, we address this knowledge gap by providing a conceptual framework for the experimental work undertaken.

Other knowledge gaps addressed in this thesis pertain to the poor understanding and implementation of on-ground revegetation protocols. Further, existing protocols are too general, relating to specific geographical regions. The type of revegetation activities required are often site-specific and depend on local climate, soil type, topography, previous land use and existing vegetation. While some revegetation guidelines have been developed for the Northern Tablelands, they often lack detail. Specifically, there are no set protocols that allow farm revegetation to be monitored scientifically in terms of the impacts of various stress factors on plant survival and growth. By applying robust methodology and statistical modelling, the present research demonstrates how this can be achieved. In addition, recommendations to improve establishment rates of direct-seeded native plants on the Northern Tablelands are scant despite the technique being widely used and often failing. To our knowledge, no attention has been given to poor recruitment attributed to technical failure. Finally, the potential for seed enhancement treatments, such as seed coatings containing microbial inoculants has not been fully explored. While the importance of the symbiotic relationship between eucalypts and mycorrhizal fungi is widely documented in the literature (Chilvers 1973; Brundett *et al.* 1996; Bougher 2007), using microbial inoculants for revegetation purposes has not gained traction and has not has not previously been examined.

1.9 Thesis outline

Chapter 2 determines if existing native-tree shelterbelts can be evaluated to identify the environmental stresses influencing planted eucalypt establishment and growth on the Northern Tablelands. The study examines the effect of biotic and abiotic factors (weed control, topographic position, altitude, slope, temperature, soil type and soil moisture) on the survival and growth of 6-year old planted eucalypts (*Eucalyptus nitens, E. pauciflora* and *E. viminalis*) in upland temperate pastures on the Northern Tablelands.

Chapter 3 compares the effects of two types of tree guard (tall Corflute® guards and milk cartons) on the survival and growth of native seedlings (*Leptospermum polygalifolium, Callistemon pungens, E. viminalis, E. stellulata* and *E. acaciiformis*) planted in lower slope, mid slope and upper slope landscape positions in relation to daily and seasonal temperature.

Chapter 4 compares the effects of three direct-seeding techniques (broadcast sowing by hand, machine-sown by KB seeder, and machine-sown by modified Chatfield seeder) and three bulking materials (rice, chicken crumbles and smoked vermiculite) on acacia and eucalypt recruitment.

Chapter 5 examines the effects of eight herbicide oversprays (oxyfluorfen, prosulfocarb, imazethapyr, glufosinate, diflufenican, metolachlor, glyphosate and isoxaflutole), with different chemical modes of action, on the survival of 11 native tree and shrub species (*Acacia harpophylla, A. pendula, A. salicina, Atriplex nummularia, Casuarina cristata, Dodonaea viscosa, Einadia nutens, Eucalyptus camaldulensis, E. coolibah, Senna artemisioides* and *Geijera parviflora*) to establish herbicide tolerances and suitability of herbicide oversprays for direct seeding.

Chapter 6 examines the effects of three seed-coating treatments (coated seed, seed coated with MycoApply® and uncoated seed) under four watering regimes (30 mL/day, 30 mL/3 days and 30 mL/5 days and no watering) and two sowing methods (surface sown and buried beneath a 0.5-cm layer of vermiculite) on the recruitment of eucalypts.

Chapter 7 investigates the effects of four weed-control techniques (scalping, herbicide, and combinations of carbon – sugar and sawdust – addition and herbicide) on the recruitment of native grasses and weed emergence.

Chapter 8 synthesises the main findings of the thesis and describes the limitations of the research. Directions for future research are also discussed.

CHAPTER 2

Farmland Revegetation and Scientific Inference: Survival and Growth of Eucalypt Species in Temperate Upland Pastures

2.1 Abstract

Measuring and monitoring on-ground revegetation is essential to evaluate the success of planting techniques and to inform the future direction of revegetation practice. However, the accuracy and reliability of data from existing revegetation projects can be compromised by the unscientific ways in which many on-ground revegetation projects are designed and implemented. The aim of this study was to determine if existing native shelterbelts can be evaluated to identify the environmental stresses influencing planted eucalypt survival and growth on the Northern Tablelands of New South Wales. The study was undertaken on seven properties with engineered woodlands, twin rows of shelterbelts consisting predominantly of *Eucalyptus nitens, E. pauciflora* and *E. viminalis*. Twenty-three transects (~three per property) located in separate shelter belts were analysed. *Eucalyptus viminalis* (69% survival) survived best, followed by *E. nitens* (43% survival) and *E. pauciflora* (40% survival). Mean tree height was greatest in *E. nitens* (6.9 ± 0.28 m), followed by *E. viminalis* (5.5 ± 0.39 m) and *E. pauciflora* (4.5 ± 0.30 m). Using multimodel inference, we

determined that cold temperature and poor weed control were the primary stresses influencing survival and growth at the time of this study. The highest ranked model for tree survival included species, time, weed control, and minimum temperature, as well as the interactions between species and weed control, and between species and minimum temperature. The highest ranked model for tree height included a species × minimum temperature interaction. Finally, we address the limitations of the study and recommend ways of improving monitoring opportunities for engineered woodland-style tree plantings.

2.2 Introduction

As agriculture continues to expand and eucalypt woodland remnants become increasingly small and isolated, there is a need to reconcile productive landscapes with healthy ecosystems (Scherr and McNeely 2008; Waters *et al.* 2013). Such integrated eco-agricultural systems have the potential to generate co-benefits for primary production and biodiversity (Scherr and McNeely 2008). One way of achieving this is by planting shelterbelts consisting of native trees (Fig. 2.1). Shelterbelts are narrow lines of trees that often follow a linear landscape feature, such as a road or fence line, and are an effective way of merging agriculture and biodiversity without financial sacrifice (Dames and Moore 1999; Cleugh 2003; Eden and Cottee-Jones 2013).

Engineered woodlands consist of wide-spaced, double or triple-row shelterbelts planted on the contour. Launched in 2007 by the Northern Inland Forestry Investment Group, the aim of the Engineered Woodlands Project was to restore tree cover on the North-west slopes and Northern Tablelands of New South Wales in an agriculturally productive way. Based on a whole paddock approach to revegetation, the engineered woodlands were designed to minimise the cost of fencing and disruption to paddock management (Andrews and Thompson 2009). The woodlands provide financial incentives to landholders in terms of timber and carbon credits, but are most valued for their role in livestock protection during winter (Andrews and Thompson 2009)

Figure 2-1 Engineered woodlands at 'Kobadah' near Woolbrook, NSW, planted in summer 2007

Source: Photograph by Sharon Brown

2.2.1 The problem with farmland tree plantings

The engineered woodlands in this study were in a style typical of farmland tree plantings. This style of revegetation is mostly designed and planted by community members or volunteers with little research experience. Consequently, recording of basic scientific information and adherence to scientific design principles are often disregarded. For this reason, fitting an empirical study around the engineered woodlands was potentially difficult due to concerns about: (1) the sample size chosen for monitoring; (2) the absence of important baseline data, such as planting dates and species provenances; (3) variation in planting times; (4) potential variations in propagation techniques; (5) monitoring procedures, and (6) the absence of long-term climate data. While basing scientific research on such methodology is not ideal, it is necessary for two reasons. Firstly, due to the decline in native trees on the Northern Tablelands through clearing and eucalypt dieback, replacing trees is essential for maintaining biodiversity and increasing farm productivity. Secondly, conducting research on existing revegetation will facilitate positive changes to current revegetation practices that often result in failed outcomes.

The aim of this study, therefore, was to determine if existing tree native shelterbelts could be evaluated to identify the environmental stresses affecting the survival and growth of 6-year-old eucalypts planted in temperate upland pastures. Based on the results of previous studies demonstrating the negative effect of extreme temperatures (minimum and maximum) and lack of soil moisture on eucalypt survival and growth (Green 1969; Harwood 1980; Paton 1980; Davidson and Reid 1985; Tibbits and Reid 1987; Ball *et al.* 1991; White *et al.* 1996; Warren *et al.* 1998; Close *et al.* 2000; Tibbits and Hodge 2003; McDowell *et al.* 2008; Leslie *et al.* 2014; Matusick *et al.* 2014), we hypothesised that subzero winter temperatures and drought would be important stressors affecting eucalypt performance.

2.3 Materials and methods

2.3.1 Study region

The Northern Tablelands is a region of elevated undulating plateaus on top of the Great Dividing Range in northern NSW. The main land uses in the region are wool, lamb and beef production and forestry (Carr 2009). The region experiences mild summers and cold winters (Armidale Airport, –30.53º, 151.62º; 1079 m a.s.l.; January maximum daily temperature, 25.9ºC, minimum daily temperature, 13.2ºC; July maximum daily temperature, 12.1ºC, minimum daily temperature, 1.2ºC; Bureau of Meteorology 2015). Mean annual rainfall across the region ranges between 600 mm and 1100 mm, with 60% of rainfall occurring in summer (October–March). In the summer of 2013–2014, rainfall was substantially lower than the average rainfall recorded for all years (Bureau of Meteorology 2015) and was coincident with summer drought (Table 2.1).

This study was conducted on seven Northern Tablelands properties (Fig. 2.2, Table 2.2). The properties were chosen because they contained engineered woodlands, which were established using the same ground-preparation and planting techniques. The engineered woodlands were whole-paddock plantings approximately 10 ha in size of widely-spaced shelterbelts designed to allow grazing and the efficient use of farm machinery for normal management between the plantings (Andrews and Thompson 2009). The shelterbelts consisted of twin rows of mixed species (predominantly eucalypts) 2 m apart with 1.5 m spacing between trees. Twenty-three transects (40–50 m long) located in separate shelterbelts were analysed. Transects were selected 6 months after planting based on geographical spread across sites and the representation of species. The location of each transect was recorded with a GPS and marked (at the beginning and end of the transect) with steel posts so they could be relocated. The transects were distributed evenly across the seven properties and the replicate transects at each property were well separated to sample the range in engineered-woodland plantings. *Eucalyptus nitens* H Deane & Maiden, *E. pauciflora*

Sieber ex Spreng. and *E. viminalis* Labill. were chosen for detailed study because they were planted in each shelterbelt.

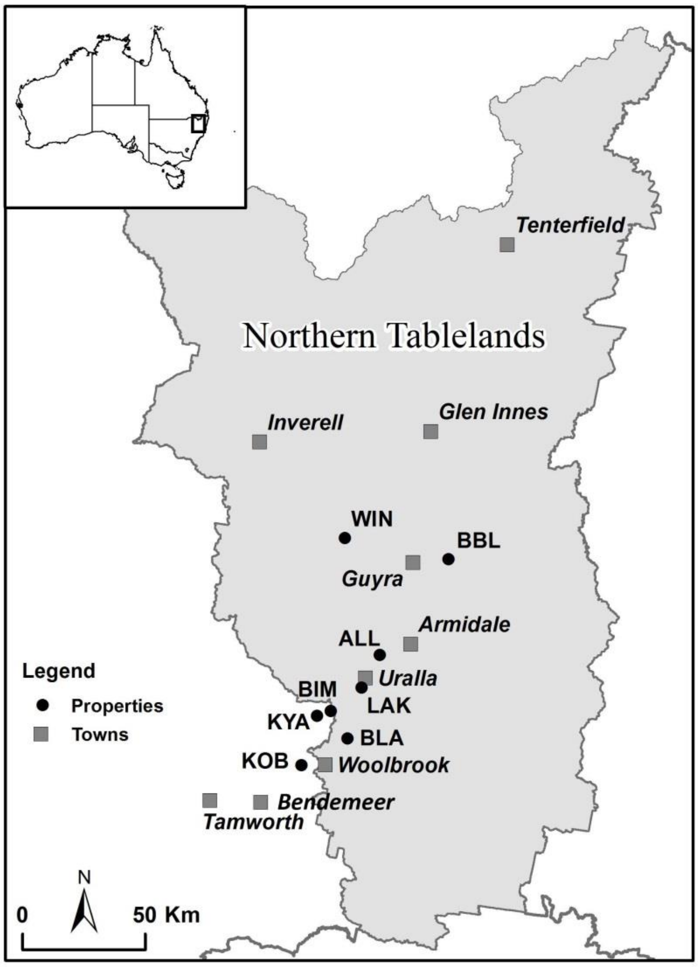

Figure 2-2 Map of the Northern Tablelands showing the location of the seven properties with engineered woodlands

(Properties abbreviated as follows: BBL = Bald Blair, ALL = Allfoxon, LAK = Lakeview, KYA = Kyabra, BLA = Blaxland, KOB = Kobadah, BIM = Bimbi-Vale.)

The trees were hand-planted between October and December 2007 using a pottiputki. Ground preparation was consistent across all properties. The planting beds were fallowed with a non-selective herbicide in February or March 2007 after slashing or heavy grazing. Repeat applications of herbicide maintained the fallow whenever weeds emerged. Rip-lines (500–600 mm deep) were established soon after the first herbicide application and mounds were formed over the rip-lines in April–May, 6 months before planting.

Table 2-1 Monthly rainfall (mm) recorded in Armidale, Uralla, Guyra, Woolbrook and Bendemeer between April 2013 and March 2014, along with long-term (year–year) mean monthly rainfall (in parentheses, mm) and the rainfall deficit (in mm) over the period for each station (Bureau of Meteorology 2015)

	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Deficit
Guyra	15.2 (48.3)	40.8 (50.4)	103.4 (61.7)	33.2 (58.8)	17.0 (54.0)	43.0 (57.3)	26.2 (79.9)	14.6 (87.5)	25.4 (100.8)	7.8 (114.0)	46.0 (92.7)	182.0 (72.6)	**−323.4**
Armidale	3.2 (30.6)	18.2 (41.5)	89.6 (50.5)	30.6 (42.2)	12.6 (47.0)	25.0 (53.7)	41.4 (70.1)	117.0 (101.4)	39.2 (98.5)	2.4 (90.6)	48.8 (93.2)	115.8 (62.2)	**−237.7**
Uralla	2.8 (40.1)	8.8 (44.1)	87.2 (55.2)	20.2 (54.0)	10.0 (51.4)	32.8 (52.1)	33.2 (70.0)	115.0 (79.6)	64.8 (88.5)	4.4 (101.0)	93.6 (82.8)	108.4 (57.9)	**−195.5**
Woolbrook	16.6 (41.4)	8.8 (46.8)	89.8 (46.3	33.4 (52.2)	8.6 (53.8)	33.6 (56.3)	16.8 (72.8)	122.7 (85.8)	60.5 (101.5)	13.8 (99.8)	75.4 (83.5)	127.8 (47.3)	**−133.4**
Bendemeer	4.7 (36.8)	13.8 (45.0)	86.7 (48.2)	32.0 (49.5)	9.9 (51.8)	29.6 (51.3)	13.0 (70.0)	115.6 (79.8)	73.5 (91.8)	0.6 (94.2)	69.4 (83.5)	170.4 (47.3)	**−130.00**

Table 3-2 Site information for the seven Northern Tablelands properties with engineered woodlands

Property	Position	Transect no.	Slope (⁰)	Topography	Soil type	Altitude (m.a.s.l.)	No. of trees at 1st monitoring		
							E. nitens	*E. pauciflora*	*E. viminalis*
Kobadah, Woolbrook	29° 45' 00"S, 151°26' 50"E	1	4	mid-slope	basalt	952	10	3	8
		2	3	upper-slope	basalt	963	12	0	4
		3	4	upper-slope	basalt	956	12	3	10
Kyabra, Kentucky	30° 44' 35"S, 151°24' 01"E	1	3	upper-slope	granite	992	14	2	5
		2	4	upper-slope	granite	979	13	4	7
		3	2	mid-slope	granite	965	13	4	8
Allfoxton, Arding	30° 29' 30"S, 151°34' 45"E	1	2	upper-slope	basalt	1042	11	4	4
		2	2	mid-slope	basalt	1041	11	3	8
		3	2	mid-slope	basalt	1035	11	1	5
Lakeview, Uralla	30° 35' 18"S, 151°32' 24"E	1	0	flat	granite	1049	11	1	5
		2	0	flat	granite	1045	1	2	0
		3	0	flat	granite	1048	7	3	4
Bimbi-vale, Kentucky	30° 44' 56"S, 151°20' 09"E	1	1	upper-slope	granite	1105	9	4	4
		2	2	upper-slope	granite	1107	10	4	4
		3	3	upper-slope	granite	1106	11	0	6
		4	2	upper-slope	granite	1105	7	3	4
Blaxland, Wollun	30° 51' 35'S, 151°27' 10"E	1	2	upper-slope	granite	1127	13	5	7
		2	3	mid-slope	granite	1126	10	4	5
		3	3	mid-slope	granite	1131	9	6	5
Bald Blair, Guyra	30° 09' 44"S, 151°49' 51"E	1	1	lower-slope	basalt	1315	12	5	6
		2	1	lower-slope	basalt	1311	13	6	6
		3	4	lower-slope	basalt	1309	12	5	5
		4	4	mid-slope	basalt	1318	8	7	4

One month before planting, a residual herbicide (terbuthylazine) was applied at the rate of 3 kg/ha in 100 L water per/ha. A multi-nutrient horticultural fertiliser was applied (50–100 g seedling^{-1}) 1 month after planting. Post-planting weed-control timing and methods varied between the seven properties, as determined by the individual landholders. The soil at the sites was derived from either basalt or granite parent materials (Carr 2009). Depth of the soil to parent material was at least 50 cm at all sites. The seed for the plantings was sourced from various locations. *E. nitens* seed was purchased from the Tasmanian seed centre, while *E. pauciflora* and *E. viminalis* seed was collected from local Northern Tableland populations across the region.

2.3.2 Data collection

Most of the data were collected prior to the commencement of this research by an employee of the Northern Inland Forestry Investment Group. The only data collected by the researchers were the site and environmental factors. Survival and growth were measured in September 2013 and again in October 2014 following landholder reports of tree deaths during the 2014 summer drought. To measure survival, trees in each transect were recorded as alive or dead and the surviving proportion was calculated for each species. The height of living trees was measured using a measuring pole. The success of weed control was classed according to the percentage of herbaceous weed cover in the tree rows 2 months after each site was planted: 1: >70% weed cover; 2: 50–70% weed cover; 3: 30–50% weed cover; 4: 10–30% weed cover, and 5: <10% weed cover. Even though weed control was measured soon after planting, we included it as a predictor variable in analyses of tree survival and growth in 2013–2014 because competition from herbaceous weeds can be detrimental to the long-term survival and growth of tree seedlings. The long-lasting effects of weed control on the performance of seedling eucalypts and other plantation species are well-known and have been documented in several studies (Balneaves *et al.* 1988; Fagg 1988; Brand 1991; Florence 1996; George and Brennan 2002). Environmental factors including topographic position, altitude, slope, temperature, soil type and soil moisture were measured in spring 2013. Topographic position was classified according to McDonald *et al.* (1998) as: F = flat; L = lower slope; M = mid slope; U = upper slope. The altitude of each site was derived from a one-second SRTM Digital Elevation Model, version1 (Gallant *et al.* 2011). Ambient temperature was measured at 1-hour intervals from 12–27 October 2013 and again from 7 August – 24 September 2014 using iButton® data loggers placed 20 cm above ground level in open pasture 10 m from the centre of each transect. Mean minimum and maximum temperature and absolute minimum and maximum temperature were calculated from combined temperature records for each transect. When temperatures from the two sampling periods were regressed against each other there were strong relationships between 2013 and 2014 for absolute minimum temperature (R^2 = 0.52) and mean minimum temperature (R^2 = 0.65), demonstrating the between-site consistency in temperatures between the two time periods. We classified parent material as either granite or basalt according to Lea *et al.* (1977). Soil samples (10 cm cores) were collected at both ends and at the midpoint of each transect (three samples/transect) in October 2013 and September 2015 to measure soil moisture content after weighing and drying the samples at 40°C for 10 days, then reweighing the soil and determining the difference in weight. Soil moisture was expressed as grams of water per gram of soil.

2.3.3 Statistical analysis

The response variables for each species were the proportion of trees surviving in 2013 and 2014, and mean tree height per transect in 2013. Exploratory plots were used to identify predictor variables (Appendix 1), and Pearson's correlation was used to check for colinearity between potential predictors (Appendix 2). Topographic position was omitted from the analyses because it was correlated with slope and altitude. Mean maximum temperature was omitted because it was correlated with absolute maximum temperature. Mean minimum temperature was omitted from analyses because it was correlated with absolute minimum temperature. Time was included to compare survival before and after the summer drought in late 2013–early 2014. Planting time was included to compare survival and growth of trees planted in October, November or December 2007 as it was speculated that differences in seasonal temperature could have had an effect on seedling performance. Site was included in models as a random variable, but was later dropped from analyses because there were no significant differences in tree survival and height among properties. We centred and scaled the continuous predictors. Multimodel inference based on the information theoretic approach (Grueber *et al.* 2011) was used to select the best fitting model for each response variable, limiting the number of predictors to one per 20 observations to avoid over-parameterisation (Burnham and Anderson 2002; Bolker *et al.* 2009). We fitted a generalised linear model with a binomial error distribution to test the effects of predictors on tree survival proportion, and a linear model to test the influence of predictors on tree height. For each response variable, we developed a set of competing models that contained different subsets of the predictor variables. Each set was restricted to models with an AIC score within 10 of the minimum AIC. Selection of the best overall model was based on the following criteria: (1) low AIC, (2) Akaike weight (ω_i) greater than 0.9, and (3) non-violation of model assumptions (Burnham and Anderson 2002; White *et al.* 2010). Model averaging was performed if the Akaike weight was less than 0.9. The best overall model for each response variable was initially run with *E. nitens* as the baseline reference and then each model was refitted with *E. pauciflora* as reference to compare it and *E. viminalis*. All analyses were performed in R Studio (Version 3.0.2; R Core Team 2015).

2.4 Results

2.4.1 Mean tree survival

Survival differed significantly among the three species. In 2013, 6 years after planting, survival (mean survival proportion ± 1 s.e., 0.81 ± 0.05) was greatest in *E. viminalis*, followed by *E. nitens* (0.65 ± 0.06), with *E. pauciflora* having the poorest survival (0.52 ± 0.08). Twelve months later, survival had declined in all three species: *E. viminalis* (0.69 ± 0.05), *E. nitens* (0.43 ± 0.06) and *E. pauciflora* (0.40 ± 0.07). For survival, our final set of predictor variables included species, absolute minimum temperature and weed control. The highest ranking model (Table 2.3) had an Akaike weight of 0.90 and model assumptions were valid. This model included species, time, weed control and absolute minimum temperature as well as species × weed and species × minimum temperature interactions. Tree survival varied with minimum temperature depending on the species (Fig. 2.3A, Table 2.4). As minimum temperature increased, so did *E. nitens* survival, with the rate of increase in survival of *E. nitens* and *E. viminalis* in relation to minimum temperature not differing significantly (Table 2.4).

Table 2-3 Comparison of candidate models for the predictor variables affecting survival up to and including 2014

Model	AICc	ΔAICc	ω_i
Survival model			
Species, time, min.temp, weed, species x min.temp, species x weed	422	0.00	0.90
Species, time, min.temp, weed, species x time, species x min.temp, species x weed	427	4.90	0.08
Species, time, min.temp, weed, species x min.temp	430	7.88	0.02

Models ranked by Akaike's Information Criterion adjusted for sample size (AICc), ΔAICc is the difference of each model's AICc value from that of the highest ranking model and ω_i is the Akaike weight (sum of all weights = 1.00). (Variables abbreviated as follows: min.temp = minimum temperature; weed = weed control)

However, there was a significant difference in the rate of change of survival with minimum temperature between *E. nitens* and *E. pauciflora* (Fig. 2.3A, Table 2.4). Similarly, the difference in the rate of change of survival with increasing minimum temperature between *E. viminalis* and *E. pauciflora* was significant with survival of *E. pauciflora* decreasing as minimum temperature increased (95% CI of the difference: 0.48, 1.70, after relevelling).

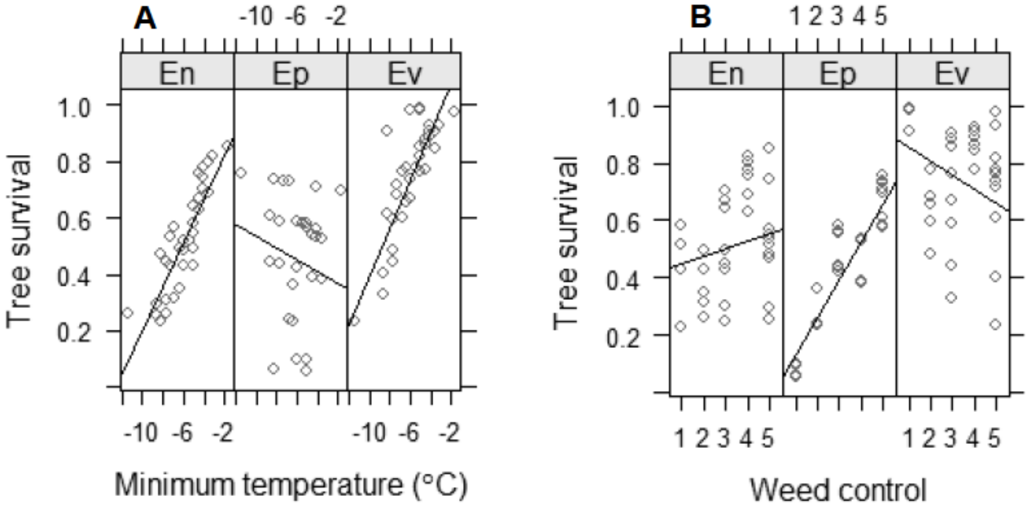

Figure 2-3 Plots showing predicted (lines) and actual (points) tree survival for the interactions between (A) species and minimum temperature, and (B) species and weed control

(Variables abbreviated: En = *E. nitens*, Ep = *E. pauciflora*, Ev = *E. viminalis*)

Tree survival varied with the efficacy of post-planting weed control among species (Fig. 2.3B). There was no significant difference in *E. nitens* survival with varying levels of weed control holding minimum temperature constant (Table 2.5).

Table 2-4 Survival model coefficients for the estimates, standard errors (SE) and 95% confidence intervals (CIs) for the variables in the highest ranking model

Variables	Coefficient	SE	95% CIs	
			Lower	Upper
Intercept	0.10	0.30	−0.50	0.70
Time 2014	−0.74	0.21	−1.16	−0.32
SpeciesEp	−2.36	0.81	−4.28	−0.94
SpeciesEv	3.76	1.11	1.96	6.73
Min.temp	0.57	0.14	0.31	0.84
Weed2	0.03	0.43	−0.81	0.87
Weed3	0.26	0.36	−0.44	0.96
Weed4	0.68	0.41	−0.11	1.49
Weed5	0.48	0.35	−0.22	1.18
SpeciesEp:Min.temp	−0.62	0.24	−1.09	−0.15
SpeciesEv:Min.temp	0.46	0.28	−0.06	1.08
SpeciesEp:Weed2	1.59	1.10	−0.55	3.92
SpeciesEv:Weed2	−2.60	1.21	−5.53	−0.52
SpeciesEp:Weed3	2.23	0.94	0.53	4.33
SpeciesEv:Weed3	−3.02	1.12	−6.02	−1.14
SpeciesEp:Weed4	1.70	0.95	−0.04	3.82
SpeciesEv:Weed4	−3.62	1.26	−6.76	−1.41
SpeciesEp:Weed5	2.66	0.87	1.14	4.67
SpeciesEv:Weed5	−9.20	1.29	−5.90	−1.40

(Variables abbreviated: Min. temp = minimum temperature; Alt = altitude; Weed = weed control, SpeciesEp = *E. pauciflora*, SpeciesEv = *E. viminalis)*

Similarly, there were no significant differences in survival of *E. pauciflora* between sites using weed control 1 (>70% weed cover), 2 (50–70%) or 4 (10–30%) (Table 2.4) However, there was a significant increase in survival of *E. pauciflora* in sites where effective weed control had been applied (weed control 3, 30–50% and 5, <10%; Table 2.5). Conversely, survival of *E. viminalis* was somewhat reduced at sites using effective weed control 2–5 (0–70% compared to transects with the least effective weed control (>70% weed cover) (Table 2.4).

2.4.2 Mean tree height

Mean tree height (mean ± 1 s.e.) after 6 years was greatest in *E. nitens* (6.9 ± 0.28 m), intermediate in *E. viminalis* (5.5 ± 0.39 m) and least in *E. pauciflora* (4.5 ± 0.30 m). For height, the highest ranked model had an Akaike weight of 0.72 (Table 2.5) and included planting time, species, minimum temperature and the species × minimum temperature interaction. The next highest ranked model had an Akaike weight of 0.26 and included planting time, species and minimum temperature.

Table 2-5. Comparison of candidate models for the predictor variables affecting tree height

Model	AICc	ΔAICc	ω_i
Height model			
Plant time + Species + Min.temp + Species x Min. temp	179	0.00	0.72
Plant time + Species + Min. temp	181	2.04	0.26
Plant time + Species	187	7.73	0.015
Species x Min. temp	189	9.77	0.005

Models ranked by Akaike's Information Criterion adjusted for sample size (AIC*c*). ΔAIC*c* is the difference of each model's AIC*c* value from that of the highest ranking model and ω_i is the Akaike weight for each model (sum of all weights = 1.00). (Variable abbreviated as follows: Min. temp = minimum temperature)

There were significant differences in height between planting times. Mean height was greater for trees planted in November (6.46 ± 0.26 m) compared to trees planted in October (4.77 ± 0.44 m; Table 2.4), but there was no significant difference in height between trees planted October and December (4.73 ± 0.45 m) after relevelling. The increase in mean tree height with increasing minimum temperature was significantly greater for *E. viminalis* than for *E. pauciflora* (95% CI of the difference: 0.19, 1.68 after revelling) or *E. nitens* (Fig. 2.4, Table 2.6). However, there was no significant difference in the rate of increase in minimum temperature with increasing minimum temperature between *E. pauciflora* and *E. nitens* (Fig. 2.4, Table 2.6).

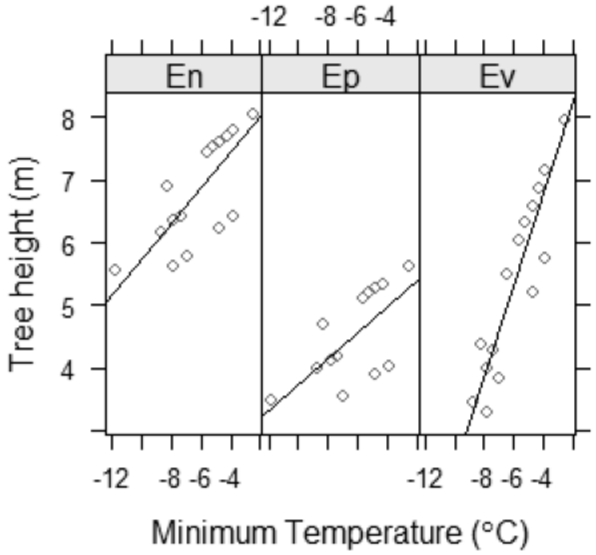

Figure 2-4 Plot showing the interaction between species and minimum temperature in relation to tree height

(Variables abbreviated as follows: En = *E. nitens,* Ep = *E. pauciflora,* Ev = *E. viminalis*)

Table 2-6 Height model coefficients for the estimates, standard errors (SE) and 95% confidence intervals (CIs) for the variables in the highest ranking models

Variables	Coefficient	SE	95% CIs Lower	 Upper
Intercept	5.96	0.35	5.27	6.66
Plant time Nov	1.39	0.36	0.69	2.10
Plant time Dec	0.74	0.52	−0.29	1.76
Min. temp	0.45	0.26	−0.07	0.96
SpeciesEp	−2.28	0.36	−3.00	−1.57
SpeciesEv	−1.55	0.34	−2.20	−0.87
SpeciesEp : Min. temp	−0.08	0.33	−0.74	0.57
SpeciesEv : Min. temp	0.85	0.37	1.12	1.57

(Variables abbreviated as follows: Min. temp = minimum temperature, SpeciesEp = *E. pauciflora*, SpeciesEv = *E. viminalis*)

2.5 Discussion

Monitoring on-ground revegetation is essential to evaluate the success of planting techniques and to inform the future direction of revegetation practice (Field *et al.* 2007). It is widely acknowledged in the literature that insufficient monitoring is currently being done (Freudenberger and Harvey 2003; Field *et al.* 2007; Munro *et al.* 2007: Smith 2008; Lindenmayer and Likens 2009). To address this issue, the present study investigated the potential for established farm revegetation to provide reliable empirical data upon which to draw scientific conclusions and guide revegetation practice. Using multimodel inference, statistically and ecological sensible models were developed to identify factors influencing the survival and growth of eucalypts that occur naturally in the region. This study also revealed flaws in the design and monitoring of this farmland revegetation project, specifically: (1) exact dates of planting and species provenance; (2) variable planting times; (3) small sample sizes; (4) the non-random selection of transects; (5) the uneven representation of eucalypt species in each transect; (6) the lack of monitoring during early establishment, and (7) the absence of long-term climate data. Addressing these issues will be important for improving the quality of data collected in the future. To achieve this, experimental design and monitoring protocols must be underpinned by methodologies that are statistically robust and allow for rigorous data analyses so that changes in the variables being measured can be detected (Field *et al.* 2007). Meaningful results and conclusions must be able to be drawn to defend the decision to undertake restoration work and justify the funding directed towards it. This highlights the need for collaboration between scientists, community members and landholders involved in tree planting to increase opportunities for meaningful monitoring and learning by doing. Based on observations from the engineered-woodland tree plantings, improvements to planting, the collection of baseline data and monitoring protocols can be recommended (Table 2.7).

Table 2-7 Recommendations for improving monitoring opportunities of engineered-woodland plantings

Planting design	Specification of baseline data	Monitoring
Random selection of study sites	Record planting dates	Conduct monthly monitoring in first 3 months
Ensure large sample size	Record seed provenance information	Conduct longitudinal monitoring from planting to maturity
Eliminate confounding variables	Record nursery propagation techniques	Reduce intervals between monitoring (6–12 months)
Consult with statistician pre-planting to ensure appropriate statistical power	Record weather and soil conditions at planting time	Ensure sites are clearly marked for easy identification

One area of scientific enquiry where collaboration between scientists and the community has been successful is that of citizen science. Citizen science engages non-professionals in authentic research ranging from long-standing large-scale projects to personalised research experiences (Dickinson *et al.* 2012). The overall view of citizen science is that, if done correctly, it can be a powerful way of increasing public environmental awareness and for fostering Earth stewardship, with the potential to create opportunities for ecological research at unprecedented spatial and temporal scales (Cooper *et al.* 2007; Dickinson *et al.* 2012). In recent years, citizen science has gained such a large following that it has been the most productive means of generating peer-reviewed publications in the field of ecology (Cooper *et al.* 2007). Its success is due to adherence to simple ground rules developed in collaboration with professional researchers to ensure the reliability of the data collected (Cooper *et al.* 2007). These ground rules mandate that: (1) research design must incorporate study protocols that take citizen science into account; (2)

researchers directing studies involving citizen scientists must be prepared to scrutinise data carefully and be willing to discard suspect or unreliable data, and (3) measures to test results for reliability, such as pairing trained researchers with citizen scientists to compare data, must be put in place (Cohn 2008). If similar ground rules were applied to farmland tree plantings, effective monitoring resulting in reliable scientific research could be more easily achieved.

2.5.1 Species performance

This study measured the performance of three eucalypt species. Both *E. viminalis* and *E. pauciflora* were grown from local provenance (Northern Tablelands) seed. *Eucalyptus nitens*, on the other hand, was grown from seed of a Tasmanian provenance, even though populations exist along the eastern escarpment of the Northern Tablelands where the rainfall is higher than in the study region. *Eucalyptus viminalis* survived best, with survival rates 1.6 times greater than *E. nitens* and 1.7 times greater than *E. pauciflora* (2014 data). Survival of all three species declined significantly between 2013 and 2014 due to summer drought. Mortality was twice as high in *E. nitens* compared to *E. pauciflora* and *E. viminalis*. *Eucalyptus nitens* was the fastest growing species with a mean height 1.5 times greater than that of *E. pauciflora* and 1.3 times greater than *E. viminalis* (2013 data). This species is well known for rapid growth and is widely grown in plantations in Australia and overseas (King *et al.* 1980; Chesterfield *et al.* 1991; Close *et al.* 2010; Harwood 2011). Height was greater for trees planted in November 2007 than October and December 2007. Examination of 2007 climate data revealed that November experienced higher rainfall and lower maximum temperatures (monthly averages: 141.6 mm and 20.7°C, respectively) than October (90.8 mm and 23.1°C) and December (117.6 mm and 22.9°C) (Armidale Airport Station; Bureau of Meteorology 2016). Tree growth is important from an ecological and management perspective. Taller individuals have a competitive advantage over shorter trees because they are able to intercept more light for photosynthesis. In addition, height has been shown to be positively correlated with seed size (Wright *et al.* 2007). Larger seeds are less prone to desiccation and contain more endosperm, which supports the survival of early-phase germinants. Taller trees also tend to have thicker bark than shorter trees, which provides better protection against extreme temperatures, fire, livestock browsing and boring insects. From a management viewpoint, fast growth is important if the trees have been planted for the purposes of crop, pasture and livestock protection, or to provide wood products within a commercial time frame.

2.5.2 Effect of cold temperature and drought on tree performance

Our results supported the hypothesis that extreme temperatures contribute to declines in survival and growth of planted eucalypts. Cold temperatures and frost cause serious disturbances in cool-temperate ecosystems, including reduced tree growth, changes in growth form, and susceptibility to pests and tree mortality (Troeng and Linder 1982; Matusick *et al.* 2016). Many eucalypts are susceptible to low temperatures below −10°C (King *et al.* 1980). Consequently, there has been interest in the selection of cold-tolerant and frost-resistant species for cold-temperate climates. *Eucalyptus nitens,* in particular, has been the focus of many trials because it is suitable for planting at high altitude where severe frosts and snow occur, and is superior in terms of rapid growth (King *et al.* 1980; Turnbull and Eldridge 1983; Byrne *et al.* 1997; Tibbits and Hodge 2003; Close *et al.* 2010). Although *E. pauciflora* and *E. viminalis* have not been as extensively trialled, several cold-tolerance studies have been performed.

In this study, *E. pauciflora* was more tolerant to cold stress compared to *E. nitens* and *E. viminalis* as it exhibited greater survival at low temperatures. Previous acclimation experiments conducted in frost chambers have demonstrated good cold tolerance and frost resistance in *E. nitens, E. viminalis* and *E. pauciflora* after hardening (Paton 1972; 1980; Harwood 1980; Tibbits and Reid 1987; Warren *et al.* 1998; Navarrete-Campos *et al.* 2013). For example, Paton (1980) demonstrated that *E. viminalis* seedlings exposed to temperatures of 2°C over 2 days were able to withstand subsequent temperatures of −6.5°C, and by progressively lowering hardening temperatures to −2.5°C, some seedlings could tolerate temperatures of up to −14°C. *Eucalyptus nitens* and *E. pauciflora* have been shown to tolerate temperatures as low as −10.7°C under similar hardening regimes (Harwood 1980; Tibbits and Reid 1987; Warren *et al.* 1998). However, *E. pauciflora* may have a survival advantage because it does not suffer from freeze-induced dehydration (Cochrane and Slatyer 1988; Ball *et al.* 1991) and recovers quickly from cold-induced photodamage, consistent with its subalpine distribution (Warren *et al.* 1998).

Landscape position was not included in this study as a predictor variable because it was correlated with slope and altitude. However, we strongly suspect that landscape position influenced the survival and growth of these eucalypts because frost hollows are a common feature of undulating tableland environments (Farrell and Ashton 1973; Moore and Williams 1976; Smethurst and Walker 2011). Frost hollows form as a result of cold air drainage when cold air mass flows downhill and pools in local depressions as well as on valley floors. Frost hollows exacerbate the severity of cold temperatures and increase the risk of freeze injury and cold-induced photoinhibition, and are the reason for the absence of trees on flat valley bottoms in subalpine regions (Moore and Williams 1976; Smethurst and Walker 2011).

Cold temperatures also had a direct impact on growth, as tree height increased with increasing minimum temperature in all three species. In eucalypts, continuous exposure to cold temperatures and frosts retards growth, often resulting in a persistent juvenile form (Farrell and Ashton 1973; Wardle 1974; Gilfedder 1988). The literature offers several explanations as to why the height of subalpine trees may be negatively influenced by low temperatures. Firstly, low soil temperatures may limit nutrient uptake by negatively influencing mycorrhizal symbionts, which enhance resource capture for their hosts (Chesterfield *et al.* 1991). Secondly, cold temperature may influence tree growth directly through repeated frost damage to meristematic tissue and buds, which due to shortened growing seasons are often not replaced (Körner 1998). Loss of foliage may also reduce photosynthetic activity resulting in insufficient carbon to support maintenance and minimum growth (Reid and Palazzo 1990; Körner 1998; Wiley and Helliker 2012). Finally, reduced height may be the trade-off for energy expenditure on cold-tolerance mechanisms, to the detriment of tree growth (Stott and Loehle 1998).

Our results supported the hypothesis that drought would be an important stressor affecting eucalypt performance on the Northern Tablelands. In this study, tree survival declined significantly following the summer drought in 2013–2014 (Fig. 2.5). Drought-induced mortality seemed to be exacerbated in locations with a northerly aspect, in well-drained soils and on ridgetops.

Glasshouse and field studies have demonstrated that high rates of productivity characteristic of fast-growing eucalypts such as *E. nitens* are often associated with high rates of water use (White *et al.* 2011; Duan *et al.* 2013; Mitchell *et al.* 2013; Booth *et al.* 2015). This has a detrimental impact on tree survival under drought conditions and may explain our observations of increased drought-induced mortality in *E. nitens* compared to the other two species.

Figure 2-5 The same transect at 'Blaxland', Wollun, in (A) early 2013, and (B) following the 2013–2014 drought

Source: Photographs by Sharon Brown

Reid *et al.* (2013) predicted that mature stands of *E. nitens* planted in upper-slope positions in the study region may exploit all the available soil water under drought conditions, resulting in reduced growth and increased mortality.

The susceptibility for *E. nitens* to drought may also be attributed to its poorer tolerance to water stress than other species of eucalypts (White *et al.* 1996). While, to our knowledge, there are no other studies comparing levels of water stress among the species examined in this study, previous work comparing water stress between *E. nitens* and *E. globulus* demonstrated that the effects of water stress in *E. nitens* persist for longer periods of time resulting in slower recovery from drought (White *et al.* 1996; Whitehead and Beadle 2004). This was because *E. globulus* could reduce stomatal conductance more slowly and maintain positive turgor over a range of relative leaf water content in comparison to *E. nitens* (White *et al.* 1996). Prolonged periods of stomatal closure reduce the rate of photosynthesis and can lead to carbon starvation and increased mortality under drought conditions (McDowell *et al.* 2008).

2.5.3 The effects of weed control on tree performance

Weeds reduce establishment, survival and growth of native trees by competing for light, water and nutrients (Wagner *et al.* 1989; Garau *et al.* 2009). Furthermore, cold-climate eucalypts stressed by weed competition are often more susceptible to freeze injury compared to trees uninhibited by weeds (Kellison *et al.* 2013). Eucalypts are vulnerable to weed competition, and low survival due to poor weed control has been noted in several studies (King *et al.* 1980; Garau *et al.* 2009; Kellison *et al.* 2013). Consequently, effective weed control is essential for successful revegetation. Our study showed that the three eucalypt species responded differently to varying levels of weed control. *Eucalyptus pauciflora* survived better at sites where weed control was effective and weed cover was low shortly after planting. *Eucalyptus viminalis* appeared to survive better at sites with high weed cover, while *E. nitens* survival was not influenced by weed cover. As tree survival is usually associated with low weed cover (George and Brennan 2002; Andrews *et al.* 2004), the *E. viminalis* result was unexpected, but could be related to different strategies employed by each species to minimise the effects of competition (Grime 1977; Goldberg and Barton 1992). Given that *E. pauciflora* inhabits cold subalpine environments, this species may allocate more energy to cold tolerance than competition avoidance and be more vulnerable to competition from weeds (Grime 1977; Tilman 1982). In comparison, *E. viminalis* might be adapted to high levels of interspecific competition due to attributes that maximise resource capture in the presence of competitors, while *E. nitens,* the fastest growing species, may avoid competition by out-growing competing weeds (Grime 1977; Goldberg and Barton 1992).

While this study identified cold temperature, drought and competition with weeds as significant stresses of Northern Tablelands eucalypts, it is important to note that the causes of tree decline are complex and involve a multitide of factors not measured here. One drawback of whole paddock restoration is the inevitable reintroduction of livestock. Of the seven properties investigated, most landholders allowed livestock to return to engineered woodland paddocks within 3 years of planting. Varying degrees of browse damage were sustained, but on some properties (Lakeview, Allfoxton and Kobadah) tree loss due to the snapping off or pushing over of young saplings was more severe. Grazing by cloven-footed livestock also has a major impact on soil structure and the soil regulatory processes that provide plants with water and nutrients (Yates *et al.* 2000). Soil compaction beneath eucalypt trees used as refuges against the weather and as stock camps is a common problem. Soil compaction interrupts important hydrological processes at the landscape scale by reducing infiltration and increasing run-off (Yates *et al.* 2000). Furthermore, it reduces the size of soil pores that function as important sites for concentrated microbial activity, including mycorrhizae (Tommerup and Bougher 2000).

In the absence of mycorrhizae, the capacity for eucalypts to capture water and nutrients from the surrounding soil is limited and can negatively impact on tree health (Tommerup and Bougher 2000). Mycorrhizal populations are further compromised by the addition of agricultural fertilisers to enhance pasture growth (Grove *et al.* 1991). Northern Tablelands gazing properties have typically applied superphosphate to pastures since the 1950s and many of the properties studied continue this practice. There is accumulating evidence to suggest that soil nutrient enrichment increases the abundance of defoliating insects, particularly scarab beetles and chrysomelid leaf beetles, as well as the palatability of eucalypt leaves in terms of increased nitrogen and water content (Landsberg 1990; Landsberg *et al.* 1990; Reid and Landsberg 2000). Insect-mediated rural dieback is considered to be a major cause of tree decline in the study region and elsewhere where livestock production is the principal land use (Carter *et al.* 1981; Mackay *et al.* 1984; Reid and Landsberg 2000; Jurskis and Turner 2002).

Habitat fragmentation also contributes to the decline of eucalypts in cleared agricultural regions (Butcher *et al.* 2005). Reduced size and increased spatial isolation of populations occupying fragmented remnants often leads to an erosion of genetic variation due to increased random genetic drift, reduced gene flow between populations and elevated inbreeding depression (Young *et al.* 1996). This may manifest as a decline in seed production, seed viability, germination and growth (Butcher *et al.* 2005), and has been reported in several species of *Eucalyptus* (Griffin and Cotterill 1988; Hardner and Potts 1995; Kennington *et al.* 1997; Butcher *et al.* 2005; Broadhurst 2013).

2.6 Conclusions

Minimum temperature, post-planting weed control and summer drought were important determinants of the survival and growth of planted eucalypts in upland temperate pastures. Our recommendations for planting eucalypts in cool-temperate climates are to avoid planting in cold-air drainage lines and frost hollows, in addition to effective and timely pre-planting and post-planting weed control. This study was limited by the non-scientific design of the plantings, monitoring and record keeping. Detecting change in ecological systems is a difficult technical and logistical challenge (Field *et al.* 2007), but monitoring is essential if we are to learn from successes and failures and apply this knowledge to guide future revegetation activities. We demonstrated in the absence of rigorous scientific design that snapshot monitoring protocols and ad-hoc repeat monitoring, while not ideal, were sufficient to detect change in planted shelterbelts and provide insights into the environmental and management factors responsible for species differences in survival and growth in such plantings. Although it may not be possible to 'science-up' already established revegetation, it is possible to obtain informative data from existing plantings. This is a positive result, given that the majority of farmland tree plantings currently focus more on getting trees in the ground than keeping them there.

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We, the PhD candidate and the candidate's Principal Supervisor, certify that all co-authors have consented to their work being included in the thesis and they have accepted the candidate's contribution as indicated in the *Statement of Originality*.

CHAPTER 2 Farmland Revegetation and Scientific Inference: Survival and Growth of Eucalypt Species in Temperate Upland Pastures

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CHAPTER 3

Twin-Walled Polypropylene Tree Guards Enhance the Establishment and Early Growth of Native Trees and Shrubs in a Temperate Upland Pasture

3.1 Abstract

Establishing native trees in temperate regions for revegetation can be challenging given the variations in local climate. Tree guards have been shown to consistently increase survival and growth in a variety of species. This study compared the survival and growth of five native tree and shrub species (*Leptospermum polygalifolium, Callistemon pungens, Eucalyptus viminalis, E. stellulata* and *E. acaciiformis*) planted in two different guard types at three landscape positions in an upland pasture. Seedlings were planted in summer and autumn at lower-slope, mid-slope and upper-slope landscape positions. Half of the seedlings were allocated a tall Corflute® guard (60 cm high) and the remainder were allocated a milk carton (30 cm). Seedling survival and height were measured between March and November 2014. Hourly temperature readings were recorded using iButtons®. Seedlings in tall guards survived better than seedlings in milk cartons at mid and upper-slope positions. Seedling height for all species was greater in tall guards than milk cartons

at all landscape positions. Eucalypts in particular, benefited from tall guards, with heights up to three times greater than in milk cartons. Average maximum temperature inside tall guards was consistently higher than that inside milk cartons or ambient conditions between March and November at all landscape positions. Average minimum temperature was coldest at the lower-slope site, while average daily temperature and average maximum temperature did not differ between landscape positions. Seedling performance in tall guards was most likely enhanced by increased temperature inside the guard, which extended the growing period. Therefore, tall Corflute® tree guards were beneficial to seedling establishment and growth in this cool-temperate environment, and have the potential to increase revegetation success using planted tube stock seedlings.

3.2 Introduction

Tree guards increase the survival and growth of planted seedlings by preventing animal browsing, reducing transplant shock, mitigating herbicide drift, and protecting seedlings against adverse climates (Potter 1991; Bellot *et al.* 2002; Lai and Wong 2005; Sharew and Hairston-Strang 2005; Chaar *et al.* 2008; Keeton 2008). As a result, they are widely used in horticulture, and urban, rural and amenity plantings (Potter 1991; Costello *et al.* 1996; Lai and Wong 2005; Keeton 2008). Tree guards modify the microclimate surrounding the growing seedling by increasing temperature and humidity, decreasing air movement and altering CO_2 concentrations, and reducing light interception (Potter 1991; Sharew and Hairston-Strang 2005; Devine and Harrington 2008). They have also been shown to harvest large quantities of dew and relieve drought stress (del Campo *et al.* 2006). Microclimatic modification is largely dependent on local climate and tree guard design (Devine and Harrington

2008). In Mediterranean climates, tree guards have the potential to aggravate heat stress and increase seedling mortality (Costello *et al.* 1996; Ladd *et al.* 2010), whereas in cooler climates, they have been shown to benefit survival (West *et al.* 1999; Sharrow 2001; Chaar *et al.* 2008). For example, in cold environments tree guards act as a buffer against cold-induced photoinhibition (Egerton *et al.* 2000; Close *et al.* 2002). This phenomenon occurs through exposure to freezing temperatures, which increase a plant's sensitivity to light and inhibit the synthesis of protein pigments that would normally reverse the effects of photodamage (Ball *et al.*1991; Blennow and Lindkvist 2000; Murata *et al.* 2007). Investigations of tree guard design in relation to material, colour, height and ventilation, and their effect on microclimate and seedling establishment have yielded varying results (Kjelgren *et al.* 1997; Dupraz and Bergez 1999; West *et al.* 1999; Sharrow 2001; Bellot *et al.* 2002; Sharew and Hairston-Strang 2005; del Campo *et al.* 2006; Chaar *et al.* 2008; Devine and Harrington 2008; Close *et al.* 2009). Given the range of potential advantages and disadvantages, comparisons of guard types in different environments are useful to identify designs that optimise seedling establishment and growth.

Establishing trees and shrubs in exposed landscapes with cold climates can be challenging (Ladd *et al.* 2010; Leslie *et al.* 2013; Reid *et al.* 2013). The Northern, Central and Southern Tablelands of New South Wales, Australia, experience cold winters characterised by sub-zero temperatures and severe frosts between March and November. Conditions are exacerbated by cold-air drainage in low-lying valleys and basins, which further impede tree establishment and prevent government revegetation targets from being met (Egerton *et al.* 2000; Close *et al.* 2002). Milk cartons have been widely used on the Northern Tablelands to establish native trees in pasture environments due to availability and price, but the results are often disappointing. Practitioners and landholders may benefit from using superior guard types to increase the success of revegetation efforts.

This paper describes an experimental field comparison of the effects of two types of tree guards on the establishment and early growth of five species of sclerophyll trees and shrubs in open pasture on the Northern Tablelands of NSW. Based on the literature (Potter 1991; West *et al.* 1999; Sharrow 2001; Bellot *et al.* 2002; Sharew and Hairston-Strang 2005; del Campo *et al.* 2006; Danby and Hik 2007; Chaar *et al.* 2008; Devine and Harrington 2008; Ladd *et al.* 2010), we tested the hypothesis that seedling survival and growth would be greater in tall guards at all landscape positions. The aims of this study were to: (1) compare the survival and growth of native seedlings planted in tall Corflute® guards and milk cartons at three landscape positions in a temperate environment, and (2) examine the influence of guard types on ambient temperature.

3.3 Materials and methods

3.3.1 Study area

This study was conducted at ‘Lakeview’, a grazing property 5 km south of Uralla, NSW, Australia (30°35′S, 151°32′E). The property is situated on the Northern Tablelands, a stepped plateau of hills and plains between 600 and 1500 m a.s.l. The region experiences mild summers and cold winters (January mean maximum daily temperature, 25.9°C; July mean minimum daily temperature, 1.2°C, Bureau of Meteorology 2016). The mean annual rainfall of Uralla is 795 mm with 60% of Uralla’s average rainfall occurring in summer (October–March, Bureau of Meteorology 2016). The study area was a pasture on a gently inclined slope with a southerly aspect. Three sites were established in different landscape positions: lower slope (1037 m a.s.l.), mid slope (1043 m) and upper slope (1047 m). The lower and mid-slope sites were 520 m apart, while the mid and upper-slope sites were separated by 836 m. Winter temperatures frequently fell below 0°C at the site and were accompanied by severe frosts. The lowest recorded temperature at the site during the study was –11.5°C. Prior to European settlement, the study site would have been woodland dominated by *Eucalyptus melliodora* and *E. blakelyi*. At the time of this study, the vegetation was primarily sown, naturalised and volunteer pasture species with scattered native trees. The topsoil was sandy loam derived from granite parent materials (Li *et al.* 2007; NSW Office of Environment and Heritage 2011).

3.3.2 Experimental design

Field work was conducted between December 2013 and November 2014. Five species were chosen for this study, *Leptospermum polygalifolium* Salisb., *Callistemon pungens* Lumley & R.D. Spencer, *Eucalyptus viminalis* Labill., *E. stellulata* Sieber ex DC. and *E. acaciiformis* H. Deane & Maiden, based upon their ability to tolerate cold climates. Site preparation began in August 2013 with deep ripping and mounding followed by application of a non-selective herbicide (glyphosate) in September 2013. Mid and upper-slope sites received an additional application of non-selective herbicide in March 2014. Planting of the lower-slope site occurred in December 2013. Due to hot, dry weather, the mid and upper-slope sites were not planted until March 2014. At the time of planting, all seedlings received 700 mL of water. The lower-slope site incorporated five pairs of each species randomly placed in a single planting row, with a tall guard and a milk carton arbitrarily allocated to the seedlings in each pair (Fig. 3.1). The robustness of the design was improved by incorporating ten replicates of paired guard treatments of each species in the mid and upper-slope plantings. The seedlings, spaced 2 m apart, were planted into the top of the mound using a pottiputki. Tall guards were a rigid enclosed triangle of UV-stabilised green translucent plastic Corflute® (60 cm high × 20 cm wide) secured with a hardwood stake. Milk cartons were a square tube of thin waxed cardboard (30 cm high × 7 cm wide) secured with bamboo stakes. A 'no guard' control was not used in this experiment. While controls are fundamental to sound experimental design, partnerships with landholders sometimes require concessions. In this case, the landholder did not want to plant seedlings without tree guards because he had experienced high seedling mortality in previous unguarded plantings and he was not prepared to risk similar losses again.

Using iButtons®, ambient temperature was measured 20 cm above ground level at the beginning, middle and end of each planting line (1) between planted seedlings in the planting line, (2) inside tall guards, and (3) inside milk cartons. Temperature was recorded at hourly intervals between March and November 2014. The survival and height of seedlings were measured in November 2014. Seedlings were recorded as dead or alive and percent survival calculated. Seedlings that resprouted after complete stem dieback were counted as alive. Height was measured from the base to the top of the living stem using a steel tape measure. The sites received follow-up weed control treatments of glyphosate in April and October 2014. Plantings in the lower-slope site were watered (700 mL per seedling) twice in January 2014, while the mid and upper-slope plantings were watered twice in October and once in November 2014, due to dry conditions.

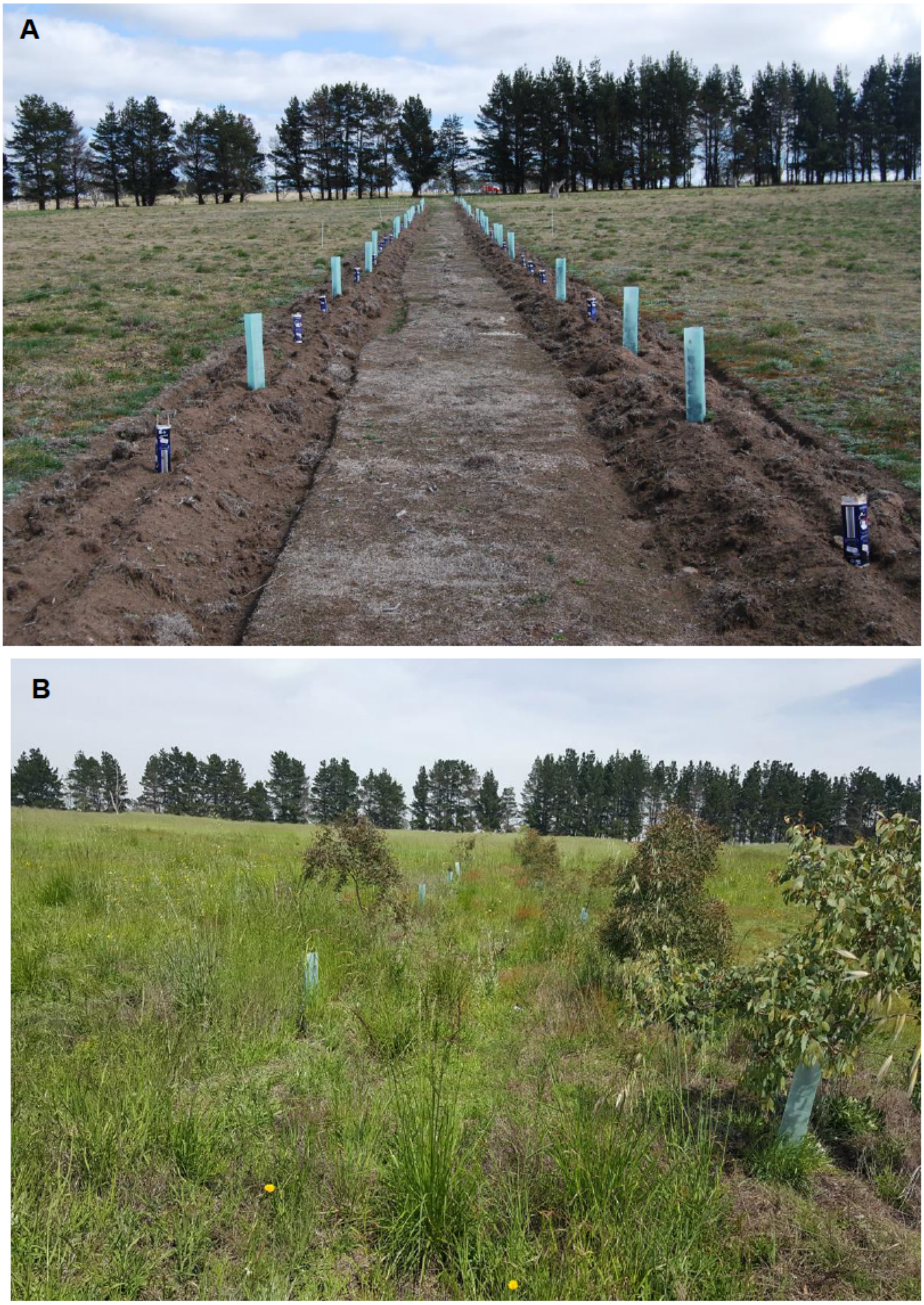


Figure 3-1 Mid-slope site at 'Lakeview' (A) 5 months post-planting (August 2014), and (B) 3.5 years post planting (November 2016)

Source: Photographs by Sharon Brown

3.3.3 Statistical analyses

Given the difference in planting times, analyses of the performance of the lower-slope plantings were undertaken separately to the combined analyses of the mid and upper-slope sites. November 2014 data were used for all analyses. Seedling survival was analysed using Fisher's exact tests comparing the survival of all plants between guard treatments. To examine differences in seedling height between species and guard treatment, we used a linear model with species, treatment and their interaction as fixed effects. For the analysis of seedling height in the mid and upper-slope sites, position in the landscape was added as a blocking factor. Seedling height was square-root-transformed prior to analysis and model diagnostics were checked to ensure goodness of fit. Models were fitted with the lm package using the R statistical computing environment (version 3.0.2; R Development Core Team 2015). Where the species by treatment interaction was significant, we used the lsmeans routine to compute pairwise comparisons between means, using the Tukey *P*-value adjustment for multiple comparisons. Hourly temperature data were partitioned into weekly intervals to compute the (weekly) average daily temperature, the average maximum temperature and the average minimum temperature per week for each iButton®. The March–November distribution of the weekly values of each temperature response variable were compared between each pair of guard treatments (ambient vs tall, ambient vs milk, and tall vs milk) within position in the landscape using a bootstrap version of the Kolmogorov–Smirnov test to compare the equality of two distributions (*D* statistic; Quinn and Keough 2002). We also compared the distribution of weekly temperatures for ambient conditions between each position in the landscape using the Kolmogorov–Smirnov test.

3.4 Results

3.4.1 Seedling performance

Survival of seedlings in tall guards and milk cartons in the lower-slope site was 88% and 80%, respectively, in November 2014. The difference in seedling survival between guard treatments at the lower-slope site was not significant (Fisher's exact, $P = 0.70$). However, at the mid and upper-slope sites survival was 97% (tall guards) and 84% (milk cartons), respectively, differing significantly (Fisher's exact, $P = 0.003$). Mean height (mean ±1 s.e.m.) of seedlings in tall guards and milk cartons at the lower-slope site was 93.8 ± 4.33 cm and 34.8 ± 2.62 cm, respectively, in November 2014. There was a highly significant interaction between species and guard type at the lower-slope site ($F = 4.69$, $df = 4$, $P < 0.005$). Mean seedling height was greater in tall guards than milk cartons for all species, particularly for eucalypts (Fig. 3.2), but the difference was not significant for *L. polygalifolium* (Fig. 3.3A).

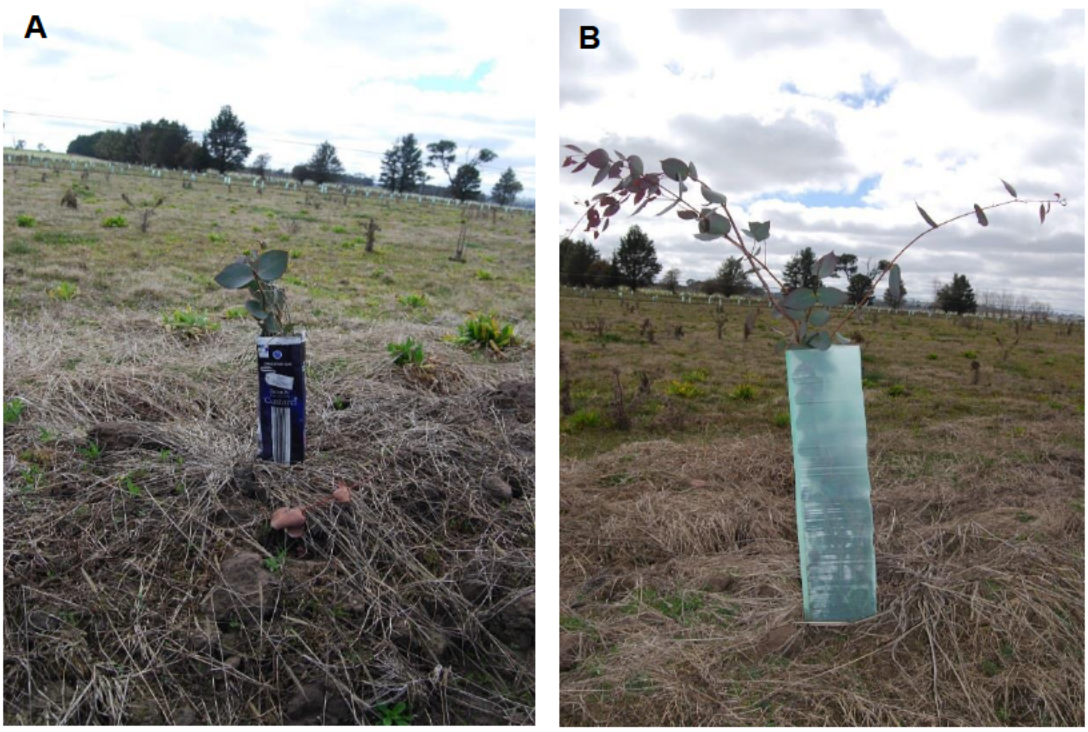


Figure 3-2 ***Eucalyptus stellulata*** **seedlings in (A) milk carton, and (B) tall guard, 5 months post-planting**

Source: Photographs by Sharon Brown

In mid and upper-slope positions, the effect of landscape position was significant ($F = 7.17$, $df = 1$, $P < 0.005$) (Table 3.1), with taller seedlings higher in the landscape (upper slope: 55.5 ± 3.9 cm, vs mid slope: 51.3 ± 3.8 cm). There was a highly significant interaction between guard type and species (Table 3.1).

At the mid-slope site, tall guards significantly outperformed milk cartons in terms of seedling height of *E. viminalis* and *E. stellulata* (Fig. 3.3B). Tall guards also outperformed milk cartons at the upper-slope site, this difference being significant for *E. viminalis*, *E. stellulata* and *E. acaciiformis* (Fig. 3.3C). The difference in seedling height between guard types was not strongly significant in the two shrub species (*L. polygalifolium* and *C. pungens*).

Table 3-1 Results of one-way and two-way ANOVA for the effect of guard treatment and species on seedling height at lower-slope, mid- and upper-slope landscape positions

Seedling height	**Degrees of freedom**	**F value**	***P* value**
Lower slope			
Species	4	18.54	$5.7e^{-08}$
Guard treatment	1	108.75	$8.0e^{-12}$
Species:guard treatment	4	4.69	$4.3e^{-03}$
Mid and upper slope			
Site	1	7.17	$8.1e^{-03}$
Species	4	87.34	$2.2e^{-16}$
Guard treatment	1	155.18	$2.2e^{-16}$
Species:guard treatment	4	9.93	$3.0e^{-07}$

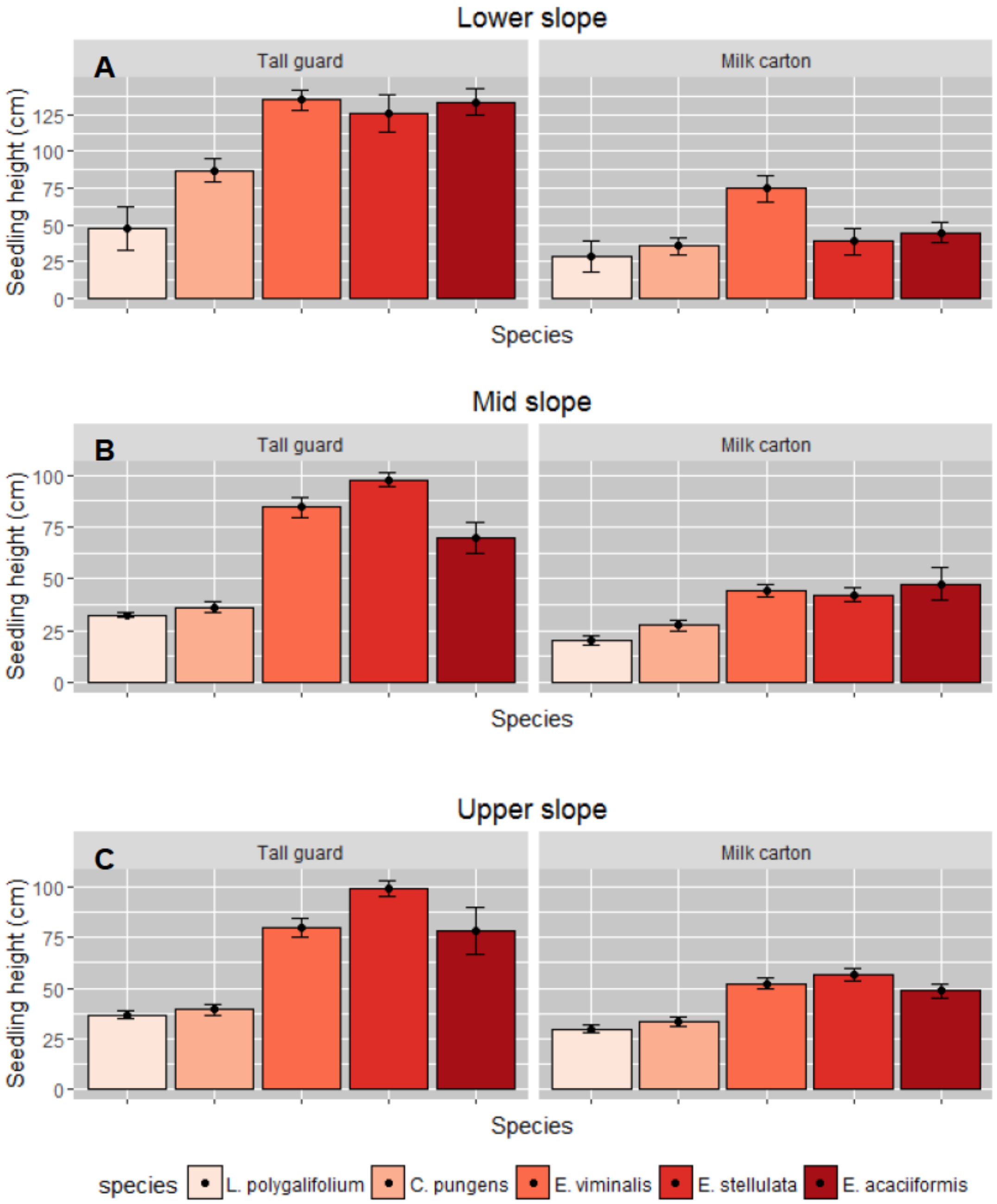
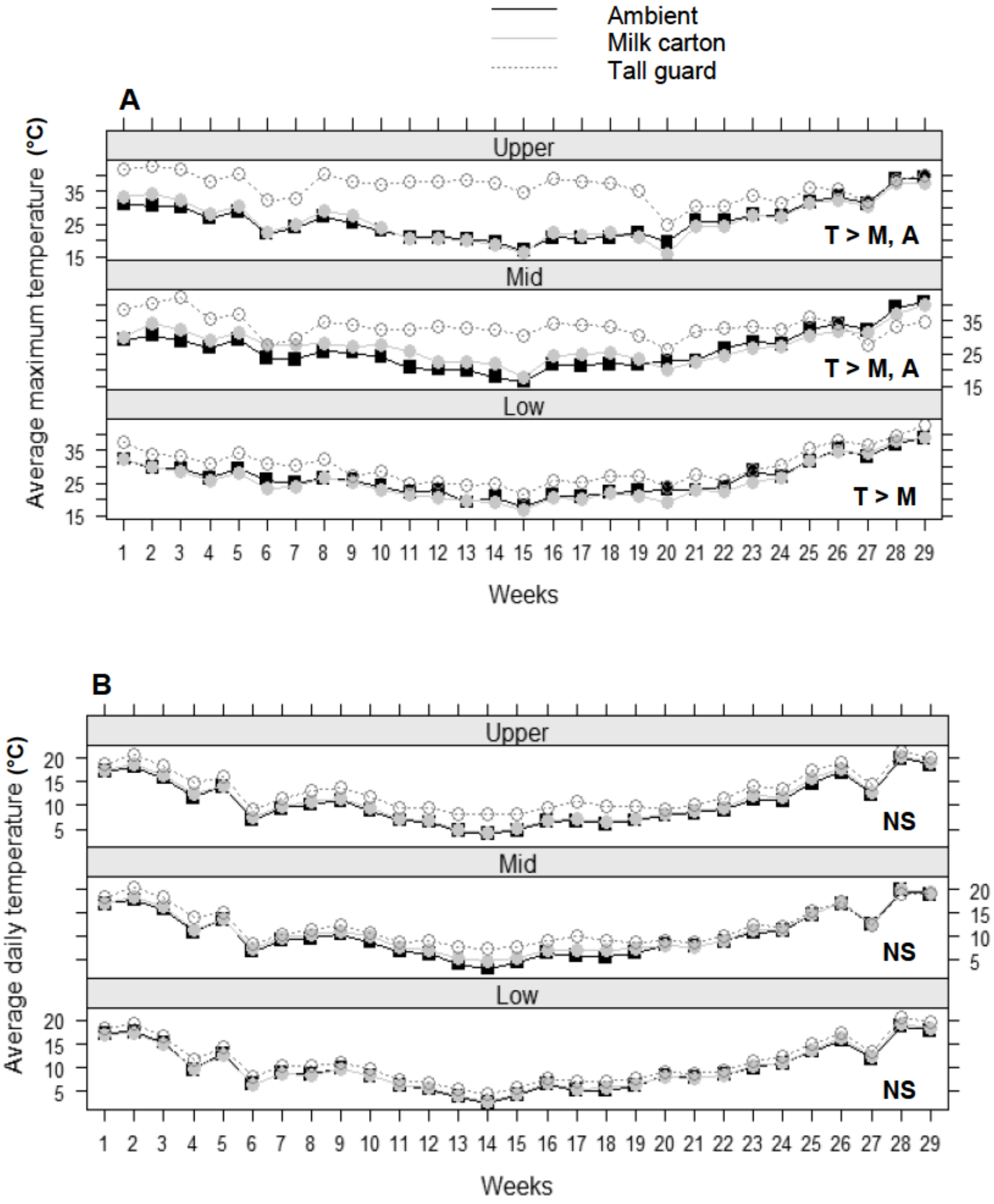


Figure 3-3 Seedling height (mean ± 1 s.e.) for tall guards and milk cartons for (A) lower-slope, (B) mid-slope, and (C) upper-slope landscape positions

3.4.2 Temperature

Average maximum temperature was higher in tall guards than milk cartons over the monitoring period (March–November) regardless of landscape position (Fig. 3.4A). Average daily temperature (Fig. 3.4B) and average minimum temperature (Fig. 3.4C) did not differ between guard types and ambient conditions in any landscape position. The effect of landscape position on ambient temperature did not vary significantly except for average minimum temperature, for which there was a marginally significant difference between the upper and lower landscape positions (−0.8 ± 0.8°C vs −4.0 ± 0.9°C, respectively, $D = 0.34$, $P = 0.06$).



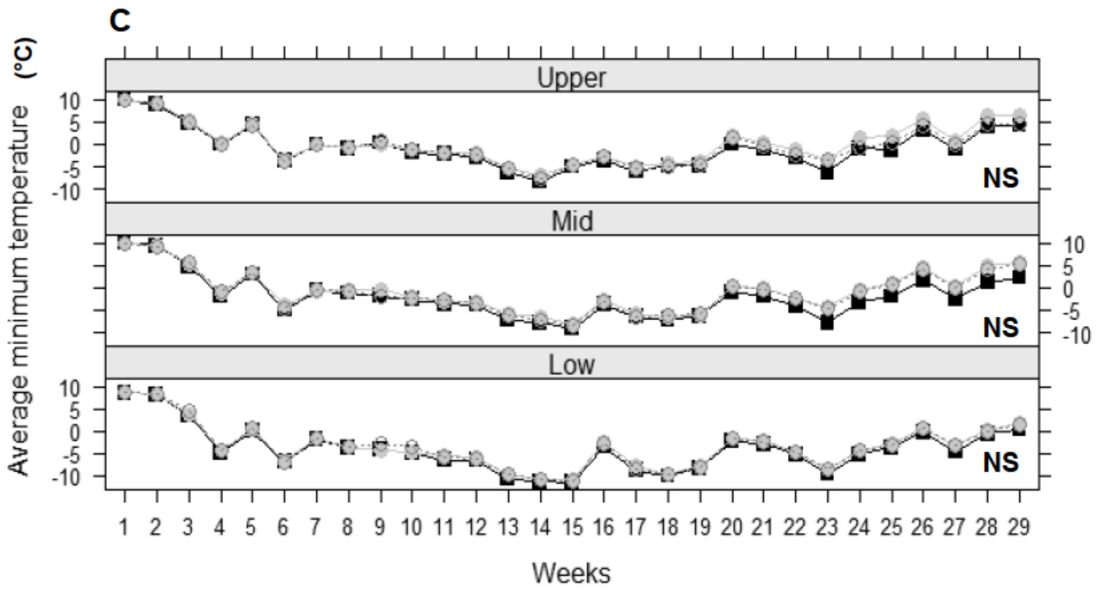


Figure 3-4 (A) Average maximum temperature, (B) average daily temperature, and (C) average minimum temperature in tall guards, milk cartons and ambient conditions at three landscape positions

NS = no significant difference between guard types and ambient conditions; T > M,A = ave. max. temp. in tall guards significantly greater than that in milk cartons and ambient conditions ($P \leq 0.05$), with no significant differences between the latter

Temperatures between guard treatments varied diurnally (Fig. 3.5). Temperatures between 5.00 pm and 7.00 am were similar for tall guards, milk cartons and ambient temperature, but during the day, temperatures increased more quickly and reached higher levels in tall guards compared to milk cartons and ambient temperature.

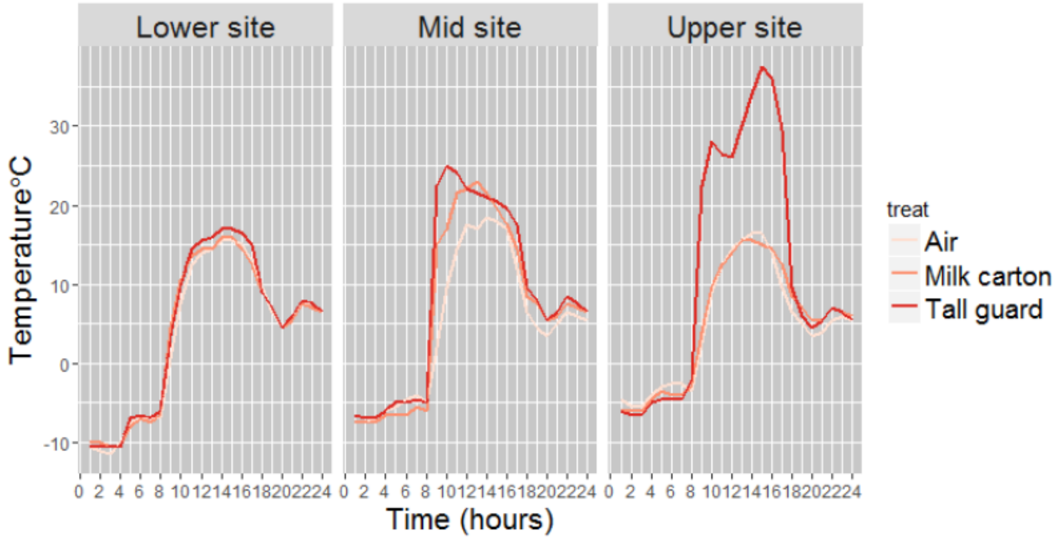


Figure 3-5 Recorded temperature over 24 hours on 9 July 2014 inside tall guards, milk cartons and ambient conditions at lower-slope, mid-slope and upper-slope landscape positions

3.5 Discussion

3.5.1 Seedling response to tree guard treatments

Our results supported the hypothesis that seedling survival and height would be greater in tall guards than milk cartons, and were consistent with past research documenting improved seedling performance in translucent plastic guards compared to other guard types (Potter 1991; Sharrow 2001; Bellot *et al.* 2002; Sharew and Hairston-Strang 2005; Chaar *et al.* 2008; Devine and Harrington 2008). Seedling survival was significantly higher in tall guards compared to milk cartons at mid and upper-slope landscape positions, but not at the lower-slope landscape position. Seedlings in the lower-slope site benefitted from being planted in spring after the last of the frosts, and had the chance to establish a well-developed root system before the following winter. The damaging effects of frost on plant growth and development have been widely reported (Paton 1972; Farrell and Ashton 1973; Wardle 1974; Harwood 1980; Gilfedder 1988; Egerton 2000; Janská *et al.* 2010). Frost causes injury to plant cells, either through the mechanical rupture of the cell membrane and cell wall, or through an imbalance in electrolytes as freezing removes water from solution (Hayden *et al.* 1986; Ball *et al.* 1991; Blennow and Lindkvist 2000).

Numerous studies highlight the benefits of different guard types to seedling survival (Dunn *et al.* 1994; Sharpe *et al.* 1999; West *et al.* 1999; Sharrow 2001; Chaar *et al.* 2008), while other studies report the absence of an effect (Costello *et al.* 1996; Bellot *et al.* 2002; Devine and Harrington 2008) or a negative effect of tree guards (Chaar *et al.* 2008; Devine and Harrington 2008; Close *et al.* 2009). In our study, seedlings planted in tall guards were generally taller than seedlings in milk cartons in all three landscape positions. Tree seedlings outperformed shrub seedlings at all landscape positions, which we attributed to differences in life form, genetic growth potential and adaptation. At the lower-slope site, guard type had little effect on the height of *L. polygalifolium* seedlings, and at the mid and upper-slope sites the height of *L. polygalifolium* and *C. pungens* seedlings was not affected by guard type. These two species naturally inhabit swamp hollows and riverbanks, and therefore are presumably well adapted to cold conditions.

Previous research suggests that increased seedling height may be facilitated by low light conditions in unventilated guards over 1 m tall (Applegate and Bragg 1989; Dupraz and Bergez 1999; Sharrow 2001; Chaar *et al.* 2008; Devine and Harrington 2008). Under such conditions, seedlings exhibit tall spindley growth accompanied by a decrease in basal diameter as more resources are allocated to stem elongation in preference to support structures (Sharpe *et al.* 1999; Devine and Harrington 2008). This disproportionate growth may result in bent posture (Chaar *et al.* 2008). However, once the crown emerges from the guard and is exposed to ambient conditions, the allocation to growth in basal diameter and height is corrected allowing trees to stand upright within a few years. Nevertheless, shorter guards, such as those used in our study are recommended (Dupraz and Bergez 1999; Hemery and Savill 2001; Chaar *et al.* 2008).

3.5.2 Effects of tree guards on microclimate

The positive effects of tree guards on seedling establishment are widely attributed to changed microclimatic conditions inside the guard (Tuley 1983; Potter 1991; Svihra *et al.* 1993; Costello *et al.* 1996; Sharew and Hairston-Strang 2005; Devine and Harrington 2008; Close *et al.* 2009). Tree guards modify the climate inside guards by increasing humidity, reducing air movements and light transmission, changing CO_2

concentrations, enhancing radiative cooler and dew harvesting, and increasing temperature. In our study, only the effects of temperature were investigated due to the finding in the previous chapter that cold temperatures significantly influence eucalypt survival and growth. However, studies of other microclimatic variables would have been useful and further research is needed to examine additional benefits of tree guards in relation to altered microclimate. Our study indicated that tall guards outperformed milk cartons in terms of seedling survival (except at the lower-slope landscape position) and growth because day-time temperatures inside tall guards were warmer than inside milk cartons, and presumably extended the growing season. The tall guards were constructed from Corflute®, or corrugated polypropylene sheeting comprised of two outer sheets separated by internal vertical channels. The twin-walled design of Corflute® had an advantage over the single-layer plastic-coated thin cardboard design of the milk cartons because it acted as a thermal insulator by allowing air to move through the channels, transferring heat from the warmer outer surface to the cooler inner surface without mixing the two streams of air (Singh *et al.* 2008; Ferguson 2009). Thermal conductivity is the ease with which heat flows through a material, thus the higher the conductivity, the greater the heat flow (Singh *et al.* 2008). Comparison of the thermal conductivity of commonly used insulation materials shows that plastic polymers, including twin-walled corrugated polypropylene have a high thermal conductivity, which is proportional to temperature (Al-Ajlan 2006; Singh *et al.* 2008).

Average maximum temperature was consistently greater in tall guards at all landscape positions, but average daily temperature and average minimum temperature did not differ significantly between guard types and ambient conditions or landscape position. These results were consistent with previous studies that report increased maximum daily temperature in translucent plastic tree guards compared to ambient temperature (Potter 1991; Kjelgren *et al.* 1997; Bellot *et al.* 2002; del Campo *et al.* 2006). The fact that minimum temperatures inside tall guards were no different to minimum temperatures inside milk cartons was an unexpected finding. A possible explanation is the radiative condensing properties of the tall tree guard walls, which has been reported in other tree guard studies (Kjelgren *et al.* 1997; del Campo *et al.* 2006). In addition, when heat is lost from the guard walls on a cold night, the air temperature inside the guards and under ambient conditions will equalise eventually, regardless of the materials the guards are made from.

3.5.3 Effects of landscape position on temperature

Temperature inside tree guards and ambient temperature varied with landscape position. The coldest temperatures were recorded at the lower-slope site, followed by the mid-slope site and the upper-slope site. The correlation between landscape position and temperature variation is well documented, particularly in temperate regions. High altitudes and minor variations in local topography combined with extensive tree clearing expose the landscape and facilitate cold-air drainage (Reid *et al.* 2013). Cold-air drainage occurs on still nights when heavier cold air moves downhill under the influence of gravity to collect in low-lying valleys and basins (Clements *et al.* 2003; Pypker *et al.* 2007). Changes in temperature from the base to the ridge of a slope by as much as 7.3°C have been recorded along natural landscape gradients with increases in seedling height corresponding to increasing temperature up-slope (Harwood 1976; Davidson and Reid 1985).

3.5.4 Are tall Corflute® guards worth the investment?

The benefits of tree guards for establishing native trees in farmland and forestry are well-known. However, tree guards increase the cost of revegetation substantially, so it is important to know whether they are worth the investment. Based on evaluations from past revegetation trials planted in the region, tree survival is severely compromised by cold temperatures and frost when guards are not used (Fig.3.6).

Fig 3-6 Two engineered woodlands (from Chapter 2) at (A) Allfoxton and (B) Kallaroo planted in exposed frost-prone landscapes (Kallaroo had so few surviving trees that it was omitted from the study)

Source: Photographs by Sharon Brown

The question, then, is which type of tree guard is best? On the Northern Tablelands, milk cartons are the most widely used tree guard, made popular in the 1980s by Landcare and bush regeneration groups. However, we observed during this study that milk cartons were inferior to tall guards for reasons other than those identified in the results. The disadvantages of using milk cartons for tree guards are: (1) they blow away easily; (2) they are attractive to rabbits, foxes and kangaroos, which chew the cartons and pull them off seedlings; (3) they degrade relatively quickly, and (4) they offer minimal protection to seedlings, which quickly grow beyond the top of the carton. As a consequence, taller, sturdier guards made from Corflute® are becoming more widely used. These guards are more expensive that milk cartons, but they solve the above problems. In addition, tall guards can be re-used and could become the preferred choice as milk carton production is phased out in favour of plastic milk bottles. A partial cost–benefit analysis (based on planting 1000 Heiko seedlings) revealed that Corflute® guards ($4.35 per seedling) are almost twice as expensive to use as milk cartons ($2.40 per seedling), based on the following: Heiko seedling cost ($1.10 per unit), guard cost (milk cartons $0.30 per unit; Corflute® guards $1.50 per unit), and labour costs (milk cartons $1.00 per seedling; Corflute® guards $1.75 per seedling). While tall guards improve seedling survival, such improvements were not substantial enough to warrant investing in these guards in the short-term. This was because the cost of using tall guards exceeded the cost associated with the seedling losses in milk cartons. However, the capacity of tall guards to increase seedling height has potential long-term economic benefits in terms of improved primary productivity (through the protection of livestock, pasture and crops) and the provision of ecosystem services (discussed in Chapter 1). These long-term benefits compensate for the initial purchase of the guards.

3.6 Conclusions

The higher survival and growth rates of seedlings grown in tall guards compared to milk cartons emphasise the value of selecting the optimal tree guard in revegetation to suit local conditions. Our study showed that tall Corflute® guards create a favourable microclimate inside the guard by increasing daytime temperatures between autumn and spring, extending the plant growing period. Therefore, these guards are a better option than milk cartons for reintroducing native trees to upland pastures in temperate climates because of their ability to accelerate growth in seedling height, which contributes to a shorter establishment period. This will have financial benefits in terms of livestock, crop and pasture protection. Based on these results, we recommend the use of Corflute® guards to improve the success of future revegetation activities on the Northern Tablelands. Further research investigating the ability of tree guards to harvest dew and reduce the effects of cold-induced photoinhibition is required to identify the full gamut of potential benefits of Corflute® guards.

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STATEMENT OF AUTHORS' CONTRIBUTION

(To appear at the end of each thesis chapter submitted as an article/paper)

We, the PhD candidate and the candidate's Principal Supervisor, certify that all co-authors have consented to their work being included in the thesis and they have accepted the candidate's contribution as indicated in the *Statement of Originality*.

CHAPTER 3 Twin-Walled Polypropylene Tree Guards Enhance the Establishment and Early Growth of Native Trees and Shrubs in a Temperate Upland Pasture

	Author's Name (please print clearly)	**% of contribution**
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STATEMENT OF ORIGINALITY

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We, the PhD candidate and the candidate's Principal Supervisor, certify that the following text, figures and diagrams are the candidate's original work.

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CHAPTER 4

Different Seeder Technologies Affect the Recruitment of Direct-seeded Acacias and Eucalypts in Northern NSW

4.1 Abstract

Direct seeding is a practical and cost-effective method for establishing native vegetation in agricultural landscapes in parts of south-eastern Australia. However, the technique is unreliable and often results in failure on the Northern Tablelands of New South Wales. This study aimed to investigate the effects of three sowing methods (a KB seeder, a Chatfield seeder and hand-sown surface seeding) and three bulking materials (rice, smoked vermiculite and chicken crumbles) on the recruitment of acacias and eucalypts in an upland grazed pasture near Walcha, NSW. Recruitment varied significantly among sowing treatments. Recruitment for the KB seeder was significantly greater than for the Chatfield seeder and the hand-sown methods, but there was no significant difference in recruitment between the Chatfield seeder and the hand-sown treatments. There were no significant differences in recruitment among bulking materials. Recruitment was significantly higher for acacias compared to eucalypts. Survival of *Acacia* recruits declined substantially between May–August 2016, while survival of *Eucalyptus* recruits declined between

July–August 2016. Seedling mortality was due to a combination of factors, including frost, waterlogging (from above-average rainfall in June and August) and infestation of rat's tail fescue in late winter. These results demonstrate the importance of using the right seeder to place seed into microsites beneath the soil to reduce seed desiccation and mortality and minimise ant predation. Competition from weed infestations that emerge along direct-seeded rip-lines is a major and on-going cause of seedling mortality and requires further investigation.

4.2 Introduction

Due to a brief, but intense history of landscape modification and degradation, the need to revegetate Australia's farming regions has never been greater. Widespread clearing of native vegetation has not only resulted in the loss of biodiversity through increased extinction rates, but also the loss of vital ecosystem services, upon which primary productivity depends (Daily 1997; Yates and Hobbs 2000). Consequently, broad-acre revegetation practices using native species, such as direct seeding have been widely implemented in Australia (Hobbs 1993; Schneemann 2008).

Direct seeding usually involves sowing a seed mix directly into a seed line and allowing natural germination and establishment to take place *in situ* (Schneemann 2008). The technique has practical, cost-effective applications to broad-scale revegetation in agricultural settings (Piggott *et al.* 1987; Schneemann and McElhinny 2012). Other advantages of direct seeding include: (1) the speed at which revegetation can be accomplished, (2) more structurally diverse and naturally spaced vegetation, and (3) germinants that are better adapted to local conditions (Knight *et al.* 1997; Rawlings *et al.* 2010). The disadvantages of direct seeding are that copious quantities of native seed are required (seed quantities will vary depending on the scale of the revegetation so it is difficult to quote an exact figure), direct seeding is slower to establish than planting seedlings, and seedlings are vulnerable to variations in climate, particularly during the critical period between germination and root development (Rawlings *et al.* 2010; Palma and Laurance 2015).

In Australia, direct seeding has had varying success (Piggott *et al.* 1987; Campbell 2001; Carr *et al.* 2009; England *et al.* 2013; Hallett *et al.* 2014). The success of direct seeding is often difficult to determine, given there are few published accounts of the outcome of direct seeding in Australia in the last few decades (Ruiz-Jaen and Aide 2005). Nevertheless, the studies that have monitored the results of direct seeding over time, generally base success on the diversity of species that have established, the structure of newly established vegetation (percent ground cover and plant density), the propensity for the site to shift along a trajectory towards resilience, and the capacity to be self-regulating (Ruiz-Jaen and Aide 2005; Pryde and Duncan 2015).

In South Australia and Victoria, where this technique was first used for planting native species, direct seeding is the predominant method of revegetation and repeated success has achieved with a broad range of species. In other states, direct seeding has not been as successful and there is scope to improve the technique (Carr *et al.* 2009). Considerable research has been conducted to identify issues likely to impede the successful implementation of direct seeding for native revegetation (Piggott *et al.* 1987; Campbell 2001; Schneemann 2008; Carr *et al.* 2009; Hallett *et al.* 2014). Failure of direct seeding has been linked to low germination and establishment of recruits (Piggott *et al.* 1987), sowing outside of optimal growing seasons (Carr *et al.* 2007; Gibson-Roy *et al.* 2010), lack of soil moisture availability (Hagon and Chan

1977; Dalton 1992; Knight *et al.* 1997; Hallett *et al.* 2014), inadequate ground preparation (Piggott *et al.* 1987; Andrews *et al.* 2004; England *et al.* 2013), inadequate knowledge of species selection (Piggott *et al.* 1987; Schneemann 2008), seed predation (Curtis 1990a; Rawlings *et al.* 2010; St-Denis *et al.* 2013), competition with exotic weeds and pasture grasses (Geeves *et al.* 2008; Carr *et al.* 2009; van Andel and Aronson 2012; Palma and Laurance 2015), and legacy effects of land use history and climate variability (England *et al.* 2013; Hallett *et al.* 2014).

While these factors are important, the inability to consistently establish native plants from seed may indicate that current seeding technologies may be impairing recruitment in the progression from seed to established plant (Madsen *et al.* 2016). Our observations from monitoring direct seeding on the Northern Tablelands and North-west Slopes and Plains over the past 3 years suggested that low numbers of recruits may also be attributed the differences in the sowing techniques among mechanical seeders. Bulking materials commonly added to seed mixes to increase volume, may also influence the flow of seed depending upon the size of the granules (Greening Australia 2003). Past research comparing the efficacy of different mechanical seeders and bulking materials is sparse, a knowledge gap that this study addresses. Understanding the ways in which sowing techniques may influence the recruitment of native species will increase our ability to design successful direct-seeding practices for broad-scale revegetation. The aims of the present study were to assess three sowing methods (hand sowing, machine sowing with a KB seeder, and machine sowing with a Chatfield seeder) and three bulking materials (rice, smoked vermiculite and chicken crumbles) on the recruitment of acacias and eucalypts in an upland temperate grazed pasture near Walcha, NSW. Based on previous research, which shows that the placement of seed into safe microsites is important for germination (Harper *et al.* 1965: Dowling *et al.* 1971; Sheldon 1974: Semple and Koen 1997), and that direct seeding favours the germination of large-seeded species over small-seeded species (Moles and Westoby 2001; Hallet *et al.* 2011; St-Denis *et al.* 2013), we hypothesised that recruitment would vary among sowing methods and between species. In addition, we hypothesised that overall recruitment would be greater in seed sown with smoked vermiculite, based on the findings of previous studies (Roche *et al.* 1997; Read *et al.* 2000; Kulkarni *et al.* 2007). It has been confirmed that a butenolide compound characteristic of plant smoke actively promotes the germination of many plant species (Kulkarni *et al.* 2007).

4.3 Materials and methods

4.3.1 Study site

The study was conducted at ‘Eastlake’, a grazing property about 20 km north-north-east of Walcha, New South Wales, Australia (30°48′15”S, 151°38′40”E, Fig. 4.1). The property is situated on the Northern Tablelands, an undulating plateau landscape of low hills and plains, 1000 m a.s.l. The region experiences mild summers and cold winters (January mean maximum daily temperature, 25.9°C; July mean minimum daily temperature, 1.2°C, Bureau of Meteorology 2016). The mean annual rainfall at Walcha is 799 mm with more (60%) rainfall occurring in summer (October–March) than winter (Bureau of Meteorology 2016).

The study site was a fenced area of approximately 622 × 15 m of pasture on a gently inclined slope with a north-westerly aspect. Prior to European settlement, the study site would have been temperate woodland dominated by *Eucalyptus dalrympleana, E. stellulata, E. viminalis* and *E. nova-anglica*. At the time of this study, the vegetation was primarily sown improved pasture of fescue and rye grass with scattered native trees of the aforementioned species.

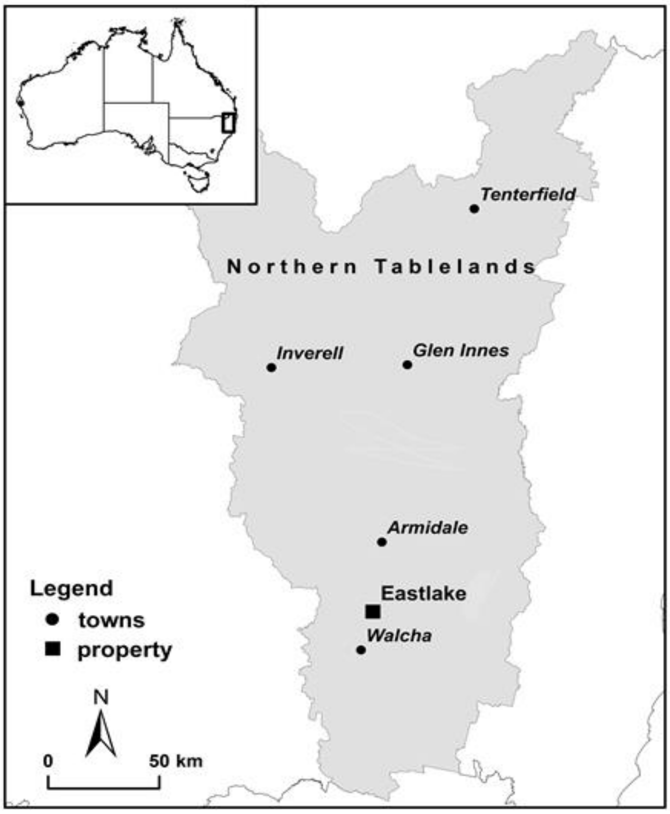

Figure 4-1 Map of the Northern Tablelands showing the location of 'Eastlake'

The topsoil was sandy loam derived from granite and metasedimentary parent materials (Li *et al.* 2007; NSW Office of Environment and Heritage 2011). The site experienced above-average rainfall between May and September 2016 following sowing, with the highest rainfall recorded in June for more than a decade (Gordon Williams pers.comm.).

4.3.2 Experimental design

Site preparation began in September 2015 with the deep ripping of three 600-m seeding lines spaced 3 m apart to a depth of 40 cm, followed by an application of a non-selective herbicide (glyphosate) over each rip-line in a band, 1.2 m wide. Glyphosate was reapplied in late February 2016. Each line was divided into 3 × 200-

m plots. Seed was sown using three different methods, two mechanical seeders, a KB seeder and a Chatfield seeder, and by hand-broadcasting seed on the surface. Three bulking materials, used to increase volume and equalise the flow of seed through the machines, were compared: white rice, smoked vermiculite and chicken crumbles (a finely pelleted commercial food for young chickens). Each of the nine combinations of sowing treatment and bulking material was randomly assigned to a 200-m plot and 5 × 10-m transects were randomly allocated to each 200-m plot for sampling. The site was divided into three blocks with each block incorporating 3 × 200 m of parallel seeded lines. Six species belonging to two genera were chosen for this experiment: *Acacia rubida* A.Cunn., *A. boormanii* Maiden, *A. filicifolia* Cheel & M.B.Welch, *A. dealbata* Link, *Eucalyptus acaciiformis* H.Deane & Maiden and *E. stellulata* Sieber ex DC. The seed was collected from local Northern Tablelands populations. Sowing occurred on 21 March 2016. *Acacia* seeds were placed in boiling water for 30 seconds the day before sowing to break dormancy. All seeds were coated with Diazinon® powder to deter ant predation and graphite powder to aid in moisture retention prior to sowing. The seeders were calibrated for each bulking material to deliver seed at a rate of 200 g/km. Recruits were counted every week between April and August 2016, and were identified to genus. Weeds (rat's tail fescue *Vulpia myuros*) began to overtake the site in late winter making the counting of recruits difficult. The site was sprayed with Fluazifop-P (Salvo®) at a rate of 212 g/L of active ingredient (plus a wetting agent) with a hand-held 1.2-m boom spray in early September 2016, but the treatment was not effective. Consequently, monitoring was discontinued.

4.3.3 Mechanical seeders

The mechanical seeders used for this experiment were chosen because of their different seed delivery and ground preparation mechanisms. The KB seeder was a purpose-built towbar-drawn commercial seeder used widely for revegetation. It was comprised of a tyne to rip the soil, two seed boxes (one containing *Acacia* seed and the other containing *Eucalyptus* seed) from which the seed was released onto a ground-driven rotating sponge disc, a tube that delivered the seed from the rotating disc into the ripline, and a press-wheel to firm down the soil ensuring good contact between the seed and the soil. *Acacia* seed was sown at a depth of 1.5 cm and *Eucalyptus* seed was delivered onto the soil surface. The Chatfield seeder employed a similar sowing system. The seed box had a fluted roller that was ground-driven by a wheel and chain. Seed was delivered from the roller onto a wide metal chute and scattered on the soil surface either side of a ripline, which was harrowed by a grader towing the seeder. To bury the seed, a quadbike was driven along the seeded lines, pressing the seed into the soil. The main difference between the two seeders was a V-shaped blade fitted to the front of the Chatfield seeder, which removed the top 5–7.5 cm of soil prior to sowing.

4.3.4 Statistical analysis

To examine differences in recruitment between sowing method and bulking material, a generalised linear model was fitted with a quasi-Poisson error distribution to allow for over-dispersion. Data from the final monitoring on 18 August 2016 were used for these analyses. Sowing method, bulking material, species and block were included in the model as fixed effects. The models were initially run with the KB seeder and the rice bulking material as baseline references. Block was later dropped from the analysis as it was not significant. The models were refitted with different levels of sowing treatment and bulking material as the reference group to obtain treatment comparisons. Model diagnostics were checked to ensure goodness of fit. All analyses were performed in R Studio (version 3.0.2; R Core Development Team 2015).

4.4 Results

4.4.1 Effect of sowing treatment and bulking material on recruitment

Recruitment varied significantly among sowing treatment and between genera, but not among bulking material or the interactions among genera, sowing treatment and bulking material. Mean recruitment (mean ± 1 s.e.) varied among sowing treatments: KB seeder (25.0 ± 10.4 germinants/150 m), Chatfield seeder (10.0 ± 3.4 germinants/150 m) and hand-sown (5.0 ± 1.7 germinants/150 m) (Fig. 4.2A). Recruitment for the KB seeder was significantly greater than the Chatfield seeder and the hand-sown methods (Table 4.1), but there was no significant difference in recruitment between the Chatfield seeder and the hand-sown methods after relevelling. There were no significant differences in recruitment among bulking materials (Fig. 4.2B). Recruitment differed significantly between genera with greater recruitment of acacias (20.7 ± 7.21 germinants/450 m) than eucalypts (6.0 ±1.91 germinants/ 450 m).

Table 4-1 Model coefficients for the estimates, standard errors (SEs) and 95% confidence intervals (CIs) for recruitment

Variables	Coefficient	SE	95% CIs Lower	95% CIs Upper
Intercept	4.79	0.72	3.35	6.21
Genera	−1.24	0.49	−2.30	−0.35
Chatfield seeder	−0.92	0.48	−1.94	−0.02
Handsown	−1.61	0.63	−3.07	−0.51
Chicken crumble	−0.31	0.57	−1.48	0.81
Vermiculite	0.47	0.47	−0.44	1.45

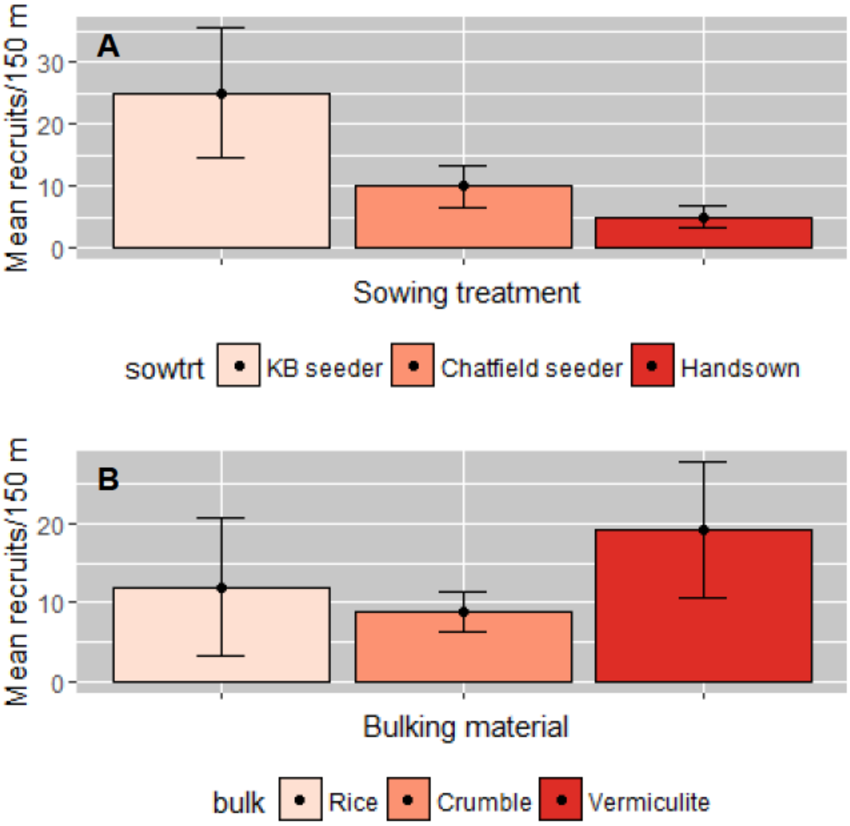

Figure 4-2 Combined *Acacia* and eucalypt recruitment (mean ± 1 s.e.)/150 m for (A) sowing method (sowtrt), and (B) bulking material (bulk)

4.4.2 Survival of *Acacia* and *Eucalyptus* recruits

Recruitment of acacias commenced 3 weeks after sowing and peaked 8 weeks after sowing on 19 May 2016 when a total of 837 recruits were recorded. For acacias, there was a substantial decline in recruits after 8–10 weeks post-sowing (between 19 May and 2 June 2016), resulting in the loss of more than half of the recruits.

Thereafter, survival continued to decline with a total of 183 recruits recorded at the end of the monitoring period (18 August 2016) (Fig. 4.3). Initial losses 8 weeks post-sowing coincided with the first winter frost (Fig. 4.4A), while subsequent losses in the following 2 weeks were due to a combination of frost and above average monthly rainfall in June (126.8 mm), particularly in lower areas that became waterlogged. Above-average rainfall was also experienced in August (124.8 mm) (Fig. 4.4B). In addition, there was some erosion of the riplines where the KB seeder was used resulting in the removal of some of the seedlings. The decline in recruits between 15 and 19 weeks was primarily due to heavy infestations of rat's tail fescue (*Vulpia myuros*) across the site (Fig. 4.5).

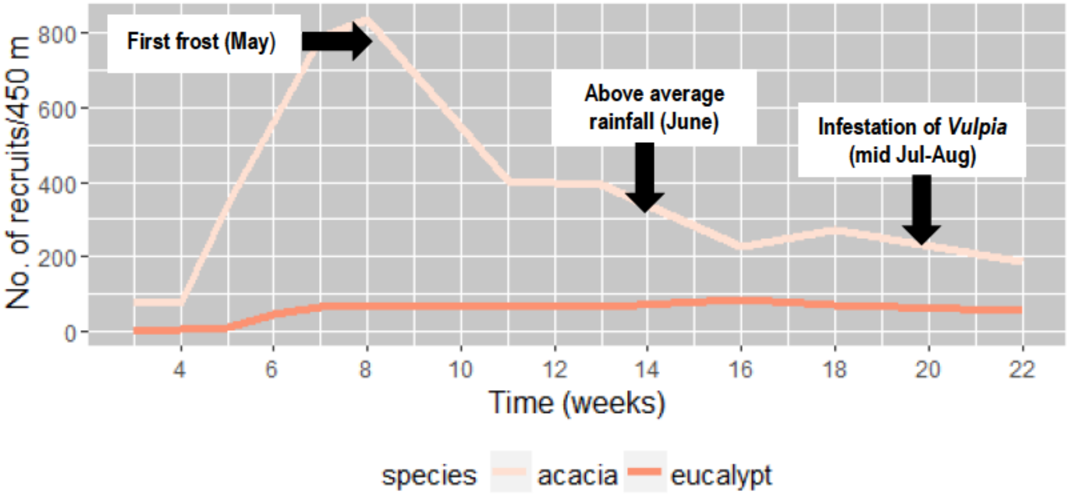

Figure 4-3 Time line for recruitment of acacias and eucalypts pooled across treatments 3–22 weeks post-sowing (between 14 April and 18 August 2016)

Eucalypt recruitment commenced between 4–5 weeks post-sowing and increased steadily until 15 weeks after sowing (7 July 2016) when 83 recruits were recorded. By week 22 at the end of the monitoring period, survival had declined to a total of 52 recruits (Fig. 4.3). Eucalypts appeared to be more tolerant of the frost and moist conditions than acacias as the number of recruits varied little between May and July

(Fig. 4.4B). However, all recruits were intolerant of weed invasion as evidenced by the decline in survival from weeks 16–22 (Fig. 4.3).

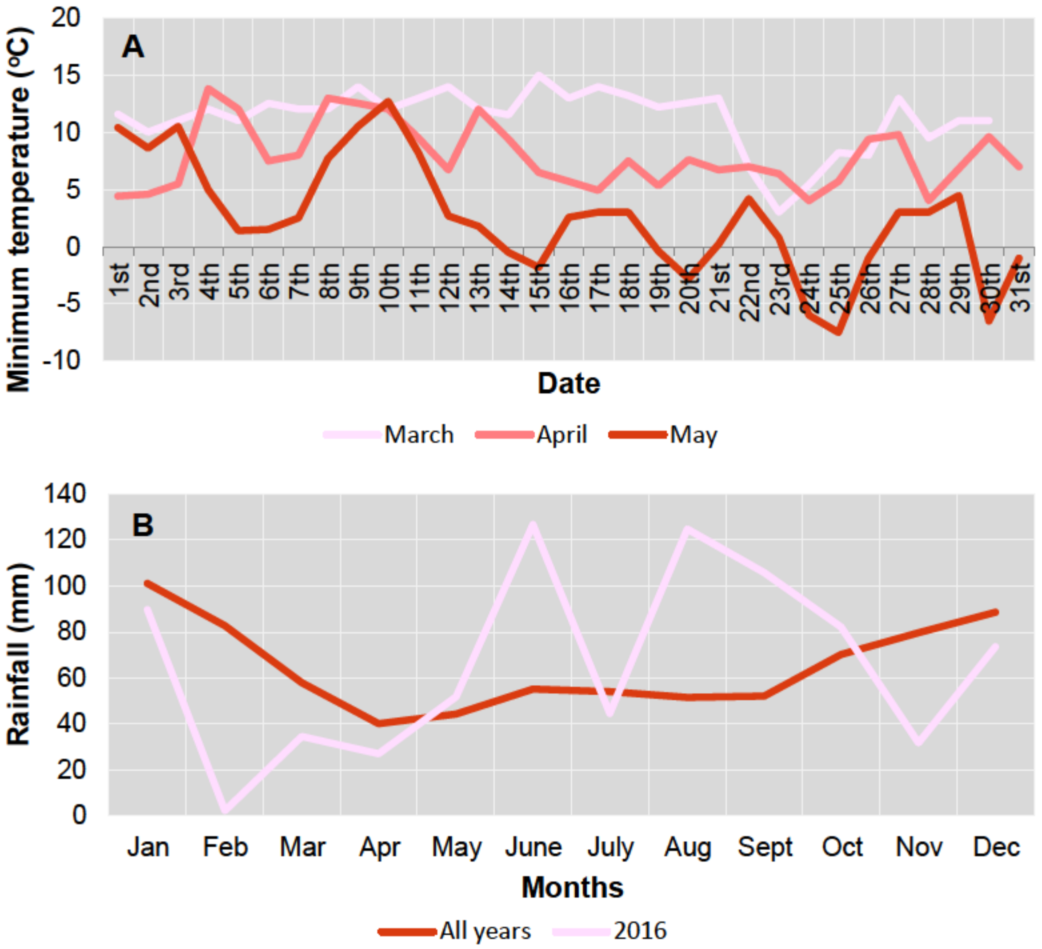

Figure 4-4 (A) Minimum daily temperature in March, April –May 2016, and (B) mean monthly rainfall for all years (1865-2015) and monthly rainfall in 2016 (Bureau of Meteorology 2017)

4.5 Discussion

Given that direct seeding is cheaper than planting seedlings and more effective for revegetating farmland on a large scale (Knight *et al.* 1997), it is widely used for farmland plantings throughout Australia (Carr *et al.* 2009). Cost comparisons indicate that direct seeding and tubestock planting cost about $770/ha and $1800/ha, respectively (Schirmer and Field 2002). However, direct seeding is unreliable in some regions, despite trials of a range of techniques and equipment (Carr *et al.* 2009) that has been trialled in the past. Direct seeding has sometimes failed completely or had very low survival (<5%) of native recruits (Schneemann 2008)

Figure 4-5 Direct-seeded site in (A) March 2016 following seeding, and (B) September 2016 showing heavy infestation of *Vulpia myuros*

Source: Photographs by Sharon Brown

As a consequence, the value of direct seeding for native revegetation has often been questioned (Schneemann 2008), despite it being the cheaper option. The best scenario would be to improve the reliability of direct seeding by increasing establishment and survival of native recruits through more on-ground experimentation.

This study demonstrated that the KB seeder was a more reliable method of direct seeding than the Chatfield seeder or hand sowing, supporting our hypothesis that recruitment would vary among sowing treatments. However, our hypothesis that recruitment would be improved using smoked vermiculite as a bulking material was not supported, given that there were no significant differences in recruitment among the three bulking materials.

4.5.1 The effect of sowing treatment on recruitment

Recruitment with the KB seeder was almost 2.5 times higher than the Chatfield seeder and 5 times higher than hand sowing. Increased recruitment was most likely attributable to the placement of the seed into safe microsites beneath the soil surface. Many studies have shown the impact of microsite limitation on plant recruitment (Harper *et al.* 1965; Eriksson and Ehrlén 1992; Münzbergová and Herben 2005; Calviño-Cancela 2007). Microsites are created by small-scale microtopography at the soil surface (Harper *et al.* 1965). They act as a buffer against the outside environment by reducing seed exposure to the atmosphere and maintaining soil moisture (Harper *et al.* 1965). Soil moisture is essential for seed germination. The KB seeder most likely enhanced germination by placing seed 1.5 cm beneath the soil surface, ensuring good seed contact with moisture-laden soil. Adequate seed–soil contact is essential for germination because it provides a route through which water can enter a seed (Brown *et al.* 1996; Woodstock 1988). Germination occurs when the seed is sufficiently hydrated to stimulate cell division in the embryo (Bell 1999). A further advantage of the KB seeder was its ability to manipulate the soil to enhance porosity and water infiltration by shallow ripping. The riplines served to channel rainwater through the soil profile into air spaces surrounding the seed, which promoted imbibition and germination. By comparison, the flat surface created by the Chatfield planter, as well as the compression of soil by the quadbike wheel probably had a negative effect on germination through soil compaction and elimination of microtopography.

Conditions for the germination of surface-sown seed are more severe than for buried seed, increasing the risk of seed desiccation and mortality (Dowling *et al.* 1971). This partly explains why hand sowing was the least effective seeding method in our study. Similar results have been obtained with various surface-sown pasture and crop species, and attributed to rapid fluctuations in moisture and humidity at the soil–air interface as well as an inability of the emerging radicle to penetrate the surface soil crust (Dowling *et al.* 1971; Sheldon 1974). Another possible explanation for the low recruitment of broadcast seed in our study was the removal of seed from the soil surface by seed predators. Even though the seeds were treated with an insecticide prior to sowing, it is possible that the chemical could have been washed from seed by the rain. Seed predation is common and reduces the amount of seed available for germination (Calviño-Cancela 2007). Microsites provide safe refuges for seed against seed predation and it is anticipated that most unprotected seed will be consumed (Calviño-Cancela 2007). This is particularly the case for *Acacia* and *Eucalyptus* seed, which are an important food source for various seed-harvesting insects (Ashton 1979; Bashford 1993; Bennet and Krebs 1987).

Our results showed that the recruitment of eucalypts was low compared to the recruitment of acacias. The poor recruitment of eucalypts *in situ* is well-documented (Curtis 1990a; Reid and Landsberg 2000; Li *et al.* 2003). New recruits, regardless of species, are vulnerable to desiccation in the early post-germination phase when emerging roots must secure an external supply of nutrients and water before seed reserves are exhausted (Fenner 1987). However, for small-seeded species, such as eucalypts, immediate access to external resources is critical because of the lack of endosperm in the seed (Fenner 1987). Consequently, there is a tendency for large-seeded species to have higher recruitment and survival percentages than small-seeded species (Moles and Westoby 2002).

4.5.2 *Acacia* and *Eucalyptus* recruitment

The number of new recruits declined over the course of this experiment after reaching a maximum 8 weeks (*Acacia*) and 15 weeks (*Eucalyptus*) after sowing. For acacias and eucalypts, the survival of recruits subsequently decreased by 78% and 37%, respectively. The main factors affecting the survival of *Acacia* recruits appeared to be frost, excessive rain and weed invasion, while the latter affected the survival of *Eucalyptus* recruits. Planting in autumn is risky in cold climates, but the risk may be rewarded if recruits are able to survive winter and develop a sufficiently deep root system before the onset of dry summer conditions. Nevertheless, autumn and winter sowing is common in temperate regions because it takes advantage of winter rains, thus minimising water deficit and heat stress (Campbell 2001; Greening Australia 2003). Previous direct-seeding trials in the southern highlands of NSW have reported success in winter sowing over the past 30 years (Campbell 2001). Unfortunately, the climate during the present experiment was different from the long-term average due to unusually high rainfall in June and August (Fig. 4.4B). This, combined with cold temperatures, had a significant negative impact on the germination and survival of acacias. The soil at the site was continuously wet from June onwards, so any seeds remaining in the soil seed bank would most likely have rotted, preventing future recruitment.

A further problem associated with autumn sowing was the limited ability to control cool-season annuals without damaging native recruits. Herbaceous weeds are vigorous competitors that inhibit germination, establishment and growth of native woody species by depleting important resources, such as water, nutrients and sunlight (Dalton 1993; Semple and Koen 2006). Invasion of rat’s tail fescue was a persistent problem at the study site, emerging in mid June and forming dense swards by mid July. Rat’s tail fescue is difficult to manage and has been a major cause of seedling mortality in other direct-seeding trials (Campbell 2001). Despite the weed control implemented at the site, the fescue was not suppressed. The two applications of glyphosate prior to sowing were ineffective in controlling emergence, and there was no discernible difference in weed cover between the two mechanical seeding treatments, suggesting that scalping was also ineffective. We attempted to suppress the growth of rat’s tail fescue with an overspray of Salvo® (fluazifop-P) in early spring, which previous studies have shown is safe to use on native seedlings (Hall 1985; Moore 1999). However, this was unsuccessful in controlling the weed burden. Due to time constraints, weed cover was not monitored during the experiment, weakening our ability to verify the claim that increasing competition from exotic weeds was related to the declining survival of direct-seeded recruits from June onwards, as opposed to the deleterious effects of frost and waterlogged soils.

4.6 Conclusions

These results support the growing body of evidence that the direct seeding of native vegetation on the Northern Tablelands of NSW often fails. While there has been some progress in determining best practice for some aspects of direct seeding, for example, site preparation and maintaining a full soil moisture profile, direct seeding cannot currently be considered a reliable revegetation technique for the region. Our results demonstrate that attention to the sowing process is needed to improve current practices. However, attention is rarely given to this aspect of direct seeding. We recommend mechanical seeders that place the seed beneath the soil surface. The present study also partly dispels the myth that recruitment is limited by poor germination of native species, given that *Acacia* germination was high. However, because eucalypt germination was low, the results support previous work that the technique is better suited to large-seeded species (St-Denis *et al.* 2013).

The fundamental challenge for improving direct seeding lies in the ability to improve establishment and survival of recruits. This is difficult, given that the technique is entirely dependent on prevailing and future climate patterns, and windows of opportunity for recruitment are often very narrow. It was unfortunate that higher than average rainfall impeded the success of this experiment. Despite initial losses of *Acacia* recruits to frost, we remain hopeful that autumn sowing can be successful on the Northern Tablelands provided weed infestations in late winter and early spring can be controlled. Pre-emergent herbicide oversprays have been used successfully to control invasive weeds at direct-seeded sites in the past. We believe there is merit in this technique, but further research investigating the tolerances of a range of native species to various selective herbicides is needed.

Journal-Article Format for PhD Theses at the University of New England

STATEMENT OF AUTHORS' CONTRIBUTION

(To appear at the end of each thesis chapter submitted as an article/paper)

We, the PhD candidate and the candidate's Principal Supervisor, certify that all co-authors have consented to their work being included in the thesis and they have accepted the candidate's contribution as indicated in the *Statement of Originality*.

CHAPTER 4 Different Seeder Technologies Affect the Recruitment of Direct-seeded Acacias and Eucalypts in Northern NSW

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CHAPTER 5

Determining Herbicide Tolerance in Native Woody Species with Application to Weed Control in Direct Seeding

5.1 Abstract

The germination and survival of native seedlings resulting from direct seeding are hindered by weeds that quickly emerge along sown riplines and compete for resources in some parts of south-eastern Australia. Better weed control methods are needed if direct seeding is to be a cost-effective alternative to planting tubestock in these regions. A potential solution is the use of herbicides as oversprays. The aim of this study was to determine the tolerance of 11 native tree and shrub species to eight herbicides, each with a different mode of action. This investigation incorporated a completely randomised block design with three replicates of nine trays, each tray containing ten pots (one species per pot) of germinated seedlings. The proportion of native woody seedlings surviving 8 weeks after spraying varied significantly among herbicide treatments. Survival proportion was greatest in seedlings treated with imazethapyr (0.62 ± 0.08) and isoxaflutole (0.59 ± 0.07), and lowest in seedlings treated with diflufenican (0.01 ± 0.02) and glyphosate (0.03 ± 0.02). There were also significant differences in survival proportion between species, with *Dodonaea*

viscosa (0.59 ± 0.08), *Acacia pendula* (0.50 ± 0.09) and *Senna artemisioides* (0.44 ± 0.09) exhibiting the highest tolerance to the greatest range of herbicides, and *Atriplex nummularia* (0.20 ± 0.06), *Casuarina cristata* (0.29 ± 0.08) and *Geijera parviflora* (0.29 ± 0.08) showing least tolerance. Although we were unable to fit a species × treatment interaction due to over-dispersion of the data, species responded in different ways to most herbicide treatments. Herbicide oversprays may be useful for controlling weeds at direct-seeded sites, particularly if planting designs incorporate species in separate rows according to their tolerance of different herbicides. Further research is needed to determine herbicide tolerance in a broader range of native species and the effectiveness of these herbicides on weed control *in situ*.

5.2 Introduction

Direct seeding is often a preferred method of revegetation because it is cost-effective and less labour intensive than planting seedlings, making it ideal for large-scale projects (Engel and Parrotta 2001; Gibson-Roy *et al.* 2007). In South Australia and Victoria, direct seeding is the predominant method of revegetation, with high levels of success achieved with a broad range of native species (Semple and Koen 2006). However, in northern New South Wales, the success of direct seeding is often below expectation and there is considerable scope for improvement (Carr *et al.* 2009). A possible explanation for the inferior results with direct seeding in northern NSW is that seeding in spring, which is recommended state-wide (Piggott *et al.* 1987; Carr *et al.* 2009), often coincides with rainfall and increasing temperatures, promoting the germination of dormant weed seeds in the soil seed bank. Spring weed infestations compete vigorously with the newly emergent seedlings and deplete important resources such as water, nutrients and sunlight (Dalton 1993; Semple and Koen

2006). Despite adequate pre-sowing and post-sowing weed control, weed infestations may quickly establish along riplines under the right conditions (Taylor 2013).

The control of weeds after seedling emergence is a challenging problem for which an effective solution has yet to be found. Manual removal is an effective strategy because weeds can be selected and removed without damaging surrounding native seedlings (Carr *et al.* 2009). However, manual weeding is labour-intensive, costly and impractical for broad-scale direct-seeded sites (Hall and Burns 1991; Graham *et al.* 2009). Conversely, chemical control using pre or post-emergent herbicides is efficient and economical, but it is difficult to selectively target weeds without damaging the direct-seeded recruits. A potential solution is to use herbicide over-sprays to which the native species are tolerant, in order to control weeds (Hall 1985).

While some herbicide screening has been carried out on native species (Hall 1985; Moore 1999; Semple and Koen 2006; Moore *et al.* 2010), this work has been limited. More research is needed to develop a list of potential herbicide oversprays suitable for a range of native species in regions where direct seeding has been unsuccessful. One such region is north-west New South Wales, where extensive agricultural development for cropping, intense disturbance and weed invasion, as well as a predominance of hostile cracking-clay soils, restrict natural regeneration and successful establishment of direct-seeded woody perennials (Carr *et al.* 2009; Azam *et al.* 2012). Cracking-clay soils develop deep fissures within the upper horizon upon drying after a rain event, which can damage plant roots and bury seed at depths that are not conducive to germination (Carr *et al.* 2009; Ruiz Talonia *et al.* 2016). Furthermore, the effectiveness of pre-emergent herbicides on cracking-clay soils is often compromised because of the large number of clay particle binding sites, which reduce the amount of herbicide available for uptake in the soil water (Congreve and Cameron 2014). Given these challenges, the aim of our study was to investigate the impact of a selection of herbicide oversprays on a taxonomically diverse range of native trees and woody perennial species suitable for direct seeding in grey cracking vertosols in north-west NSW. Herbicides with different modes of action were selected for this study. The mode of action refers to the sequence of events in a plant exposed to herbicide from initial contact through to plant death (Streibig 2010). Different mode-of-action groups elicit various physiological responses in plants by targeting specific molecular sites (Duke 1990; Gunsolus and Curran 2007). The objectives of this study were to: (1) examine the effects of a range of herbicide oversprays on seedling survival (selectivity), and (2) examine the differential tolerance of native woody species to a range of herbicide treatments. Based on previous work (Hall 1985; Hall and Burns 1991; Taylor 2013), we hypothesised that seedling survival and the tolerance of individual species would vary among herbicides due to varying chemical selectivity. The research aimed to contribute to the advancement of effective methods of weed control, which is of key importance to the successful direct seeding of native woody seedlings.

5.3 Materials and methods

This investigation was conducted in a glasshouse at the University of New England in Armidale, NSW, between July 2014 and February 2015. Eleven tree and shrub species were chosen for this study because they were representative of the main plant families indigenous to the north-west plains of NSW. They were *Acacia harpophylla* F.Muell. ex Benth., *A. pendula* A.Cunn. & G.Don., *A. salicina* Lindl., *Eucalyptus coolibah* Blakely & Jacobs, *E. camaldulensis* Dehnh., *Casuarina cristata* Miq., *Senna artemisioides* (DC.) Randell, *Atriplex nummularia* Lindl., *Einadia nutans* (R.Br.) A.J.Scott, *Dodonaea viscosa* Jacq. and *Geijera parviflora* Lindl. The seed was locally sourced in the region. The seedlings were germinated and grown under a day/night regime of 12:12 hours of light and dark and glasshouse temperatures of 30°C/18°C. Seeds of each species were sown into a 7.5 × 7.5 × 15.0 cm pot (25 seeds per pot) containing cracking grey vertosols to a depth of 5 mm.

The soil was collected from a fallow paddock near Bellata in north-western NSW (29°48'28" S, 149°38'27" E) and was typical of the predominant soil type used for cropping in the region. The soil was watered and dried in the glasshouse three times over a period of 7 weeks to remove emergent weeds before being passed through a 2-mm sieve. A template was used to ensure uniform distribution of seeds. The pots were randomly arranged in trays with each tray representing one of nine herbicide treatments. The herbicides in this experiment (oxyfluorfen, prosulfocarb, imazethapyr, glufosinate, diflufenican, metolachlor, glyphosate and isoxaflutole) were selected based on their mode of action, safety of use and their effectiveness in controlling both grassy and broad-leaf weeds (Table 5.1). All were selective herbicides, except for glyphosate. A non-ionic wetting agent was added to imazethapyr as recommended on the label. The ninth tray was a control and received no herbicide spray. Trays were arranged in a completely randomised block design with three replicates (blocks) per herbicide treatment. Seedlings were watered every 3 days at a rate of 50 mL per pot. The herbicides were applied at recommended application rates (Table 5.1) over the top of 5-month old seedlings, using a hand-held sprayer. To avoid spray drift, the trays receiving each herbicide treatment were removed from the glasshouse and treated in isolation of the remaining trays. Seedling survival was measured at 1, 2, 7 and 8 weeks post-spraying. Seedlings were recorded as dead or alive and the proportion surviving was calculated for each species 8 weeks after spraying. We fitted a linear model to test the effects of predictors (species and herbicide treatment) on the proportion of seedlings surviving. Block was included in the model as a fixed effect. Due to inflation of the data with values 0 and 1, a model incorporating the species × treatment interaction could not be fitted. All analyses were performed using the R statistical package (version 3.0.2; R Development Core Team 2015).

Table 5-1 Herbicides used in this experiment, their mode of action, activity and rates of application

Herbicide (Trade name)	Mode of action	Herbicide activity	Rate (kg a.i./ha)	No. of seedlings/ treatment
Imazethapyr (*Spinnaker*)	B	Inhibits acetolactate synthase (ASL)	0.70	166
Diflufenican (*Jaguar*)*	F	Inhibits phytoene desaturase	0.20	154
Oxyfluorfen (*Goal*)	G	Inhibits photoporphyrinogen oxidase (PPO)	0.18	170
Isoxaflutole (*Balance*)	H	Inhibits 4-hydroxyphenyl-pyruvate dioxygenase (HPPD)	0.75	156
Prosulfocarb (*Boxer gold*)	J	Inhibits Acetyl-CoA carboxylase (ACC)	2.30	163
Metolachlor (*Dual gold*)	K	Inhibits very-long-chain fatty acid synthase	1.44	159
Glyphosate (*Roundup*)	M	Inhibits 5-enolpyruvylshikimate-3-phosphate (EPSP)	0.45	177
Glufosinate (*Basta*)	N	Inhibits glutamine synthase	0.20	166

* Bromoxynil present as octanoate

5.4 Results

Seedling survival 8 weeks after spraying differed significantly between herbicide treatments ($F = 30.0$, $df = 8$, $P < 0.001$; Figs. 5.1, 5.2). The proportion of seedlings surviving (mean ± 1 s.e.) was greatest for seedlings treated with imazethapyr (0.62 ± 0.08), isoxaflutole (0.59 ± 0.07), intermediate for seedlings treated with prosulfocarb

(0.44 ± 0.06) and oxyfluorfen (0.43 ± 0.07), and poor in seedlings treated with glyphosate (0.03 ± 0.02) and diflufenican (0.01 ± 0.02) (Fig. 5.2, Table 5.2). Seedling survival also differed significantly between species (*F* = 3.0, *df* = 10, *P* = 0.0013). *D. viscosa* (0.59 ± 0.08), *A. pendula* (0.50 ± 0.09) and *S. artemisiodes* (0.44 ± 0.09) exhibited the greatest tolerance to more herbicides than other species, while *A. nummularia* (0.20 ± 0.06), *C. cristata* (0.29 ± 0.08) and *G. parviflora* (29 ± 0.08) exhibited least tolerance. The block effect was not significant (*F* = 0.8, *df* = 2, *P* = 0.5). Although we could not fit a model with the species × treatment interaction due to over-dispersion, it is evident from Table 5.2 and Figure 5.1 that while most species were tolerant of oxyfluorfen, prosulfocarb and imazethapyr, *S. artemisioides*, *A. nummularia* and *C. cristata* were sensitive to oxyfluorfen and prosulfocarb, and *E. nutans* was sensitive to imazethapyr.

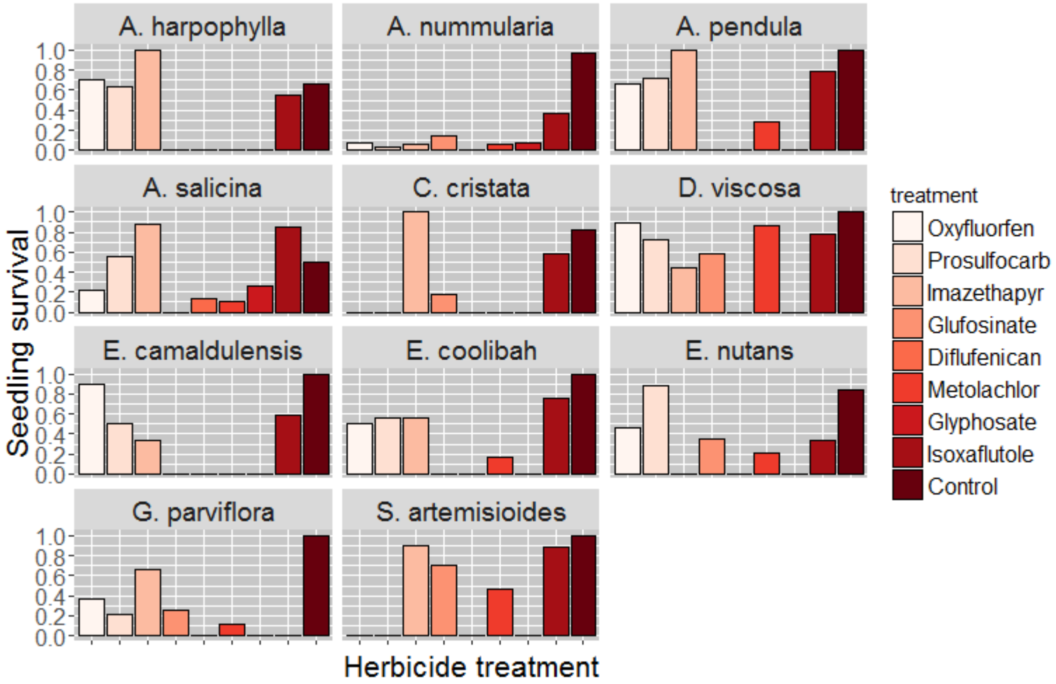

Figure 5-1 Interaction plot of the effect of species and herbicide treatment on proportion of seedlings surviving

Geijera parviflora, *S. artemisioides*, *E. nutans* and *D. viscosa* were somewhat tolerant of glufosinate, and *A. pendula*, *S. artemisioides* and *D. viscosa* were tolerant of metolachlor to varying degrees compared to other species. *G. parviflora* was the only species that did not tolerate isoxaflutole, while *A. salicina* was the only species tolerant of diflufenican (to a minor degree). In the control tray, the proportion of *A. salicina* and *A. harpophylla* seedlings surviving was only 0.50–0.66. This was unexpected and cannot readily be explained. The random placement of pots may have contributed if conditions in the glasshouse were uneven.

Figure 5-2 Seedlings treated with oversprays of (A) imazethapyr, (B) isoxaflutole, (C) diflufenican, (D) glyphosate compared to (E) the control 7 weeks post-spraying

Source: Photographs by Sharon Brown

Table 5-2 Proportion of surviving seedlings for each combination of native plant species and herbicide treatment (mean ± 1 s.e.)

Herbicide	Species											Average survival
	A. harpophylla	*A. pendula*	*A. salicina*	*A. nummularia*	*C. cristata*	*D. viscosa*	*E. nutans*	*E. camaldulensis*	*E. coolibah*	*G. parviflora*	*S. artemisioides*	
Oxyfluorfen	0.71(±0.04)	0.67(±0.03)	0.21(±0.15)	0.07(±0.04)	0	0.89(±0.11)	0.47(±0.29)	0.89(±0.05)	0.50(±0.30)	0.36(±0.22)	0	**0.41**(±0.07)
Prosulfocarb	0.63(±0.13)	0.72(±0.15)	0.56(±0.11)	0.04(±0.04)	0	0.72(±0.15)	0.89(±0.11)	0.50(±0.27)	0.06(±0.06)	0.22(±0.12)	0	**0.44**(±0.06)
Imazethapyr	1.0	1.0	0.88(±0.06)	0.06(±0.06)	1.0	0.44(±0.29)	0	0.33(±0.33)	0.06(±0.15)	0.67(±0.33)	0.90(±0.10)	**0.62**(±0.08)
Glufosinate	0	0	0	0.14(±0.07)	0.18(±0.11)	0.58(±0.30)	0.34(±0.90)	0	0	0.35(±0.25)	0.70(±0.11)	**0.20**(±0.05)
Diflufenican	0	0	0.13(±0.13)	0	0	0	0	0	0	0	0	**0.01**(±0.01)
Metolachlor	0	0.28(±0.03)	0.11(±0.11)	0.06(±0.06)	0	0.87(±0.13)	0.21(±0.21)	0	0.17(±0.17)	0.11(±0.11)	0.47(±0.26)	**0.21**(±0.06)
Glyphosate	0	0	0.25(±0.13)	0.07(±0.04)	0	0	0	0	0	0	0	**0.03**(±0.02)
Isoxaflutole	0.56(±0.29)	0.79(±0.12)	0.85(±0.10)	0.38(±0.10)	0.58(±0.23)	0.78(±0.11)	0	0.58(±0.30)	0.75(±0.14)	0	0.88(±0.07)	**0.59**(±0.07)
Control	0.66(±0.33)	1.0	0.50(±2.9)	0.97(±0.03)	1.0	1.0	0.83(±0.17)	1.0	1.0	1.0	1.0	**0.89**(±0.05)
Average survival	**0.38**(±0.09)	**0.50**(±0.09)	**0.38**(±0.07)	**0.20**(±0.06)	**0.29**(±0.08)	**0.59**(±0.08)	**0.34**(±0.08)	**0.37**(±0.09)	**0.39**(±0.08)	**0.29**(±0.08)	**0.44**(±0.09)	

Survival proportion (Moore 1999):

<0.50 survival = poor tolerance

0.50–0.75 = requires further research

>0.75 survival = adequate tolerance

5.5 Discussion

Managing weeds in riplines is one of the main impediments to successful direct seeding (Taylor 2013). After native germinants emerge, standard weed control practice on direct-seeded sites is to spray either side of the ripline to minimise competition from weeds and maximise resource availability for emerging germinants. However, the effect is temporary and further recolonisation by weeds inevitably occurs (Taylor 2013). This study was conducted under controlled conditions in a glasshouse and, while the results contribute to the scant body of knowledge on the use of herbicide oversprays on native seedlings, the true test of the effectiveness of this technique will be on direct-seeded recruits in the field. Our results supported the hypothesis that tolerance of native woody species to herbicide treatments would vary. Survival was significantly higher in seedlings treated with imazethapyr (0.62 ± 0.08) and isoxaflutole (0.59 ± 0.07). For these herbicides, adequate tolerance (>75% survival) was observed in almost half of the species. Survival was low in seedlings treated with diflufenican (0.01 ± 0.01) and glyphosate (0.03 ± 0.02), all species exhibiting poor tolerance (<50% survival) to these herbicides. Glyphosate is not selective, explaining its high toxicity. Diflufenican is a selective herbicide, but its effect was uniformly potent. Very little resistance has been recorded to diflufenican and other herbicides with the same mode of action (Catchpole *et al.* 1993; Beckie and Tardif 2012).

Some species were more tolerant to herbicide oversprays than others. *Dodonaea viscosa, A. pendula* and *S. artemisioides* were most tolerant, while *A. nummularia, C. cristata* and *E. nutans* were least tolerant, on average. In addition, species responded differently to some herbicides. For example, the rank order in proportion of seedlings surviving glufosinate from high to low tolerance was *S. artemisioides* (0.70 ± 0.11) > *D. viscosa* (0.58 ± 0.30) > *G. parviflora* (0.35 ± 0.25) > *E. nutans* (0.34 ± 0.90), with acacias and eucalypts exhibiting zero tolerance. Similar patterns of species tolerance were observed for metolachlor: *D. viscosa* (0.87 ± 0.13) > *S. artemisioides* (0.47 ± 0.26) > *A. pendula* (0.28 ± 0.03) > *E. nutans* (0.21 ± 0.21) > *E. coolibah* (0.17 ± 0.17). *A. harpophylla, C. cristata* and *E. camadulensis* exhibited zero tolerance to metolachlor. *Acacia salicina* was the only species exhibiting any tolerance to glyphosate (0.25 ± 0.13) and diflufenican (0.13 ± 0.13).

Our results agree with previous work demonstrating strong selectivity in Australian native species to a range of herbicides. Selectivity is achieved by exploiting the dissimilarities between susceptible and tolerant species (Carvalho *et al.* 2009; Cobb and Reade 2010). It is primarily governed by the mode of action of individual herbicides and differential uptake, translocation and metabolism of herbicides among species. Moore (1999) reported tolerance levels in seedlings of 39 Australian native species (13 genera) to 38 pre-emergent herbicide oversprays 14 weeks post-spraying. More than 50% of species exhibited adequate tolerance (>75% survival) to imazethapyr, oxyfluorfen, metolachlor, glufosinate and glyphosate (Moore 1999). Hall (1985) reported tolerances (85–100% survival) in eucalypt, casuarina and acacia seedlings to oxadiazon (same mode of action as oxyfluorfen) and propyzimide (same mode of action as metolachlor). In the field, applications of chlorsulfuron and metsulfuron (same mode of action as imazethapyr) and oxyfluorfen to 9–12 month-old direct-seeded plantings have been shown to have little effect on the stocking rates of eucalypts and other native species (Nardon *et al.* 2005).

5.6 Conclusions

We conclude from this study that herbicide oversprays have potential for managing weeds that germinate and grow in direct-seeded riplines. The species examined exhibited varying tolerances to a range of herbicides and differential responses to particular herbicides. Consequently, the likelihood of a ‘one-size-fits-all’ selective herbicide that controls herbaceous weeds without damaging native woody seedlings is improbable. Focus on thoughtful design at the initial planning stage will be an important step towards improving the success of future direct seeding in regions where climate and the weed flora conspire to outcompete direct-seeded plantings. We recommend grouping species according to their herbicide tolerance and planting each group in a separate row for subsequent overspraying with the herbicide to which the species are tolerant. A planting design based on this research could incorporate separate rows of (1) *E. camaldulensis* and *D. viscosa* with oxyfluorfen as the overspray, (2) *C. cristata, A. harpophylla* and *A. pendula* (imazethapyr), and (3) *A. salicina, E. coolibah* and *S. artemisioides* (isoxaflutole). Planted rows should be spaced appropriately to avoid spray-drift of herbicides applied to adjacent rows. *Atriplex nummularia* was not tolerant of any of the herbicides in this study. Given it is widely planted in arid regions (for revegetation and stock fodder), identifying a herbicide to which this species is tolerant is needed.

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CHAPTER 5 Determining Herbicide Tolerance in Native Woody Species with Application to Weed Control in Direct Seeding

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Table 5.2	p. 108

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CHAPTER 6

Microbial Inoculants in Coated Seed, Soil Moisture and Seed Burial Enhance Germination of Northern New South Wales Eucalypts

6.1 Abstract

Revegetation by direct seeding has the potential to reverse land degradation and increase tree cover in agricultural landscapes. However, past direct-seeding efforts have often resulted in poor establishment due to limited germination, particularly with small-seeded eucalypts. We conducted trials with eucalypt seed from northern New South Wales to (1) test the effects of seed-coating treatments (coated seed, coated seed with MycoApply® and uncoated seed), watering regimes (30 mL per 1 day, per 3 days and per 5 days, and no water) and sowing methods (surface-sown and buried beneath 0.5 cm of vermiculite) on germination, and (2) test the effect of seed-coating treatments on seedling establishment. Coating the seed with microbial inoculants as well as daily watering significantly increased germination. Sowing method alone had no significant effect on germination, but there was a significant interaction of sowing method nested within seed-coat treatment × watering regime. Seed-coating treatments had no effect on seedling growth. Daily watering and continuous soil moisture were critical for eucalypt germination. Under conditions of

low soil moisture, mycorrhizal symbiosis and seed burial increased water capture and improved seed-soil contact, both of which prevented seed desiccation and enhanced germination.

6.2 Introduction

Native trees in agricultural landscapes confer many benefits in terms of biodiversity conservation, ecosystem services and primary productivity (Bird *et al.* 1984; Pimentel *et al.* 1992; Reid and Landsberg 2000; Scherr and McNeely 2008; Power 2010; Schneemann and McElhinny 2012). However, widespread land clearing since European settlement has resulted in significant loss of tree cover in agricultural districts and a legacy of landscape degradation (Yates and Hobbs 2000). Eucalypt woodlands are particularly vulnerable because they occupy relatively fertile soils that are valuable for cropping and grazing enterprises (Yates and Hobbs 2000).

On the Northern Tablelands of New South Wales, tree loss is exacerbated by rural dieback, a syndrome of tree ill-health that manifests as progressive dying back of the canopy (Mackay *et al.* 1984; Reid and Landsberg 2000; Nadolny 2008). Furthermore, the natural recruitment of eucalypts on farmland in the region, which is important for the replacement of aging and debilitated trees, is frequently absent due to livestock grazing, competition from fertilised pastures and other land management practices and environmental changes (Curtis 1990a; Whalley and Curtis 1991; Reid and Landsberg 2000; Li *et al.* 2007). One way of reversing the effects of dieback and landscape degradation is through the re-establishment of native trees. Direct seeding is often favoured over other revegetation methods because it is cost-effective, suitable for broad-scale applications and results in more naturally spaced communities (Rawlings *et al.* 2010; Schneemann and McElhinny 2012; Hallett *et al.*

2014). However, direct seeding of native vegetation on the Northern Tablelands has proven to be challenging as the germination and establishment of eucalypts from seed can be difficult (Curtis 1990a; Li *et al.* 2003). This creates a major bottleneck in the regeneration of these long-lived tree species (Fenner 1987).

Soil moisture is of universal importance for seed germination (Weinberg *et al.* 2011). Seed germination is regulated by the water content of the seed, which in turn, influences the way in which metabolism in various seed tissues is coordinated via the intervention of different growth regulators, such as gibberellins and cytokinins (Mayer 1986). Germination occurs when the minimum level of hydration that signals stimulation of cell division in the embryo is reached in the seed (Bell 1999). The ability for a seed to take up enough water to facilitate germination is underpinned by several factors, including seed size, the soil water and matric potentials, and the area of contact between the seed and the soil (Khurana and Singh 2004). Eucalypt seeds are particularly vulnerable to desiccation because they are small and lack an endosperm, and do not persist for long periods in the environment if the requirements for germination are not met (Weinberg *et al.* 2011). To prevent desiccation of direct-seeded eucalypts, seed burial is recommended (Curtis 1990a; Long *et al.* 2015). Seed burial is important because it places seed in an environment that provides moisture through soil contact and reduces opportunities for seed predation (Long *et al.* 2015).

Symbioses with soil microbes, in particular with mycorrhizal fungi, also play an important role in water acquisition for early-phase germinants (Gardner and Malajczuk 1988; Linderman 1988; Ajeesh *et al.* 2015). Mycorrhizae are highly evolved, mutualistic associations between soil fungi and plant roots (Brundett *et al.* 1996). Eucalypts form mycorrhizal associations predominantly with ectomycorrhizae

(EM), but also with arbuscular mycorrhizae (AM). The primary EMs that colonise eucalypt roots include species of *Laccaria*, *Schleroderma*, *Amanita*, *Pisolithus*, *Cortinaria*, *Paxillus* and *Ramaria* (Lu *et al.* 1999; Glen *et al.* 2008), while common AMs include species of *Acaulospora*, *Gigasporum*, *Glomus*, *Paraglomus* and *Scutellospora* (Carrenho *et al.* 2008).

Mycorrhizae improve seedling establishment and increase growth and productivity in many species (Allen 1982; Azcón-Aguilar and Barea 1997; Carrenho *et al.* 2008; Van Der Heijden and Horton 2009), but little is known of the benefits of mycorrhizae in the seed germination process. Understanding the factors that limit the recruitment of eucalypts is a critical first step in addressing tree decline on the Northern Tablelands. Consequently, we conducted trials to: (1) test the effects of seed-coating treatments, watering regime and sowing method on eucalypt germination, and (2) test the effect of seed-coating treatments on early seedling growth of eucalypts. Based on previous research (Curtis 1990a; Bell 1999; Khurana and Singh 2004; Van Der Heijden 2004; Van Der Heijden and Horton 2009; Long *et al.* 2015; Varga and Kytöviita 2016), we formulated the following hypotheses: (1) microbial inoculants (MycoApply®) would improve germination and growth of eucalypt seedlings; (2) eucalypt germination would increase with increasing water availability, and (3) seed burial would enhance germination through greater contact with moist soil particles and the reduced likelihood of seed desiccation.

6.3 Materials and methods

This investigation was conducted in a glasshouse at the University of New England in Armidale, NSW, between July and September 2016. Glasshouse temperatures were 25°C (day) and 15°C (night) with a 12:12 hour day/night regime. For this study, we used seed containing a mixture of nine Northern Tableland eucalypt species (*E. viminalis* Labill., *E. pauciflora* Sieber ex Spreng., *E. melliodora* A.Cunn. ex Schauer., *E. nova-anglica* H.Deane & Maiden., *E. blakelyi* Maiden, *E. laevopinea* R.T. Baker, *E. dalrympleana* Maiden, *E. camaldulensis* Dehnh. and *E. dealbata* A.Cunn. ex Schauer. By mixing the seed a great deal of insightful information was admittedly lost. However, the seed was surplus from a direct-seeding field experiment in which the seed of these species had been combined in a single hopper and sown into a ripline. When the field experiment failed (no germination), the study was transferred to the glasshouse to determine the causes of the failure.

The seed was cleaned in an air tunnel to remove the chaff, after which it was bulked and divided into three seed lots. Two of the seed lots were pelleted and coated, one lot incorporating a commercial mix of ectomycorrhizae (EM) and arbuscular mycorrhiza (AM) (MycoApply®) and the other without. MycoApply® contained ectomycorrhizae (*Rhizopogon roseolus, R. vulgaris, Laccaria bicolour, L. laccata, Pisolithus tinctorius, Scleroderma cepa* and *S. citrinum*), arbuscular mycorrhizae (*Glomus intraradices, G. etunicatum, G. aggregatum, G. mosseae, G. clarum* and *G. monosporum*), plus other beneficial organisms (*Azospirillum brasilense, A. lipoferum, Bacillus subtilis, B. licheniformis, Pseudomonas fluorescens, P. putida, Streptomyces cellulomonas, Trichoderma harzianum* and *T. viride*). The seeds were pelleted with a blend of calcium carbonate and mica, and finished with a polymer coating (Fig. 6.1). The soil used for the experiment was black clay derived from basalt parent materials collected on private property (30°31'31" S, 151°30'54" E) at Invergowrie, near Armidale, NSW. Prior to the experiment, germination trials of seed subject to each coating treatment (coated seed, coated seed with mycorrhizae and uncoated seed) were performed in climate-controlled germination cabinets.

Figure 6-1 Coated seed with MycoApply® (pink), and coated seed without MycoApply® (blue)

Source: Photograph by Ian Perryman

6.3.1 Experimental design

This experiment was a split-plot randomised block design incorporating seeds subject to three coating treatments (coated seed, coated seed with MycoApply® and uncoated seed), four watering regimes (30 mL/day, 30 mL/3 days, 30 mL/5 days and no water or control) and two sowing methods (surface-sown vs buried seed beneath a 5-mm vermiculite layer). Seedling trays (35 × 25 cm) contained 24 cells and four seeds were surface-sown in each cell, giving a total of ninety-six seeds per tray. Each combination of seed coating and watering treatment was allocated to a tray (to give 12 trays) and replicated three times (36 trays in total) in three separate blocks. The trays were sectioned into two equal halves (48 seeds/half) and a layer of vermiculite added to one half of the tray. The position of trays in each set of 12 was randomised in each block. Germination was counted and recorded daily between 19 July and 25 August. Seedling height was measured at the end of the experiment to compare the effects of seed coating treatment on early seedling growth. Soil samples (five replicates of each watering regime) were collected to compare differences in soil moisture. On 25 August (at the end of the experiment), surface topsoil (10 mm depth) was removed from five randomly selected cells (in which germination was absent) in each watering regime. Soil moisture content was measured by weighing the soil samples prior to air drying at 40°C for 10 days, then reweighing the soil and determining the difference in weight. Soil moisture was expressed in grams of water per gram of soil (w/w).

6.3.2 Statistical analysis

To examine differences in germination between coating treatment, water regime and sowing method, we fitted a generalised linear model with a quasi-binomial error distribution. Block, coating treatment, water regime and their interaction, and sowing method nested within the interaction between coating treatment and water regime were included in the model. We removed two watering levels (30 mL/5 days and the control) from the analysis as germination was absent in these treatments. Coated seed was also removed from the analyses given that there was a zero-inflation problem with this level; the mean values for coated and uncoated seed were similar. The lsmeans routine was used to compute the significance (Tukey's) of pairwise comparisons between means. Linear models with block as a fixed effect were used to examine the difference in seedling height between coating treatments, and the difference in soil moisture content between sowing methods. Models were fitted using the R statistical computing environment (version 3.0.2; R Development Core Team 2015). Diagnostics were checked to ensure goodness of fit and the validity of model assumptions.

6.4 Results

6.4.1 Soil moisture

Soil moisture varied significantly among watering regimes (Table 6.1). Soil moisture content (mean ± 1 s.e.) in the 30 mL/day watering treatment (0.32 ± 0.06 gg^{-1}) was 2.5 times higher than in the 30 mL/3 days treatment (0.13 ± 0.07 gg^{-1}), 4 times higher than in the 30 mL/5 days treatment (0.8 ± 0.04 gg^{-1}) and 12.5 times higher than the control (0.03 ± 0.01 gg^{-1}) (Fig. 6.2A). There was a significant difference in soil moisture between sowing methods (Table 6.1). Soil moisture was higher in the presence of vermiculite (0.15 ± 0.02 gg^{-1}) compared to bare soil (0.13 ± 0.02 gg^{-1}) (Fig. 6.2B). There was no significant interaction between water regime and sowing method, but the block effect was significant (Fig. 6.2C, Table 6.1).

Table 6-1 Coefficients for the estimates, standard errors (SE) and 95% confidence intervals (CIs) for the effect of water regime and sowing method on soil moisture

Variables	Coefficient	SE	95% CIs Lower	 Upper
Intercept	0.31	0.007	0.30	0.33
30 mL/3 days	−0.18	0.009	−0.20	−0.16
30 mL/ 5 days	−0.24	0.009	−0.24	−0.22
No water	−0.28	0.009	−0.30	−0.26
Buried seed	0.02	0.009	0.006	0.04
Block 2	−0.01	0.005	−0.02	−0.001
Block 3	−0.02	0.005	−0.03	−0.007
30 mL/3 days: Buried seed	−0.006	0.013	−0.03	0.02
30 mL/ 5 days: Buried seed	0.001	0.013	−0.02	0.03
No water: Buried seed	−0.02	0.01	−0.05	0.004

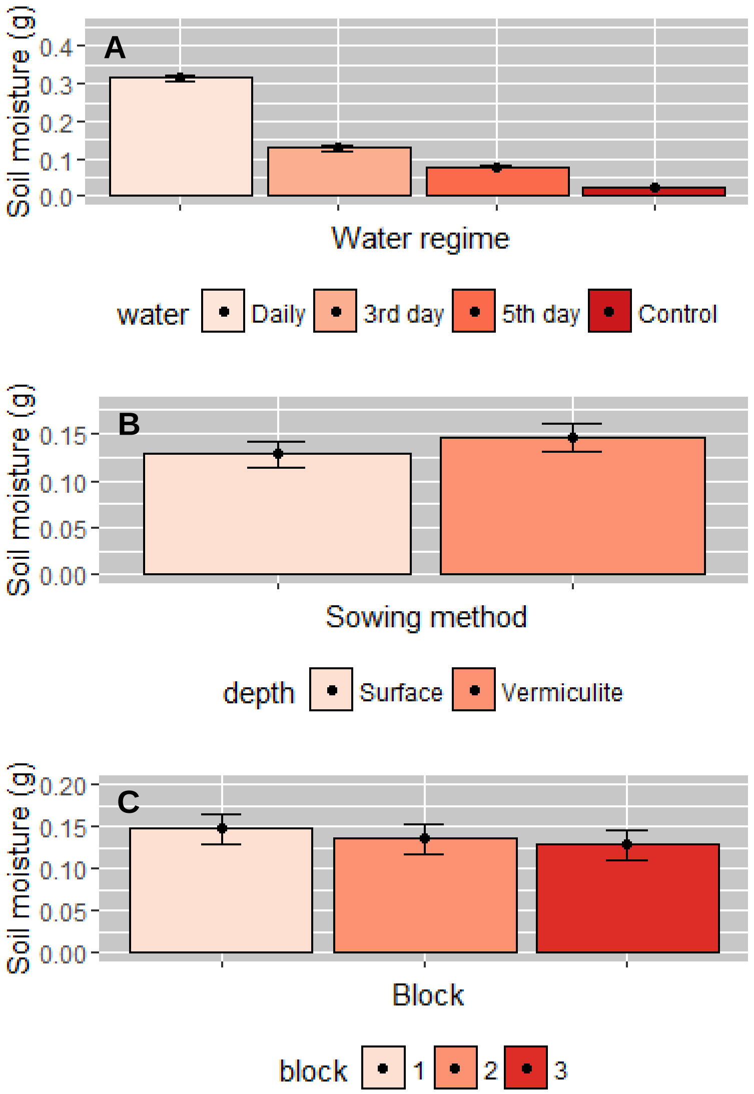

Figure 6-2 Soil moisture content (mean ± 1 s.e.) for (A) water regime, (B) sowing method, and (C) block

6.4.2 Eucalypt germination

Preliminary growth cabinet germination trials showed significantly higher germination in seed coated with MycoApply® compared to plain coated seed and uncoated seed. In the analysis of the glasshouse trial, the block effect was marginal (*P* = 0.07). The nested term (sowing method nested within the interaction of coating treatment and water regime) was significant (*P* = 0.05) (Fig. 6.3, Table 6.2). Under the 30 mL/3days watering regime, germination of seed coated with MycoApply® was greater with buried seed than surface-sown seed (*P* = 0.04), but the difference in germination in seed coated with MycoApply® and watered daily was not significant between surface-sown and buried seed ($P = 0.99$).

Table 6-2 Coefficients for the estimates, standard errors (SE) and 95% confidence intervals (CIs) for the effect of coating treatment, water regime and sowing method on germination

Variables	Coefficient	SE	95% CIs	
			Lower	Upper
Intercept	0.46	0.27	−0.06	0.99
Block 2	−0.72	0.26	−1.24	−0.21
Block 3	−0.40	0.25	−0.90	0.09
Uncoated seed	−0.92	0.33	−1.58	−0.29
30 mL/ 3 days	−5.01	1.34	−9.44	−3.10
Uncoated seed: 30 mL/ 3 days	2.75	1.47	0.37	7.20
Coated seed with MycoApply®:30 mL/ day:Buried seed	−0.23	0.31	−0.85	0.39
Uncoated seed: 30 mL/ day:Buried seed	−0.03	0.34	−0.71	0.64
Coated seed with MycoApply®:30 mL/ 3 days:Buried seed	3.03	1.37	1.03	7.39
Uncoated seed:30 mL/ 3 days:Buried seed	−0.42	0.87	−2.31	1.28

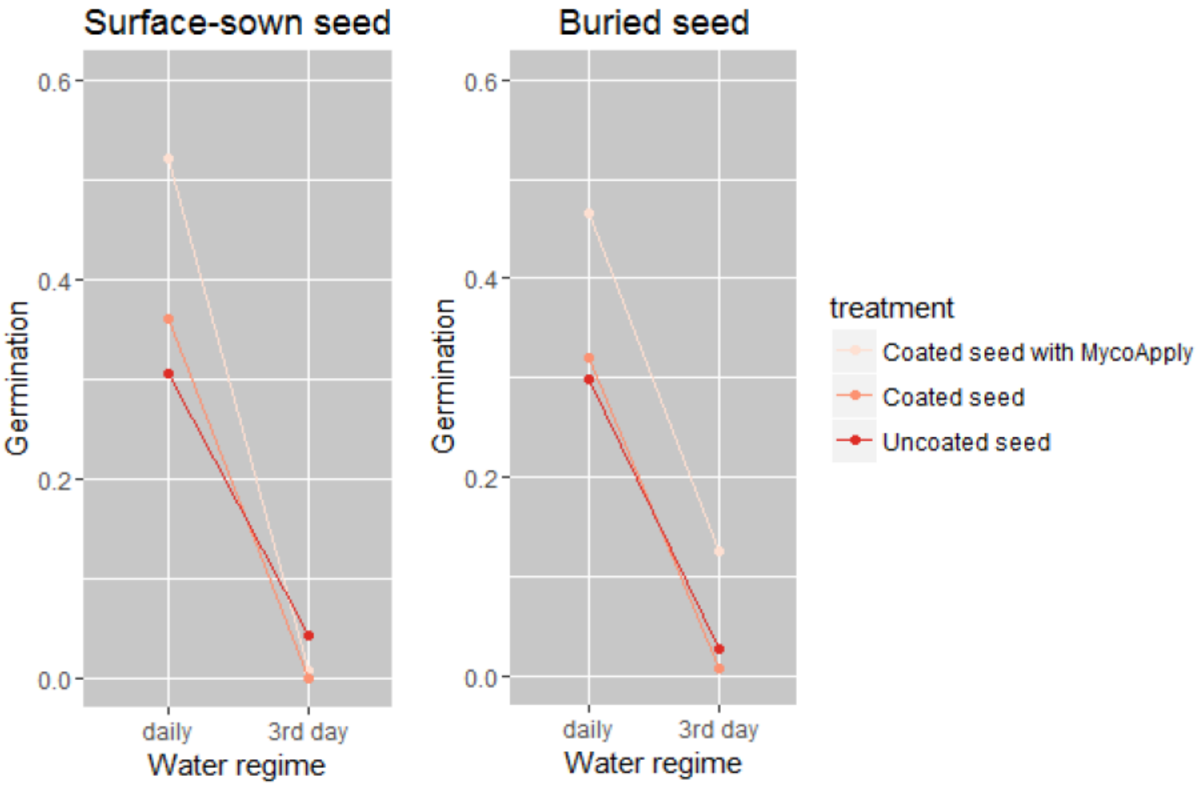

Figure 6-3 Plots showing eucalypt germination in response to the significant nested term: sowing method nested within the interaction between coating treatment and water regime

6.4.3 Seedling height

Mean seedling height differed significantly among coating treatments ($P < 0.05$) (Fig. 6.4, Table 6.3). Seedling height (mean ± 1 s.e.) was greater in the plain coated seed treatment (4.05 ± 0.20 cm) compared to the uncoated seed treatment (3.46 ± 0.14 cm) and seed coated with MycoApply® (3.38 ± 0.15 cm). Seedling height for uncoated seed was significantly less than plain coated seed (95% CI: −1.032, −0.148, after relevelling). The block effect was not significant (Table 6.3).

Table 6-3 Coefficients for the estimates, standard errors (SE) and 95% confidence intervals (CIs) for the effect of coating treatment on seedling height

Variables	Coefficient	SE	95% Cis	
			Lower	Upper
Intercept	3.43	0.21	3.02	3.85
Coat without MycoApply®	0.67	0.25	0.18	1.15
Uncoated seed	0.08	0.22	−0.35	0.51
Block 2	−0.25	0.23	−047	0.42
Block 3	−0.13	0.22	−0.56	0.30

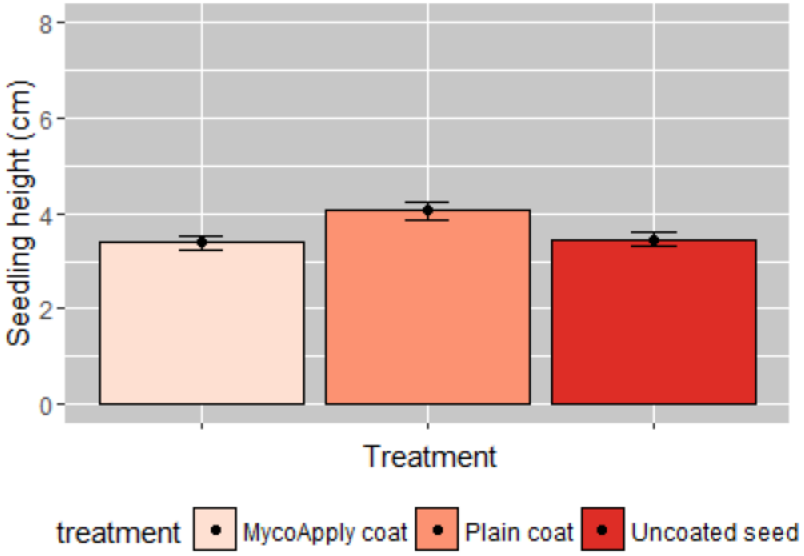

Figure 6-4 Seedling height (mean ± 1 s.e.) in response to coating treatment

6.5 Discussion

6.5.1 The effect of microbial inoculants on eucalypt germination and growth

Our hypothesis that germination would be significantly higher for seed coated with MycoApply® compared to coated seed without microbial inoculants and uncoated seed was supported. However, given the MycoApply® contained different species of micro-organisms, it could not be determined which species(s) were beneficial to seed germination. Studies demonstrating the effect of inoculated seed coating on eucalypt germination are scarce, but there is evidence in the literature that mycorrhizae benefit the germination of species of other families, particularly Orchidaceae. These studies consistently demonstrate significantly higher germination in seeds treated with mycorrhizae compared to non-mycorrhizal seed (Johnson *et al.* 2007; Porras-Alfaro and Bayman 2007; Nontachaiyapoom *et al.* 2011). Increased germination in the presence of microbial inoculants has also been reported in switchgrass (Poaceae*)* (Ghimire *et al.* 2009), coyote tobacco (Barazani *et al.* 2005) and capsicum (Solanaceae; Rueda-Puente *et al.* 2010), and harebell (Campanulaceae; Varga and Kytöviita 2016). To our knowledge, symbiotic seed germination in eucalypts has not been examined, so this research addresses an important knowledge gap.

The ability of mycorrhizae to enhance water capture by the host plant is well documented (Allen 1982; Augé 2001; Linderman 1988; Varga and Kytöviita 2016). Improved water relations are achieved through an increase in root surface area by fungal hyphae (Allen 1982), increasing water uptake and reducing resistance of water flow along the entire hydraulic pathway from the soil to the host shoots (Allen 1982; Augé 2001). Past studies have also shown that mycorrhizae confer improved levels of drought resistance in hosts by allowing photosynthetic and metabolic functions to be more effectively maintained (Subramanian and Charest 1995; Schellenbaum *et al.* 1999; Augé 2001). Further, fungal hyphae and their exudates physically and chemically bind soil aggregates and improve soil water retention properties (Augé 2001).

In this study, seedling height was not influenced by the presence of MycoApply®, but other studies have demonstrated mycorrhizal benefits to seedling growth (Bougher *et al.* 1990; Ajeesh *et al.* 2015; Varga and Kytöviita 2016). This was because germination mostly occurred when soil moisture was not limiting, so seedling establishment did not reflect the benefits of symbiosis. Mycorrhizae are mostly advantageous to plant hosts under stress or when environmental conditions are unfavourable, thus increasing host dependence on enhanced water and nutrient capture (Carrenho *et al.* 2008).

6.5.2 The effect of soil moisture on eucalypt germination

Our results supported the hypothesis that germination would increase with increasing soil moisture. Germination was 13 times higher in trays watered daily compared to trays watered every 3 days, and absent in trays watered every 5 days and in the absence of water. The results are consistent with previous studies, which document the detrimental impacts of water stress on germination in several eucalypt species, including *E. occidentalis* (Zohar *et al.* 1975), *E. delegatensis* (Battaglia 1993), *E. sieberi*, *E. pilularis*, *E. maculata* (Bachelard 1985), *E. camaldulensis*, *E. regnans* (Edgar 1977), *E. blakelyi*, *E. viminalis*, *E. melliodora* (Curtis 1990a), *E. pilligaensis* and *E. populnea* (Ruiz Talonia *et al.* 2016).

Given the sensitivity of eucalypt seeds to minor variations in soil moisture (Facelli *et al.* 1999; Humara *et al.* 2002), this study highlighted the importance of consistently high soil moisture for eucalypt germination. Generally, seeds are tolerant of short cycles of wetting and drying provided there is sufficient soil moisture to maintain the process of imbibition (Battaglia and Reid 1993). We suspect that imbibition was interrupted when the time between watering was extended. Sometimes, seeds are held in a partial state of imbibition where drying is incomplete, resulting in decreased germination. However, if imbibition progresses sufficiently before complete drying, seed death occurs (Battaglia and Reid 1993).

6.5.3 The effect of sowing method on eucalypt germination

Seed burial alone did not affect the germination of eucalypt seed. However, seed burial played a significant role in increasing germination in conjunction with seed coating treatment and watering regime. To fully understand the effects of seed burial on eucalypt germination, trials investigating germination at a range of soil depths would need to be conducted. Previous work suggests that soil depth and water regime play significant roles in the germination of eucalypt seed. For example, Curtis (1990b) reported a significant water × depth interaction in *E. blakelyi, E. melliodora* and *E. viminalis* seeds planted at depths of 2, 4, 9, 14, 19 and 23 mm, and watered at intervals of 2, 4, 7 and 14 days. The optimal depth for emergence was between 2 and 4 mm. Recent work by Ruiz Talonia *et al.* (2016) demonstrated that the germination of *E. blakelyi, E. camaldulensis, E. melanophloia, E. melliodora, E. pilligaensis* and *E. populnea* seeds was greatest in surface-sown seed under moist and flood conditions, declining with increasing depths of 6, 12 and 20 mm. Under dry conditions, however, emergence was greatest in seed sown at a depth of 6 mm. These results support the findings of the present study, and indicate that surface conditions are preferable for eucalypt germination unless soil moisture is limiting, in which case shallow burial (2–6 mm) is optimal. In the absence of a suitable growth medium to maximise seed–soil contact, seeds are susceptible to declining moisture levels (Humara *et al.* 2002).

In this study, vermiculite was used to bury eucalypt seed after sowing. Vermiculite is a clay silicate commonly used as a growth medium. The layered structure of vermiculite particles and its capacity for cation exchange allow it to hold a substantially higher volume of water than most soils (Reinholdt *et al.* 2013). Consequently, vermiculite is often used in horticulture as a growth medium and as a solid matrix primer to enhance the germination of small-seeded crops (Taylor *et al.* 1988; Pill *et al.* 1994; Jisha *et al.* 2013). Given that vermiculite has distinctly different physical and chemical properties, additional investigations to compare the germination of eucalypts in field soils would complement this study.

This experiment had its origins in a direct-seeded field trial, which was abandoned after two seeding attempts failed to produce eucalypt recruits. The experiment was then conducted under controlled glasshouse conditions to determine why the field trials might have failed. We suspected that even though 10 L of water/m^2 was applied in the field trials every 3 days, the topsoil dried out too quickly, resulting in seed desiccation. Thus a range of watering regimes was incorporated in the glasshouse experiment. The experiment should now be repeated in the field to confirm that continuous soil moisture, shallow seed burial and microbial seed coating can lead to eucalypt recruitment at the site of the failed trials.

6.6 Conclusions

The re-establishment of eucalypts in farmland through direct seeding has the potential to increase native tree cover. At present, direct seeding has yielded mixed results, with poor germination and survival of germinants often contributing to failure. Although it is well-known that eucalypts form symbiotic relationships with both AM and EM, there is a lack of research on the benefits of these fungi and other microbes for revegetating eucalypts by direct seeding. This study has demonstrated that microbial inoculation, soil moisture and seed burial interact to improve the germination of eucalypts. We recommend coating eucalypt seed with MycoApply® to improve the success of direct seeding in areas where re-establishing native trees is challenging. The enhanced capacity of mycorrhizae to capture water from the surrounding soil and confer drought-resistance to young seedlings are indicative of its potential as a useful revegetation tool. However, the insights gained from this glasshouse experiment need to be tested in the field to increase confidence in these recommendations for in-field sowing practices.

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We, the PhD candidate and the candidate's Principal Supervisor, certify that all co-authors have consented to their work being included in the thesis and they have accepted the candidate's contribution as indicated in the *Statement of Originality*.

CHAPTER 6 Microbial Inoculants in Coated Seed, Soil Moisture and Seed Burial Enhance Germination of Northern New South Wales Eucalypts

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CHAPTER 7

Top Soil Removal and Carbon Addition are Effective Weed Control Strategies for Recruiting Native Grasses on the Northern Tablelands of New South Wales

7.1 Abstract

Restoring the grassy understorey to temperate woodlands in south-eastern Australia is often disregarded due to a poor understanding of the techniques involved. The natural recruitment of native grasses is uncommon in the remnants of some of these woodlands, so the restoration and management of the grass layer is often dependent on interventions to overcome restoration barriers. Soil enrichment from agricultural fertilisers favours the invasion of exotic broadleaf weeds and grasses, and is one of the primary barriers to successful recruitment and establishment of native grasses, which dominated prior to agricultural development. This study, on the Northern Tablelands of New South Wales, investigated the effects of four weed control treatments – scalping, glyphosate (Roundup®) herbicide, and combinations of glyphosate with carbon (sugar and sawdust) addition – on native grass recruitment and weed emergence. The experimental site was a mown grass lawn consisting mainly of fescue (*Festuca arundinacea* Shreb.), cocksfoot (*Dactylis glomerata* L.)

and paspalum (*Paspalum dilatatum* Poir). Treatments were applied to 1 × 1-m plots in a completely randomised design with six replicates per treatment, including a control (nil treatment). The mean number of native grass recruits was significantly greater in scalped plots than in other treatments. Scalping is likely to be a successful restoration tool because it removes the top layer of soil containing stored weed seeds, as well as reducing elevated soil nutrient levels that favour weed emergence. This allows time for slow-growing native grasses to germinate and establish in the absence of competitive fast-growing exotic weeds. While combinations of sugar and sawdust with glyphosate were also effective in controlling weeds, they did not benefit native grass recruitment. The cost of purchasing, transporting and applying large quantities of sugar or sawdust will limit their use in broad-scale revegetation.

7.2 Introduction

Box gum grassy woodlands are an endangered community in south-eastern Australia (Hobbs and Yates 2000). They occur either as woodlands with a diverse understory of tussock grasses and native forbs or as derived native grasslands. These woodlands are typically dominated by white box (*Eucalyptus albens*), yellow box (*E. melliodora*), Blakely’s red gum (*E. blakelyi*) or grey box (*E. moluccana*) (NSW Department of Environment 2010). Having once occupied 5 011 655 ha of rural New South Wales, South Australia, Victoria and southern Queensland, box gum grassy woodlands have been reduced to remnants of varying size, quality and isolation, totalling 416 325 ha due to agricultural development (Department of the Environment and Energy 2006). Consequently, the protection and management of these communities in terms of biodiversity and the ecosystem services they provide are a priority. Box gum grassy woodlands have been listed as a critically endangered ecological community under the *Environment Protection and Biodiversity Conservation Act 1999* (Commonwealth).

In the past, restoration has focused largely on re-establishing dominant eucalypt and other woody species by planting seedlings, but little attention has been given to the native grass understorey. This has given rise to partially restored communities that are floristically impoverished with limited biodiversity conservation value (Prober *et al.* 2002b; Spooner *et al.* 2002; Cole and Lunt 2005). In disturbed and degraded ecosystems, natural recruitment of native grasses is rare, due to poor seed reserves in the soil seed bank and diminished opportunities for seed dispersal from adjacent sites (Lunt 1997; Morgan 1998; Hobbs and Yates 2000; Prober *et al.* 2002b). Therefore, the conservation of grassland communities is heavily dependent on assisted intervention strategies to overcome restoration barriers and facilitate transition into a more desirable and resilient state (Whisenant 1999). The challenge lies in knowing which strategies to apply, as grassland restoration techniques are not well-established in Australia as yet (Waters *et al.* 2001; Prober *et al.* 2002b).

Grassland restoration is unlikely to be successful in the absence of effective and timely weed control (Waters *et al.* 2001; Cole and Lunt 2005; Gibson-Roy *et al.* 2007; McIntyre 2011). Weeds affect the establishment of native understorey species by reducing emergence and restricting growth (Cole and Lunt 2005). Exotic grasses tend to dominate as they establish quickly from the soil seed bank, forming dense swards in autumn and winter, which outcompete slow-growing native grasses (Waters *et al.* 2001; Cole and Lunt 2005; Prober and Thiele 2005). One way of addressing this problem is by implementing soil-nutrient reduction treatments, which exploit the dissimilarities in nutrient requirements between fast-growing exotics and slow-growing native species (Cole *et al.* 2016). Removing nutrient-rich topsoil by scalping and adding sources of carbon such as sugar, sawdust or woodchips are two examples. Both have similar outcomes in that they reduce or reverse the restoration barrier resulting from high soil nutrient influx (Prober *et al.* 2005; Smallbone *et al.* 2008; Perry *et al.* 2010), but scalping has the added advantage of removing seeds of annual exotic weeds stored in the soil seed bank (Gibson-Roy *et al.* 2010). Non-selective herbicides, such as glyphosate, are commonly used prior to planting to remove above-ground biomass, but are often ineffective for grassland restoration because native grasses are slow-growing and weeds often reinvade before the native species re-establish (Prober and Lunt 2008; Gibson-Roy *et al.* 2010). Further, non-selective herbicides do not remove weed seeds stored in the soil seedbank, even with repeated use (D. Carr, 2016, pers. comm.).

Past research indicates that scalping and carbon addition are effective methods of weed control (Prober and Lunt 2008; Gibson-Roy *et al.* 2010). Based on these findings, the primary objective of this study was to test whether four weed control treatments – scalping, glyphosate 450 (Roundup®), sugar + glyphosate, and sawdust + glyphosate – could facilitate native grass recruitment and inhibit weed emergence.

7.3 Materials and methods

7.3.1 Study site

This study was conducted on the property of the Armidale Tree Group in Armidale, New South Wales, Australia, between August 2015 and March 2016. The study site was a gently sloping mowed lawn consisting mostly of exotic grasses, such as fescue (*Festuca arundinacea*), cocksfoot (*Dactylis glomerata*) and paspalum (*Paspalum dilatatum* Poir) For most of its history, the site was crown land and existed in its original state until the early to mid 1900s. In 1929, the Armidale Teachers College was built on nearby land and the site became an agricultural paddock until 1990 at the latest. During this time livestock were grazed on the site but it was not cultivated. There has been no fertiliser application since 1990 (Wal Whalley, 2017, pers. comm.).

The soil at the site was a ‘prairie soil’ (Jessup 1965) or an alluvial black clay vertosol in the Australian Soil Classification (Isbell 1996) derived from basaltic and metasedimentary parent materials, which was damp at the southern end of the site due to an adjacent man-made drainage pool. Prior to European settlement, the area would have been grassy woodland dominated by *Eucalyptus viminalis*, *E. pauciflora* and *E. dalrympleana*.

7.3.2 Experimental design

This study was a randomised block design comprising six replications of five treatments in 1 × 1-m plots each separated by a 0.5 m buffer. The five weed control treatments comprised (1) scalping; (2) glyphosate 450 (Roundup®); (3) glyphosate plus sugar; (4) glyphosate plus sawdust, and (5) a control (nil) treatment. In October, 2015, the glyphosate treatments were sprayed at the recommended application rate and the scalped plots were excavated to a depth of 10 cm. This depth was chosen based on the results of soil phosphorus and nitrogen analyses. Vertical drains 10 cm in diameter and 50 cm deep were constructed on the southern edge of each scalped plot and filled with roadbase to prevent flooding. In early November 2015, each plot (including the control) was hand-broadcast with 5 g of mixed native grass seed and the sugar and sawdust applied. Based on Prober *et al*. (2004), white sugar was applied manually at the rate of 0.5 kg m^{-2} and reapplied at the same rate in January,

2016. Sawdust was spread in a uniform 2-cm layer over the plot surface. The grass species used in this experiment were *Themeda australis* (R.Br.) Stapf, *Sorghum leiocladum* (Hack.) C.E.Hubb, *Poa sieberiana* Vickery, *Dichanthium setosum* S.T Blake, *Dichelachne crinita* (L.) Hook.f., *Austrostipa scabra* (Lindl.) S.W.L.Jacobs & J.Everett, *Eragrostis leptostachya* (R.Br.) Steud., *Pennisetum alopecuroides* (L.) Spreng., *Sporobolus creber* De Nardi, *Chloris truncata* R.Br. and *Rytidosperma richardsonii* (Cashmore) Connor & Edgar. Seed was harvested at Saumarez travelling stock route, near Armidale, in February 2014 and was dry-stored until sowing. The number of recruits per plot and per species was recorded monthly from November 2015 until March 2016. Weed emergence was recorded monthly by visually estimating percent weed cover and allocating a score of: 1, <5% weed cover; 2, 5–10% cover; 3, 10–25%; 4, 25–50%; 5, 50–75% and 6, 75–95% weed cover. A list of emergent weed species is provided in Table 7.1.

Soil samples were taken from each plot receiving a scalping or sugar treatment at 0–1, 5–6, 9–10 and 11–20-cm depths using a 5-cm soil core. Samples were oven-dried for 10 days at 40°C. In preparation for laboratory analysis, soil samples were ground to 2 mm (phosphorus content) and 0.5 mm (nitrogen content). Colwell phosphorus was measured using 0.5 M $NaHCO_3$ extraction (Motomiza *et al.* 1983) and total nitrogen content was determined using a TruSpec series carbon and nitrogen analyser (LECO Corporation Pty Ltd).

7.3.3 Statistical analysis

The control treatment was omitted from analyses as no recruitment was observed in control plots. A zero-inflated Poisson (ZIP) regression model was fitted to analyse the effects of weed control treatments on native grass recruitment and weed cover.

Table 7-1 List of weed species present in plots at the time of the study

Species	Common name	Family
Bromus catharticus	Prairie grass	Poaceae
Cirsium vulgare	Scotch thistle	Asteraceae
Cyperus eragrostis	Umbrella sedge	Cyperaceae
Dactylis glomerata	Cocksfoot	Poaceae
Digitaria sanguinalis	Summer grass	Poaceae
Festuca arundinaceae	Tall fescue	Poaceae
Hypochaeris radicata	Dandelion	Asteraceae
Malva parviflora	Small-flowered mallow	Malvaceae
Modiola caroliniana	Red-flowered mallow	Malvaceae
Paspalum dilatatum	Paspalum	Poaceae
Pennisetum clandestinum	Kikuyu grass	Poaceae
Plantago lanceolata	Plantain	Plantaginaceae
Portulaca olearacea	Pigweed	Portulaceae
Rumex obtusifolius	Broad leaf dock	Polygonaceae

A ZIP regression model was chosen to deal with the over-dispersion of the data due to the number of zeros caused by the absence of native grass recruitment in some treatments. The ZIP regression provided a two-part model for which the probability of the presence of native grass germinants and the abundance of germinants when present were modelled using the same data. The ZIP model was fitted with the pscl package in the R statistical computing environment (version 3.0.2; R Development Core Team 2015). To analyse the effects of weed control treatments on weed emergence, we fitted a linear model incorporating a logit transformation of the response. Model diagnostics were checked to ensure goodness of fit. As seedlings of only two native grass species were recorded (*Eragrostis leptostachya* and *Rytidosperma richardsonii*), we chose not to include species as a predictor variable.

ZIP models were initially run with the variables showing the highest recruitment as the baseline references so clear comparisons could be shown in the results. The models were refitted with different levels of weed control as the reference group to obtain treatment comparisons. All analyses were performed in R Studio (Version 3.0.2; R Core Development Team 2015).

7.4 Results

7.4.1 Soil analyses

The cracking black clay soils at the study site were high-fertility soils, containing large concentrations of N and P in the topsoil. These soils are typical of some lower slopes and valley floors on the Northern Tablelands and were described by Jessup (1965) as ‘prairie soils’. Jessup’s work showed a steep decline in nutrients in these soils with depth, with N and P concentrations levelling out at about 10 cm depth. The decision to excavate scalped plots to a depth of 10 cm was based on Jessup (1965), and verified in Fig. 7.1. If a shallower depth had been chosen, too much N and P would have been present in the seed bed. Soil tests comparing the nitrogen and phosphorus levels (mean ± 1 s.e.) between non-scalped and scalped plots showed that nitrogen levels decreased from an average of 0.6 ± 0.07% in non-scalped plots to 0.1% ± 0.02% in scalped plots, and Colwell phosphorus decreased from an average of 191.6 ± 34.9 ppm in non-scalped plots to 40.3 ± 11.3 ppm in scalped plots (Fig. 7.1).

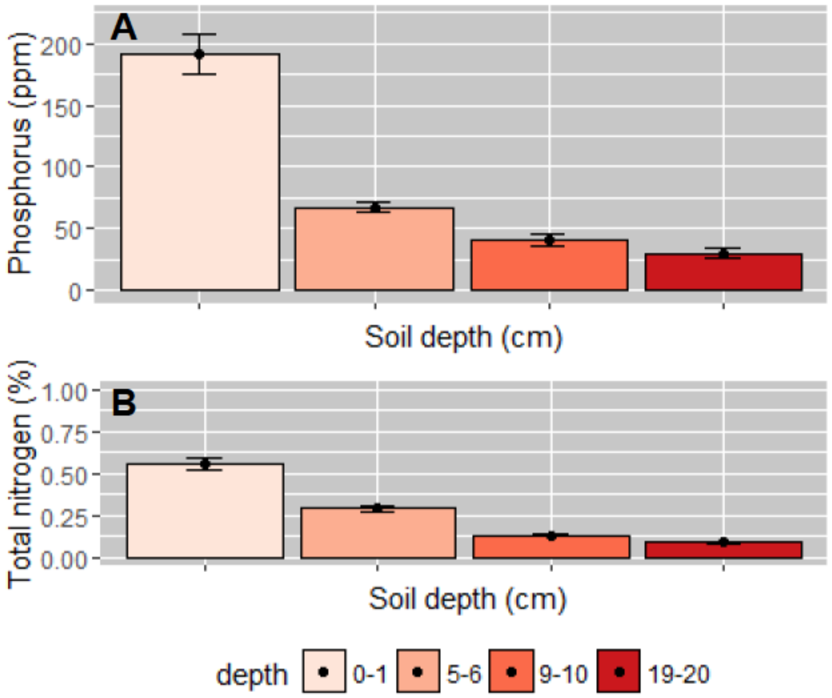

Figure 7-1 (A) Soil phosphorus (mean ± 1 s.e.) and (B) total nitrogen concentration (mean ± 1 s.e.) at different soil depths

7.4.2 Effect of weed control treatments on native grass germination

Native grass recruitment varied with weed control treatment (Fig. 7.2, Table 7.2). Recruitment was significantly greater in the scalped treatment than other weed control treatments ($P < 0.001$ for all comparisons). Not all scalped plots contained large numbers of germinants. One plot towards the lower end of the study site accounted for 76% of recruitment across all scalping treatments (Fig. 7.3A). Good soil-moisture conditions were maintained in this plot after rain. Minimal recruitment occurred in an adjacent scalped plot because it was prone to waterlogging and remained inundated for long periods of time (Fig. 7.3B). Recruitment was marginally higher ($P = 0.051$), on average, in the sawdust/glyphosate treatment than the sugar/glyphosate treatment.

Table 7-2 Coefficients for the estimates, standard errors (SE) and *P*-values for recruitment of native grasses and weed emergence compared to treatment

Variables	Coefficient	SE	*P* value
Recruitment			
Intercept	3.53	0.08	$2.0e^{-16}$
Sugar /glyphosate	−3.89	0.66	$3.5e^{-09}$
Glyphosate	−3.15	0.42	$8.3e^{-14}$
Sawdust /glyphosate	−2.44	0.39	$2.7e^{-10}$
Weed emergence			
Intercept	−0.49	0.47	0.31
Sugar /glyphosate	−1.59	0.67	0.03
Scalping	−0.73	0.67	0.28
Sawdust/glyphosate	−0.71	0.67	0.03

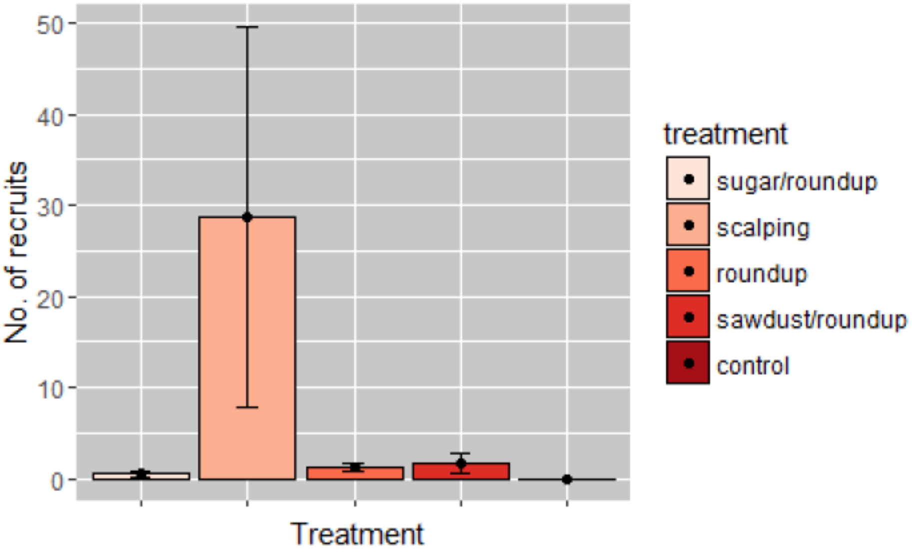

Figure 7-2 The effect of weed control treatment on the number of recruits (mean ± 1 s.e.) 16 weeks post-sowing

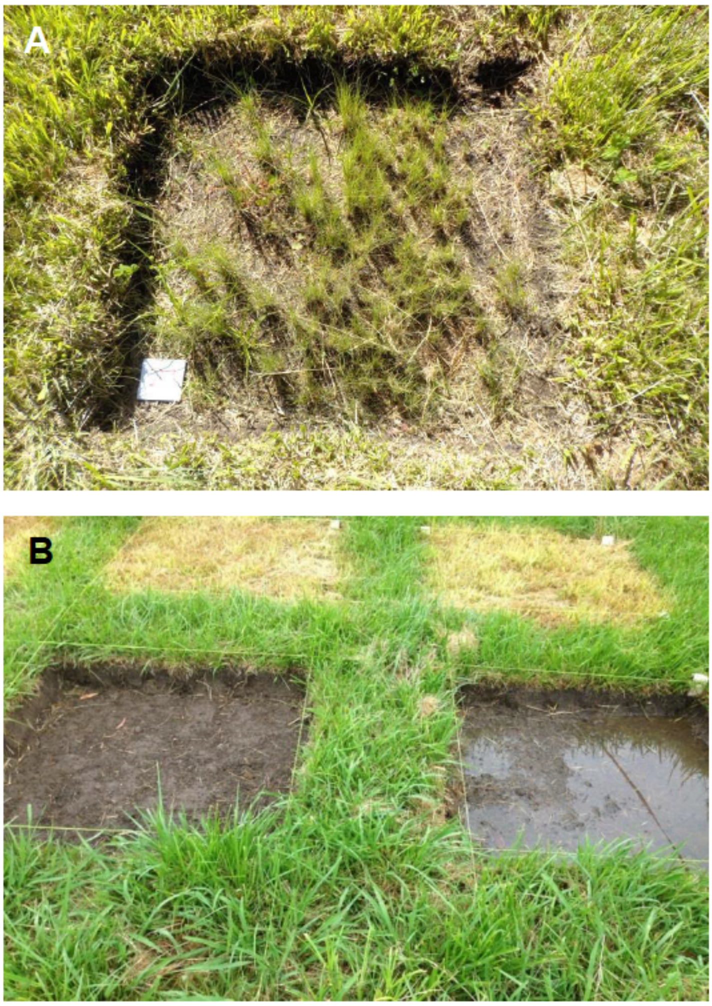

Figure 7-3 (A) Scalped plot 12 weeks post-sowing showing high germination, and (B) inundated after rain

Source: Photographs by Sharon Brown

7.4.3 Effect of treatments on weed cover

Weed cover varied among treatments (Table 7.2). Compared to the glyphosate treatment, weed cover was significantly lower in the sugar/glyphosate ($P = 0.03$) and sawdust/glyphosate treatments ($P = 0.02$) (Fig. 7.4). There were no significant differences between sugar/glyphosate and sawdust glyphosate ($P = 0.85$) or between sugar/glyphosate and scalping ($P = 0.21$). Sixteen weeks after weed control treatments were applied, the amount of weed cover in scalped plots was estimated to be 50% and consisted mostly of small broadleaf weeds. These species were present in the surrounding landscape, which suggests that the weed seeds arrived at the site by wind dispersal or could have been present as deeply buried seed. The weed cover in plots treated with glyphosate was estimated to be 75% and consisted of large mature exotic grasses.

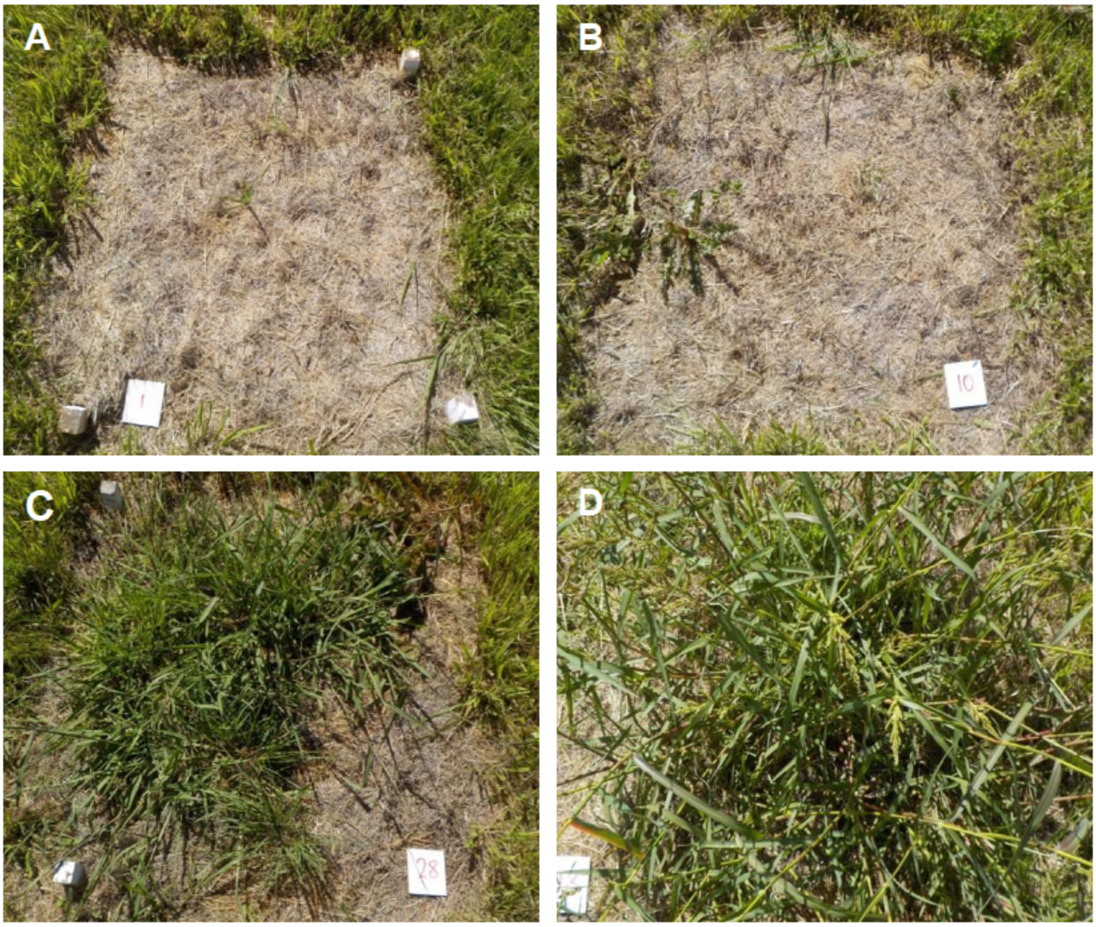

Figure 7-4 Weed cover in weed control treatments: (A) sugar and glyphosate, (B) sawdust and glyphosate, (C) glyphosate only, and (D) control

Source: Photographs by Sharon Brown

7.5 Discussion

The results reported here are from a small-scale study. Although such a study can only provide preliminary insights into the effects of scalping and carbon addition on native grass recruitment and weed emergence in field-scale revegetation trials, these weed control techniques have not been previously trialled in this region, and so the study contributes positively to the current inadequate understanding of grassland restoration on the Northern Tablelands of NSW.

The scalped plots became inundated after heavy rain, which may have confounded the results. For plots at the high end of the site, the water drained quickly, but plots at the lower end of the site retained water longer, with a negative effect on native grass recruitment in the plot where water ponded the longest but a seemingly positive effect where drainage was intermediate. While the potential drainage problem was taken into account at the planning stage of the experiment and drains were incorporated into the design, the summer rainfall overwhelmed the drainage system.

Poor weed control accounts for the vast majority of restoration failures (Landcare 2003; Carr *et al.* 2009; Willoughby and Jinks 2009; Gibson-Roy *et al.* 2010; Cole *et al.* 2016). Good weed management frees up vital resources, allows soil moisture to accumulate in the soil profile, and maintains a bare seedbed long enough to allow the recruitment and establishment of slow-growing native grasses, all of which are critical for successful grassland repair (Hagon and Chan 1977; Lloret *et al.* 1999; Windsor *et al.* 2000; Close and Davidson 2003; Engelbrecht *et al.* 2005; Mercuri *et al.* 2005; Padilla and Pugnaire 2007; Carr *et al.* 2009). Our hypothesis that scalping would favour higher native grass recruitment was supported. However, in terms of weed control, both scalping and carbon addition (sugar and sawdust) combined with glyphosate were effective in reducing weed emergence. We suspect that native grass recruitment would have been higher in the carbon addition treatments if the dead groundcover had been removed before sowing. This is because bare substrate allows greater soil–seed contact and therefore greater access to soil moisture (Khurana and Singh 2004), but also because dead organic matter cycles nitrogen back into the soil profile and favours nitrophilic weeds (Prober *et al.* 2002b).

7.5.1 The benefits of scalping for grassland restoration

Of the four weed control treatments, scalping was the most successful in terms of native grass recruitment. For the scalped treatment, recruitment was 57 times higher than the sugar/glyphosate treatment, 25 times higher than the sawdust/glyphosate treatment and 17 times higher than glyphosate alone. This was probably due to a number of factors. Scalping reduces weed seeds in the soil seed bank, given most seeds are concentrated in the top layer of soil (Morgan 1998). Soil seed banks in degraded native grassland remnants are typically dominated by annual exotic grasses and broadleaf weeds (Morgan 1998; Blumenthal 2006; Gibson-Roy *et al.* 2014). Several studies in Australia and elsewhere have reported successful restoration of grasslands following scalping due to reduced competition from weeds (Semple and Koen 1997; Allison and Ausden 2004; Buisson *et al.* 2006; Gibson-Roy 2008).

Furthermore, scalping addresses the underlying issue of soil nutrient enrichment by removing excess nitrogen and phosphorus with the topsoil (Tallowin and Smith 2001; Prober *et al.* 2002b; Prober *et al.* 2004; Härdtle *et al.* 2006; Gibson-Roy 2008; Perry *et al.* 2010). In our study, scalping decreased soil nitrogen from 0.6% to 0.1%, and Colwell phosphorus from 192 to 40 ppm. Enriched topsoil favours the invasion of exotic annuals adapted to resource-rich soils, and constitutes a major disturbance in grassland ecosystems worldwide (Allcock 2002; Prober *et al.* 2002b; Leishman *et al.* 2007; Perry *et al.* 2010; McIntyre 2011). Soil nutrient influx has been linked to several natural and anthropogenic activities, but human activities produce more nitrogen than all natural processes combined (Scherr and McNeely 2008). The main contributor to soil nutrient influx in Australian grassland communities is the application of agricultural fertilisers (Prober *et al.* 2002a; Perry *et al.* 2010). Scalping also appeared to improve soil moisture retention in this study, but this was attributed to the layout and design of the scalped plots and would not necessarily be repeated in scalping or revegetation trials with a different design.

Although scalping has yielded promising results in grassland restoration overseas (Tallowin and Smith 2001; Buisson *et al.* 2006), its implementation as a weed control strategy in Australia has been slow to gain popularity. Nevertheless, several studies implementing both scalping and topsoil inversion in south-eastern Australia have demonstrated that these techniques are superior to other methods of weed control (Gibson-Roy 2008; Gibson-Roy *et al.* 2010). The main disadvantage of scalping is that it is intrusive and has the potential to exacerbate existing disturbances by altering soil structure and chemistry and removing native propagules that may be present in the seed bank. Additional concerns are that scalping may negatively affect mycorrhizal communities that form important symbioses with native species (Perry *et al.* 2010; Gibson-Roy *et al.* 2014).

7.5.2 The benefits of carbon addition combinations with glyphosate for grassland restoration

In our study, the sugar/glyphosate and sawdust/glyphosate treatments were equally effective in reducing weed emergence. These treatments incorporated a combination of ‘top-down’ and ‘bottom-up’ techniques that simultaneously reduced existing weed biomass, while addressing the underlying issues of topsoil nutrient enrichment (Prober *et al.* 2004; Prober and Lunt 2008; Gibson-Roy *et al.* 2010). Carbon addition performed a similar function to scalping by reversing elevated soil nutrients, but it is effective only in reducing nitrogen and does not rectify high-phosphorus content should such occur. Carbon addition promotes an increase in carbon-limited micro-organisms in the soil, which take up nitrogen and reduce the amount of soil nitrogen available to exotic weeds (Perry *et al.* 2010). This provides a window of opportunity for slower-growing native species to germinate and establish.

Studies in American prairies have shown that carbon addition does not always favour desired species over invasive species (Huddleston and Young 2005; Vinton and Goergen 2006). This can vary with methods of carbon application and environmental conditions, and is probably related to variations in the nitrophilic properties of exotic weeds relative to native species (Blumenthal *et al.* 2003; Spiegelberger *et al.* 2009; Perry *et al.* 2010; Morris and de Barse 2013; Cole *et al.* 2016). Low nitrogen-requiring species, upon which carbon addition has minimal effect, have been reported in Australia and overseas (Blumenthal *et al.* 2003; Spiegelberger *et al.* 2009; Cole *et al.* 2016). In addition, purchasing, transporting and applying large quantities of sugar (5 t/ ha) is unlikely to be practical or affordable and will probably restrict its use to small remnants or highly valued conservation areas, particularly as the effects are temporary and reapplication is required every 3 months (Perry *et al.* 2010; Morris and de Barse 2013). However, this study showed that applying sawdust was equally effective in controlling weeds and could be a cost-effective alternative for large areas of grassland restoration. Spiegelberger *et al.* (2009) proposed that sawdust addition is a potent tool for mountain grassland restoration as it reduces above-ground productivity and biomass within a short period and has the added advantages of preserving the topsoil and perennial soil seed bank.

7.6 Conclusions

Effectively managing the restoration of grassland ecosystems should initially focus on identifying and reversing disturbances that lead to degradation. Elevated soil nutrients due to repeated applications of agricultural fertiliser play a major role in the disturbance history of grassland remnants in rural landscapes by favouring the invasion of weed species adapted to nutrient-rich soils (Prober *et al.* 2002b; Perry *et al.* 2010). Our results confirm that scalping and the addition of sugar and sawdust, combined with pre-sowing applications of glyphosate, are effective weed control strategies for restoring native grasslands. However, as sugar application is expensive, sawdust application would be an invaluable alternative for large areas requiring restoration. Our study showed that ample soil moisture increased the recruitment of native grasses, so ensuring a full soil moisture profile prior to sowing, correlating planting times with seasonal rainfall and irrigating when required, are important strategies for alleviating moisture stress (Hagon and Chan 1977; Carr *et al.* 2009).

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(To appear at the end of each thesis chapter submitted as an article/paper)

We, the PhD candidate and the candidate's Principal Supervisor, certify that all co-authors have consented to their work being included in the thesis and they have accepted the candidate's contribution as indicated in the *Statement of Originality*.

CHAPTER 7 Top Soil Removal and Carbon Addition are Effective Weed Control Strategies for Recruiting Native Grasses on The Northern Tablelands of New South Wales

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9 January 2017

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Type of work	Page number/s
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Fig. 7.3	p. 142
Fig. 7.4	p. 143
Table 7.1	p. 138
Table 7.2	p. 141

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CHAPTER 8

Synthesis and General Conclusions

8.1 Introduction

This thesis investigated the barriers preventing the successful revegetation of temperate upland pastures. It is framed within the conceptual framework of the threshold model for ecological restoration. The primary aim of the research was to evaluate the effectiveness of tubestock planting and direct seeding as revegetation techniques and develop on-ground practices to assist and inform the restoration of upland pastures on the Northern Tablelands of New South Wales. This research focussed on aspects of current revegetation practice on the Northern Tablelands that need improvement. The research is important because, despite substantial efforts in recent decades, planted seedlings and direct-seeded recruits have often failed to establish, resulting in poor long-term survival. Revegetation of upland pastures in the most intensively developed parts of the Northern Tablelands is critical to reverse land degradation resulting from extensive land clearing and other land-use practices, and to keep pace with tree decline associated with the dieback of eucalypts (Hobbs 1993; Nadolny 2008). Revegetation provides important ecosystem services that increase primary productivity, thus supporting production-dependent rural livelihoods (Scherr and McNeely 2008). It is also essential for increasing habitat and restoring the fine-scale connectivity between remnants of native vegetation in these fragmented and variegated landscapes. Doing so reduces the risk of extinction of native fauna and flora and provides opportunities for migration in response to climate change (Lindenmayer and Burgman 2005). Successful restoration of upland pastures on the Northern Tablelands, therefore, plays a pivotal role in reconciling agriculture with nature.

The objectives of this concluding chapter are to: (1) summarise the main findings of this research; (2) explain the significance of the research in relation to our conceptual understanding of ecosystem restoration; (3) briefly discuss the limitations of the research, and (4) provide recommendations for future research.

8.2 Summary of main findings and conclusions

Chapter 2 identified the types of biotic and abiotic stresses most likely to affect the performance of eucalypts in upland pastures across northern NSW. The survival and growth of 6-year old eucalypts planted in farm shelterbelts were evaluated and a range of environmental variables were measured in 2013 and 2014. Multimodel inference was used to select the best-fitting model from a subset of candidate models. *Eucalyptus viminalis* was the best survivor, followed by *E. pauciflora* and *E. nitens*. *Eucalyptus nitens* was the fastest growing species compared to *E. viminalis* and *E. pauciflora*, but this became a liability in the drought of summer 2013–2014. Cold temperature, weed competition and drought were important stresses influencing the survival and growth of these plantings. Survival of *E. nitens* and *E. viminalis* declined with increasingly cold temperatures, whereas *E. pauciflora* survival increased with declining minimum temperature. Both *E. nitens* and *E. viminalis* were able to tolerate competition from invasive weeds better than *E. pauciflora*, the survival of which was associated with fewer weeds. Height of all three species was negatively influenced by cold temperature.

Chapter 3 investigated the effects of two types of tree guard (tall Corflute® guards and milk cartons) on the survival and growth of five native tree and shrub species (*Leptospermum polygalifolium, Callistemon pungens, Eucalyptus viminalis, E. stellulata* and *E. viminalis*) planted in an open upland pasture near Uralla, NSW. At the lower-slope site, seedling survival was not affected by guard type. However, mean seedling height was greater in tall guards than milk cartons for all species except for the shrub *L. polygalifolium*. At the mid and upper-slope sites, survival and seedling height were significantly greater in tall guards than milk cartons. A major finding of this study was that day-time temperatures were consistently higher in tall guards than in milk cartons or under ambient conditions, resulting in increased survival and growth in the former.

Chapter 4 compared the effects of three sowing methods (KB seeder, Chatfield seeder and hand-sown surface seeding) and three bulking materials (rice, chicken crumble and smoked vermiculite) on the recruitment of direct-seeded acacias and eucalypts. The KB seeder was more effective for direct seeding than the Chatfield seeder, accounting for the majority of recruitment. Broadcasting seed by hand was unsuccessful as a direct-seeding method. Recruitment did not vary among bulking materials. The recruitment of eucalypts was low compared to the recruitment of acacias, as reported in previous work (Curtis 1990a; Li *et al.* 2003).

The survival of new recruits declined from 8 weeks post-sowing for acacias and 15 weeks post-sowing for eucalypts by 78% and 37%, respectively. The main factors responsible for declining survival through time appeared to be frost, excessive rain resulting in waterlogging and a weed invasion of rat's tail fescue in late winter–early spring.

Chapter 5 examined the potential of selective herbicides to be used as over-sprays on direct-seeded revegetation. The tolerance of 11 species of 5-month old native tree and shrub seedlings to eight herbicides was tested. Imazethapyr, isoxaflutole, prosulfocarb and oxyfluorfen showed the most promise as potential oversprays in direct seeding. The most tolerant species to the broadest range of herbicides species were *Dodonaea viscosa*, *Acacia pendula* and *Senna artemisioides*, while *Atriplex nummularia*, *Casuarina cristata* and *Geijera parviflora* were least tolerant. Individual species responded differently to each herbicide, demonstrating strong species selectivity. Notable findings included the capacity for species of *Acacia* to tolerate imazethapyr and for species of *Eucalyptus* to tolerate oxyfluorfen and prosulfocarb, because species in these genera are most commonly used in direct seeding. All species exhibited little or no tolerance to diflufenican and glyphosate, which makes them unsuitable as herbicide oversprays in direct seeding.

Chapter 6 investigated the effects of three seed coating treatments (coated seed, coated seed with MycoApply® and uncoated control seed), four water regimes (30 mL per day, 30 mL every 3 days, 30 mL per 5 days, and no watering) and two sowing methods (surface-sown seed vs seed burial beneath a 5-mm vermiculite layer) on the recruitment of eucalypts. The nested term (sowing method nested within the interaction of coating treatment and water regime) was significant, indicating the importance of all three factors for eucalypt germination. Germination was higher for seed coated with MycoApply® than uncoated seed and coated seed under the 30 mL/3days watering regime for buried seed, but not for surface seed. The difference in germination for seed coated with MycoApply® under 30 mL/day between surface-sown seed and buried seed was not significant. Coating treatment did not influence seedling growth because water was not a limiting factor so there was no opportunity for microbial inoculation to enhance water capture.

Chapter 7 examined the potential of four weed control treatments – scalping (the removal of the top 10 cm of topsoil), glyphosate (Roundup®) herbicide, and combinations of glyphosate plus carbon (sugar and sawdust) amendment – to enhance native grass recruitment and control emerging weeds. Scalping was the most successful treatment in terms of native grass recruitment. Native grass recruitment in the scalped treatment was 57 times higher than the sugar/glyphosate treatment, 25 times higher than the sawdust/glyphosate treatment and 17 times higher than the glyphosate only treatment. Weed cover varied with treatment. Scalping and carbon addition combined with glyphosate were the most effective treatments for inhibiting weed emergence.

8.3 Synthesis

The aim of the research was to evaluate the effectiveness of two revegetation techniques (tubestock planting and direct seeding) and develop on-ground practices to assist and inform the restoration of upland pastures on the Northern Tablelands of New South Wales. The threshold model was used as a theoretical framework for this work to help conceptualise a range of barriers (or thresholds) that prevented the successful establishment of native trees and shrubs in upland pastures on the Northern Tablelands of NSW. Once barriers are identified, appropriate restoration treatments can be implemented. The threshold model of ecological restoration was originally introduced to define boundaries in space and time among multiple stable communities that can exist at a single site (Briske *et al*. 2005). It accounts for the fact that that vegetation dynamics can be continuous and reversible, or discontinuous and irreversible. Continuous and reversible vegetation dynamics exist within stable vegetation states, whereas discontinuous and non-reversible dynamics occur when thresholds are crossed and a stable state transitions into an alternative state (Briske *et al.* 2005). Changes in vegetation communities can be triggered by natural or human-induced events that cause a threshold to be breached. The severity of the event dictates whether the change can be reversed. The ability of the community to revert to its pre-disturbed state is a measure of community resilience. Usually, once a threshold is crossed, restoration is needed to place the community on a positive trajectory towards recovery (Stringham *et al.* 2003; James *et al.* 2013).

Various types of ecological thresholds have been discussed in the literature. Whisenant (1999) categorised thresholds according to whether they are created by biotic or abiotic disturbances. Biotic thresholds are due to interference from organisms such as invasive weeds or introduced vertebrate pests. An example of a biotic threshold is described in a recent study by Gooden *et al.* (2009), who demonstrated a threshold of 75% vegetation cover in the invasive thicket-forming weed *Lantana camara*, above which native plant species rapidly declined. The decline was accompanied by a shift in vegetation structure from tall open schlerophyll forest to low, dense lantana-dominated shrubland. Restoration and management decisions focused on maintaining lantana infestations below the 75%-cover threshold (Gooden *et al.* 2009).

Abiotic thresholds relate to physical barriers such as dysfunctional hydrological processes or a harsh microclimate (Whisenant 1999). An example of an abiotic threshold negatively impacting the survival of native trees is reported by Cramer *et al.* (2004). The authors were able to quantify the position of a threshold in woodlands in south-western Australia by investigating the effects of small changes in elevation (and depth to a saline water table) on eucalypt health. Complete tree mortality was reported at elevations below a threshold of 0.5 m in contrast to healthy trees at higher elevations (Cramer *et al.* 2004).

The first important step towards facilitating the recovery of a vegetation community is to identify whether the threshold that has been breached is biotic or abiotic as restoration approaches differ for each. Intervention for biotic thresholds should be based upon removal of problematic species, as well as introducing appropriate species (Whisenant 1999). Addressing issues caused by abiotic disturbances is more complex and requires (1) controlling the flux or resources (e.g. soil, water, nutrients) into and out of the community, and (2) managing the physical micro-environmental conditions (Whisenant 1999).

Hugget (2005) described two different types of thresholds in degraded ecosystems – point and zone thresholds, which relate to the rate of change across a threshold. Point thresholds are usually preceded by an accumulation of factors, which upon reaching a critical tipping point, cause an abrupt change from one state to another. Zone thresholds represent a gradual or transitional shift from one state to another (Hugget 2005). According to Wiens *et al.* (2002), thresholds are regions or zones in which the rate of change is correlated to the distance of points from the threshold. For example, points within the zone of transition have similar rates of change compared to those outside the zone. This may be attributed to differences in the level of resilience of a community or the amount of disturbance that a community is capable of absorbing before transitioning into an alternative state (Wiens *et al.* 2002).

In this thesis, both biotic and abiotic barriers to farmland revegetation were identified. Each one is a limiting process with the potential to act as a critical restoration threshold resulting in the failure of direct seeding and tubestock planting in temperate upland pastures. These are depicted in Fig. 8.1 in combination with a range of revegetation strategies investigated to reverse their effects. The biotic thresholds included: (1) competition with invasive weeds along direct-seeded riplines and in planting beds; (2) the predation of surface-sown seed by harvesting ants, and (3) germination and establishment limitations arising from the small size of eucalypt seeds. The strategies implemented in this thesis to reverse degradation caused by these factors are discussed in Chapters 4, 5 and 7, and are revisited here:

1. Weed competition. Herbicide oversprays have the potential to improve revegetation efforts by exploiting the differences in herbicide tolerance between desired native species and weed species. In Chapter 2, we identified the importance of effective and timely weed control for the establishment of planted seedlings, which had a detrimental long-term effect on the survival and growth of mature trees. Scalping (topsoil removal) and carbon addition assisted native grass recruitment by reducing the abundance of weed seeds stored in the soil seed bank and depleting nitrophilic weeds of the soil nutrients they require for fast growth and establishment.

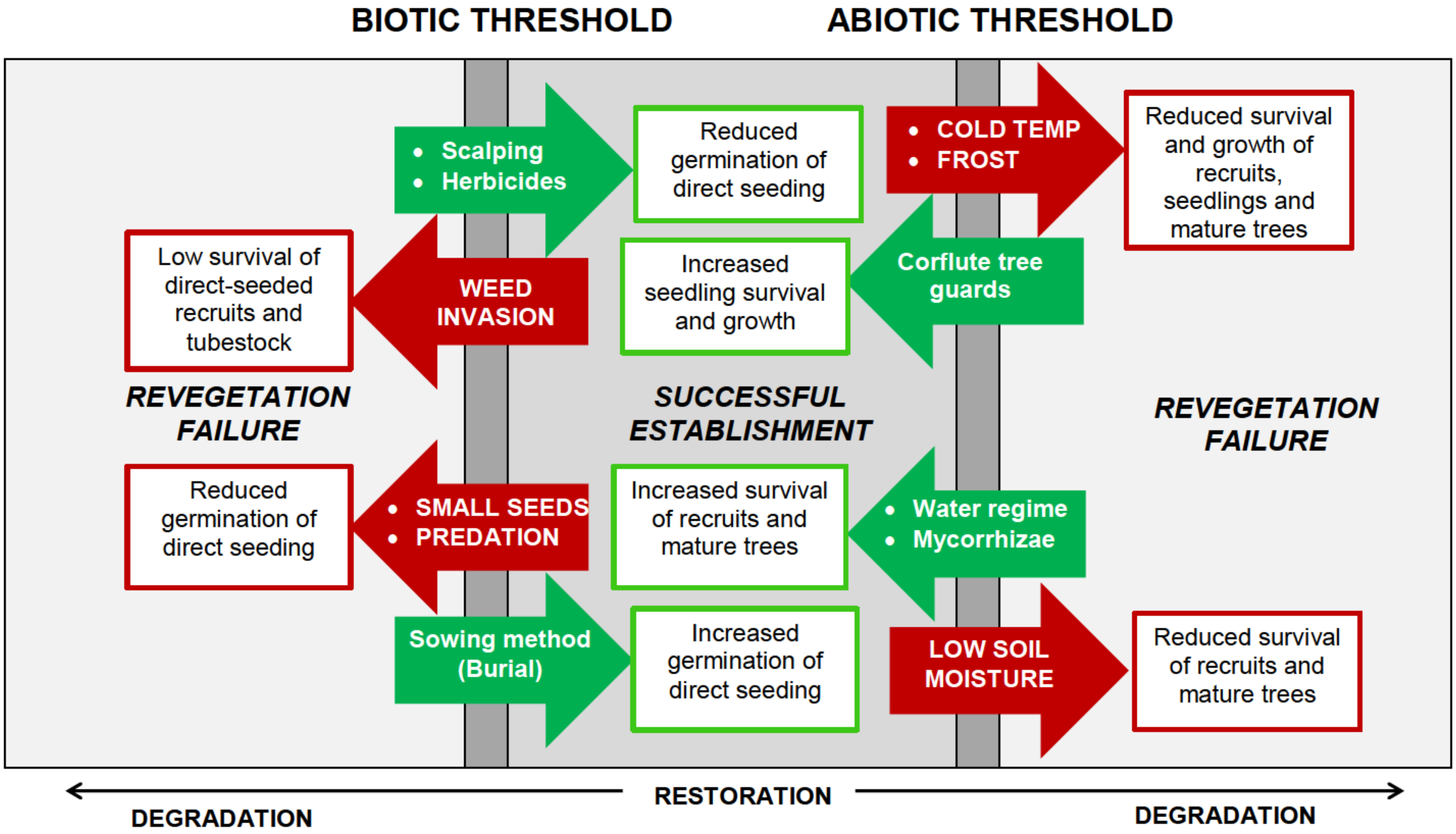

Figure 8-1 Degradation of farmland revegetation showing the effects of biotic and abiotic thresholds and the strategies implemented to overcome these thresholds

2. Seed predation. Choosing the correct sowing technique is important for maximising the germination of direct-seeded native species. Mechanical seeders that sow the seed beneath the soil surface mitigate the risk of ant predation.

3. Small seed size. Seed burial is also crucial for the establishment of small-seeded eucalypts, which due to a lack of seed endosperm, are vulnerable to desiccation during the early post-germination phase, given they are reliant on tapping into external resources immediately after the radicle emerges from the seed. Mechanical seeders that sow the seed beneath the soil surface mitigate the risk of seed desiccation due to poor seed–soil contact.

The abiotic thresholds identified in this thesis included: (1) cold temperatures and frost, and (2) lack of soil moisture. The strategies implemented in this thesis to reverse degradation caused by these factors are discussed in Chapters 3 and 6:

1. Sub-zero temperatures. Using tall Corflute® tree guards to ameliorate the effects of cold temperature and frost, as opposed to the use of milk cartons, is beneficial for the survival and growth of planted native seedlings. Tall guards increase the temperature surrounding the seedling and extend the growing period.

2. Dry soil. Implementing watering regimes to ensure continuous soil moisture during the germination and early establishment of eucalypt recruits is important for reasons discussed above. When imbibition is interrupted, seed mortality is high (Battaglia and Read 1993). One way of enhancing drought resistance in early-phase germinants is by ensuring immediate contact of the emerging radicle with beneficial microbes, such as EM and AM symbionts, which increase the surface area of host roots and enhance water capture from the surrounding soil. Coating seed with

beneficial microbial inoculants is a novel revegetation strategy demonstrated in this thesis, which has the potential to significantly increase the recruitment of direct-seeded eucalypts.

8.4 Limitations of the study

During the course of this research, potential study limitations were identified. Firstly, in Chapter 2, the study was limited by the non-scientific approach taken in the engineered woodlands project investigated, a common failing in farmland revegetation projects. The engineered woodlands were typical of the majority of revegetation occurring on the Northern Tablelands in that records were absent or incomplete, insufficient attention was given to statistical design prior to planting, and poor monitoring procedures were implemented. This made the post hoc evaluation of the project difficult. In addition, trees are long-lived and the capacity to monitor tree performance from germination to maturation is not possible in a three-year PhD study. While this study was admittedly a ‘snapshot’ in time of the trees’ total lifespan, through the implementation of sophisticated statistical modelling it was possible to identify stress factors impeding survival and growth in planted 6-year old eucalypts. Despite the limitations, it is important to evaluate whether scientifically useful information can be derived from the revegetation projects that have been carried out in the region in recent decades.

Secondly, some of the studies in this thesis were limited by experimental design. The study undertaken in Chapter 3 was limited by small sample size at the lower-slope site. This was rectified in the mid and upper-slope plantings, but the potential for false negative results for the lower slope site were greater due to the low statistical power of the analyses. The differences in planting times between the lower slope site and the mid and upper-slope sites prevented comparisons of seedling survival and height, but were beyond the control of the study.

The studies undertaken in Chapters 5 and 6 were limited because they were conducted in a glasshouse. While the glasshouse environment was useful for investigating the effects of herbicide oversprays on seedling survival (Chapter 5) and the effects of seed coating treatments, water regime and sowing method on eucalypt germination (Chapter 6) without the confounding of heterogeneous environmental factors, the controlled conditions were not representative of real-world situations. For this reason, complementary field experiments are required. The study undertaken in Chapter 7 was a pilot study and needs to be performed at field revegetation scale. Mitigating the effects of soil enrichment on native grass recruitment is an important restoration activity, given the widespread use of superphosphate and other fertilisers in the region. In addition, the scalped plots in the weed control experiment were prone to flooding. While this problem was foreseen at the experimental design stage and drains were implemented, the results were variable due to the retention of water in some plots and not others.

Finally, while revegetation has been used extensively to increase native tree cover on the Northern Tablelands, current methods have limited application in terms of re-establishing eucalypts in highly degraded landscapes. Widespread and recurrent eucalypt dieback is testimony to underlying ecosystem dysfunction. Tubestock planting and direct seeding of eucalypts over a fraction of the landscape, as currently practised, may not be dependable strategies, as revegetation may be ineffective in providing the solution to a broader, more fundamental problem. The level of intervention needed to reverse degradation and reinstate ecosystem function to shift affected areas into a trajectory of recovery, may have to involve restoration works at a much greater scale than has been considered previously in revegetation in the region and during planning of this research.

8.5 Recommendations for future research

The field of revegetation science is severely limited by the pervasive lack of rigour in analysing, reporting and responding to the results of data collected (Field *et al.* 2007). Effective monitoring allows a comprehensive assessment of revegetation successes and failures, and provides a strong platform to guide management decisions and facilitate improvements. A stronger research focus for farmland tree plantings to ensure the collection of reliable data, and better collaboration between scientists, community members and volunteers are needed.

The effects of tree guards on the growth and survival of tree seedlings is well established, particularly in forestry. However, an avenue of further study should be to investigate the potential of solid-walled polypropylene guards to modify the microclimate inside tree guards. In this thesis, only changes in temperature inside tree guards were measured. Studies quantifying the effects of other microclimatic variables on seedling survival and growth, such as humidity, light transmission and dew harvesting, are needed.

Herbicide oversprays have the potential to improve the establishment of direct-seeded recruits. Further research is required to examine the tolerance of direct-seeded native seedlings in the field, and trial the approach of sowing the seeds of species with similar tolerances to particular herbicides in separate rows, then over-spraying the various rows with the corresponding herbicide to control weeds after seedling emergence.

Finally, the glasshouse experiments performed in this body of research (Chapters 5 and 6) need to be repeated in the field to determine their usefulness potential revegetation strategies.

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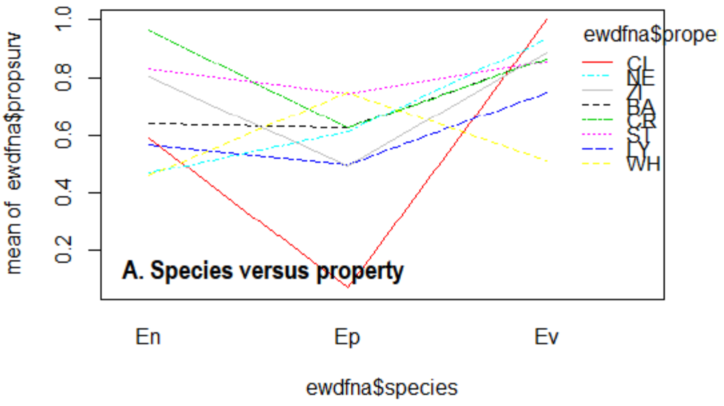
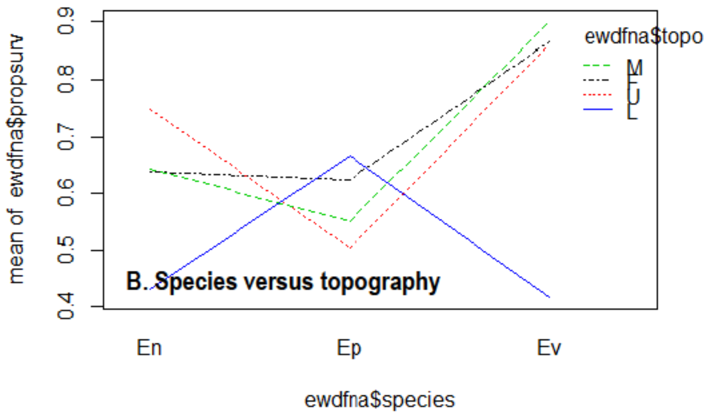
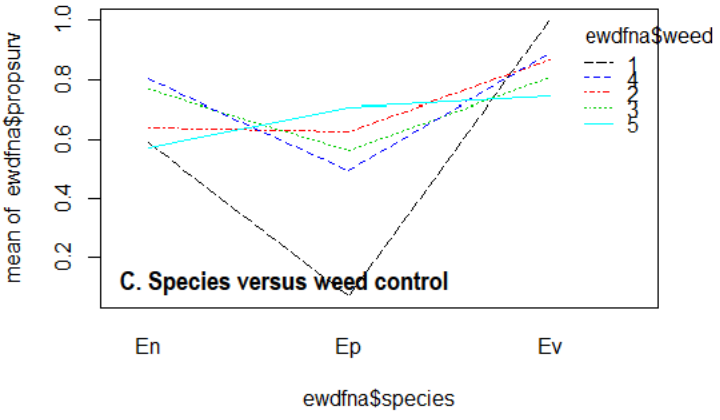
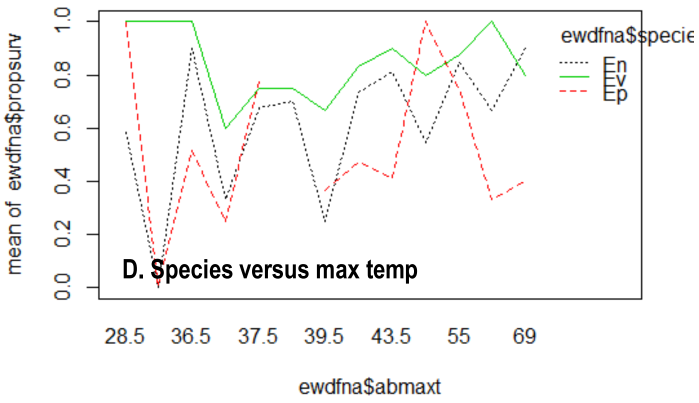
Appendix 1 Exploratory plots for Chapter 2








D. Species versus max temp
mean of ewdfna$propsurv
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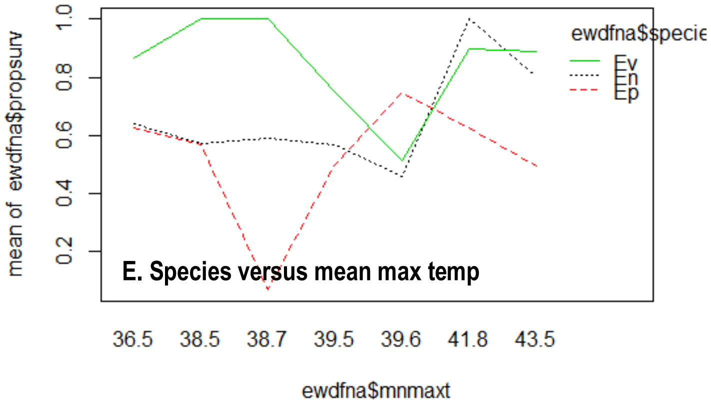
E. Species versus mean max temp
mean of ewdfna$propsurv
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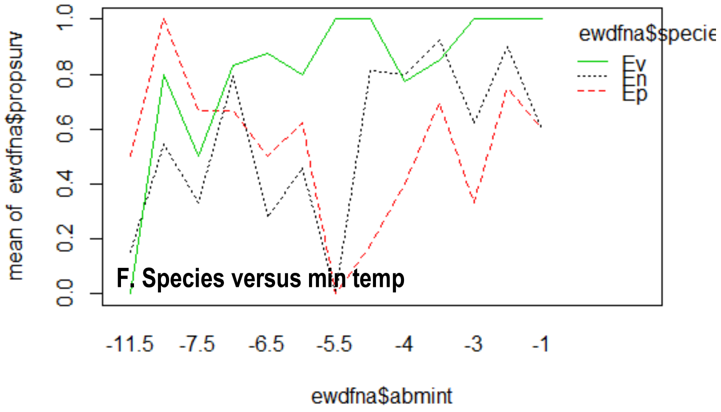
F. Species versus min temp
mean of ewdfna$propsurv
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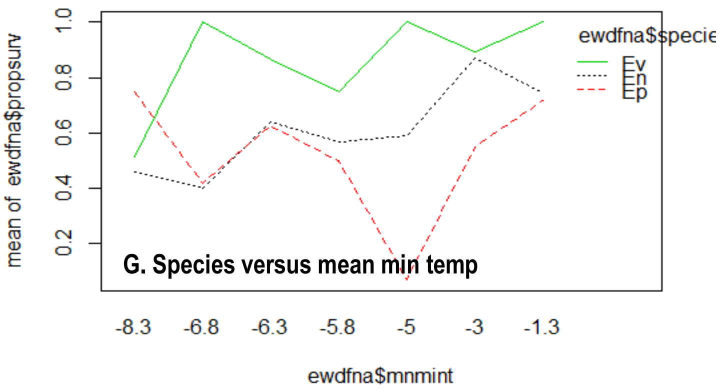
G. Species versus mean min temp
mean of ewdfna$propsurv
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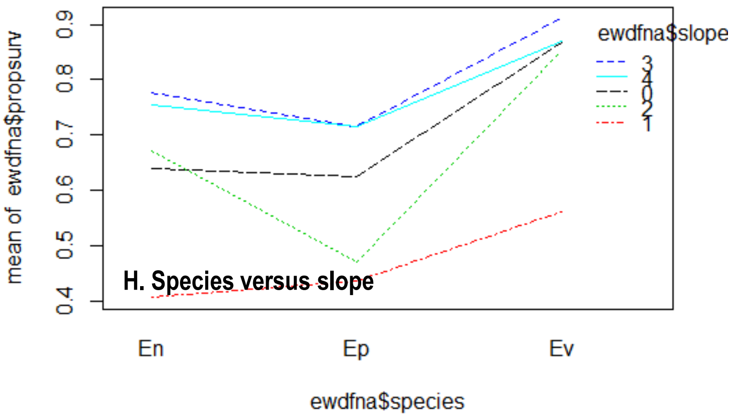
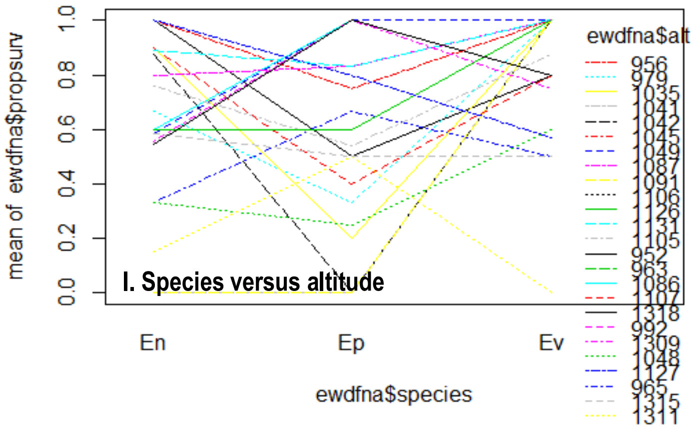
En
Ep


H. Species versus slope
mean of ewdfna$propsurv
0.4
0.5
0.6
0.7
0.8
0.9
En
Ep
Ev
ewdfna$species
ewdfna$slope
3
4
0
2
1


I. Species versus altitude
mean of ewdfna$propsurv
0.0
0.2
0.4
0.6
0.8
1.0
En
Ep
Ev
ewdfna$species
ewdfna$alt
956
979
1035
1041
1042
1045
1049
1087
1091
1106
1126
1131
1105
952
963
1086
1107
1318
992
1309
1048
1127
965
1315
1311

Appendix 2 Correlation analysis for Chapter 2

Correlations for all pairs of data series			
Pair	Pearson	Spearman	Kendall
topo;slope	0.3197	0.2505	0.2013
topo;alt	0.451	0.2561	0.1913
topo;mnmaxt	0.1762	0.1433	0.1035
topo;abmaxt	0.0069	0.0865	0.049
topo;mnmint	-0.2573	-0.1967	-0.1319
topo;abmint	-0.2597	-0.2147	-0.1479
topo;water	0.1839	0.1368	0.0991
slope;alt	-0.0625	-0.15	-0.1068
slope;mnmaxt	0.4075	0.4054	0.3244
slope;abmaxt	0.5229	0.4824	0.3752
slope;mnmint	0.2767	0.3356	0.2664
slope;abmint	0.387	0.3905	0.3051
slope;water	-0.2218	-0.2591	-0.1793
alt;mnmaxt	0.1442	0.1434	0.0294
alt;abmaxt	0.1162	-0.0371	-0.0365
alt;mnmint	-0.5729	-0.3136	-0.229
alt;abmint	-0.4777	-0.2084	-0.1667
alt;water	0.3255	0.1428	0.0845
mnmaxt;abmaxt	0.6472	0.6444	0.4985
mnmaxt;mnmint	0.3667	0.2383	0.1189
mnmaxt;abmint	0.328	0.3257	0.2324
mnmaxt;water	-0.216	-0.2184	-0.1625
abmaxt;mnmint	0.1123	0.1914	0.1342
abmaxt;abmint	0.0682	0.1477	0.1118

abmaxt;water	-0.0284	-0.1144	-0.0903
mnmint;abmint	0.8688	0.8974	0.7678
mnmint;water	-0.4781	-0.4448	-0.3131
abmint;water	-0.5316	-0.5172	-0.3463